

DÉBORAH ROMASKEVIS GOMES LOPES

**DIFERENÇAS NA COMPOSIÇÃO DA MICROBIOTA DO TRATO
GASTROINTESTINAL E DE FEZES DE NOVILHOS NELORE COM ALTA E
BAIXA EFICIÊNCIA ALIMENTAR**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 27 de fevereiro de 2018.

Cynthia Canedo da Silva
(Coorientadora)

Luciano da Silva Cabral

Edenio Detmann

Marcio de Souza Duarte

Hilário Cuquetto Mantovani
(Orientador)

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BIOGRAFIA

DÉBORAH ROMASKEVIS GOMES LOPES, filha de Geraldo Magela Lopes e Maria Auxiliadora Romaskevis Gomes Lopes, nasceu em 13 de abril de 1989, em Ponte Nova – Minas Gerais. Iniciou o curso de Ciências Biológicas, na Universidade Federal de Viçosa, em março de 2007, graduando-se em janeiro de 2012. Em março de 2012 ingressou no curso de Mestrado em Microbiologia Agrícola, na Universidade Federal de Viçosa e obteve o título de mestre em fevereiro de 2014. No mês seguinte ingressou no curso de doutorado em Microbiologia Agrícola pela Universidade Federal de Viçosa.

RESUMO

LOPES, Déborah Romaskevis Gomes Lopes, D.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Diferenças na composição da microbiota do trato gastrointestinal e de fezes de novilhos Nelore com alta e baixa eficiência alimentar.** Orientador: Hilário Cuquetto Mantovani. Coorientadores: Cynthia Canedo da Silva e Garret Suen.

A análise das interações entre o hospedeiro e o microbioma intestinal pode ser útil para entender as diferenças no fenótipo de eficiência alimentar de animais de produção. Estudos que investigam a relação entre o microbioma ruminal e a eficiência alimentar de bovinos de corte ainda são escassos, sendo que ainda não existem trabalhos com bovinos da raça Nelore, a qual se destaca pela relevância econômica na produção de carne no Brasil. Neste trabalho, os objetivos foram comparar a composição da microbiota e analisar os parâmetros bioquímicos do rúmen, intestino delgado, ceco e das fezes de 27 novilhos Nelore classificados como de alta ou baixa eficiência alimentar. Para avaliar a composição da comunidade bacteriana foi realizada a extração de DNA metagenômico das amostras do rúmen (fração sólida e líquida), intestino delgado, ceco e fezes dos animais e sequenciamento da região V4 do gene rRNA 16S. Nas amostras obtidas do rúmen também foi realizada a avaliação da composição de arqueias por meio do sequenciamento da região V6-V8 do gene rRNA 16S. Além disso, a concentração de ácidos orgânicos voláteis (ácidos acético, succínico, propiônico, valérico, isovalérico, butírico e isobutírico) foi avaliada em todos os segmentos do sistema digestivo, além da concentração de amônia e do pH no conteúdo ruminal. Foi observado que os índices de Shannon (diversidade) e Simpson (dominância) das comunidades bacterianas ruminais ($5,35 \pm 0,26$ e $0,017 \pm 0,007$, respectivamente) e fecais ($4,26 \pm 0,32$ e $0,04 \pm 0,015$, respectivamente) não foram diferentes para os dois grupos de eficiência alimentar (t-test, $P > 0,05$). Já a riqueza de arqueias no rúmen (índice de Chao) foi maior (t-test, $P < 0,05$) nos novilhos com fenótipo de alta eficiência alimentar ($45,18 \pm 11,1$) em relação aos de baixa eficiência ($36,22 \pm 8,12$). O intestino delgado foi o segmento do trato gastrointestinal com menor (Tukey test, $P < 0,05$) diversidade e riqueza de bactérias ($3,77 \pm 0,73$ e 607 ± 192 , respectivamente), bem como o de menor concentração de ácidos orgânicos voláteis ($11,57 \pm 6,65$ mmol/l). A análise de beta diversidade das comunidades microbianas não demonstrou agrupamento dos animais em função do fenótipo de eficiência alimentar (Anosim, $P > 0,01$). No entanto, foi observado agrupamento em função das

comunidades bacterianas de cada segmento do trato gastrointestinal (Anosim, $P < 0,001$). O agrupamento taxonômico das OTUs evidenciou que as famílias Lachnospiraceae ($24,61 \pm 6,58 \%$) e Ruminococcaceae ($20,87 \pm 4,22 \%$) são as mais abundantes em todo o trato digestivo de bovinos Nelore. As diferenças na composição bacteriana (*White's non-parametric t-test*, $P < 0.05$) tanto do rúmen quanto das fezes dos animais de alta e baixa eficiência alimentar foram identificadas principalmente em OTUs classificadas nessas famílias, bem como no gênero *Prevotella*. Assim, algumas estirpes específicas desses grupos parecem estar relacionadas com o aumento ou com a redução da eficiência alimentar. Foram observadas diferenças na correlação (*Sperman's rank*, $P < 0,05$) entre os parâmetros bioquímicos e a composição da microbiota ruminal dos novilhos de alta e baixa eficiência alimentar. Esses resultados demonstram que as diferenças na composição do microbioma de animais de alta e baixa eficiência alimentar se concentram em grupos microbianos com potencial fibrolítico e celulolítico, os quais são considerados relevantes para a fermentação dos componentes da dieta e, conseqüentemente, para a obtenção de energia pelo hospedeiro. Além das alterações da comunidade bacteriana ao longo do trato digestivo, evidenciaram-se algumas diferenças quanto à abundância desses grupos funcionais nas fezes dos animais. Assim, a coleta de amostras fecais pode representar uma forma não invasiva de avaliar, a partir de estudos em larga escala, a relação entre o microbioma de bovinos e fenótipos de produtividade desses animais.

ABSTRACT

LOPES, Déborah Romaskevis Gomes Lopes, D.Sc., Universidade Federal de Viçosa, February, 2018. **Differences in the microbiota composition of gastrointestinal tract and feces of Nelore steers with high and low feed efficiency.** Adviser: Hilário Cuquetto Mantovani. Co-advisers: Cynthia Canedo da Silva and Garret Suen.

The analysis of the interactions between the host and its gut microbiome may be useful to understand differences in the feed efficiency phenotype of livestock animals. Studies investigating the relationship between ruminal microbiome composition and feed efficiency of beef cattle are still scarce, being virtually nonexistent with the Nelore cattle, which is notable for its economic relevance for meat production in Brazil. In this work, we aimed to compare the composition of the microbiota and the biochemical parameters of the rumen, small intestine, cecum and feces of 27 Nelore steers with high or low feed efficiency. To access the bacterial community composition, metagenomic DNA was extracted from the rumen (solid and liquid fraction), small intestine, cecum and fecal samples and the sequencing of the V4 region of the 16S rRNA gene was performed. For the ruminal samples, we also performed the evaluation of archaeal community composition by sequencing of the V6-V8 regions of the 16S rRNA gene. In addition, the concentration of volatile fatty acids (acetic, succinic, propionic, valeric, isovaleric, butyric and isobutyric acids) was evaluated in all segments of the digestive system, as well as ammonia concentration and pH in ruminal contents. We observed that the Shannon (diversity) and Simpson (dominance) indexes of the ruminal (5.35 ± 0.26 and 0.017 ± 0.007 , respectively) and fecal (4.26 ± 0.32 and 0.04 ± 0.015 , respectively) bacterial communities were not different for the two feed efficiency groups (t-test, $P > 0.05$). On the other hand, the ruminal archaea richness (Chao index) was higher (t-test, $P < 0.05$) in high feed efficiency steers (45.18 ± 11.1) than in low feed efficiency steers (36.22 ± 8.12). The small intestine was the gastrointestinal tract segment that showed lower (Tukey test, $P < 0.05$) bacteria diversity and richness (3.77 ± 0.73 and 607 ± 192 , respectively), as well as lower concentration of volatile fatty acids (11.57 ± 6.65 mmol/l). Beta diversity analysis of the microbial communities did not show grouping of the steers according to the feed efficiency phenotype (Anosim, $P > 0.01$). However, we observed grouping according to the bacterial communities of each gastrointestinal tract segment (Anosim, $P < 0.001$). The OTUs taxonomic grouping showed that Lachnospiraceae ($24.61 \pm$

6.58 %) and Ruminococcaceae (20.87 ± 4.22 %) families are the most abundant across the digestive tract of Nelore cattle. The differences in bacterial composition (White's non-parametric t-test, $P < 0.05$) of both rumen and feces from steers with high and low feed efficiency were mainly identified in OTUs assigned in these families, as well as in the *Prevotella* genus. Thus, some specific strains of these groups appear to be related to the increase or reduction of feed efficiency. Differences in correlation (Sperman's rank, $P < 0.05$) between biochemical parameters and ruminal microbiota composition of high and low feed efficiency steers were observed. Our results demonstrate that differences in the ruminal microbiome composition of high and low feed efficiency steers are concentrated in microbial groups with fibrolytic and cellulolytic potential, which are considered relevant for the fermentation of diet components and, consequently, to the host energy-obtaining activities. Besides the changes in the bacterial community across the digestive tract, there were some differences in the abundance of these functional groups in the feces of the Nelore steers. Therefore, the collection of fecal samples may represent a non-invasive way to evaluate, by large scale studies, the relationship between bovine microbiome and productivity phenotypes.

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Introdução geral

O rebanho brasileiro de bovinos de corte é composto predominantemente pela raça Nelore, que é considerada bem adaptada às condições ambientais e ao sistema de produção “a pasto”, na qual gramíneas tropicais perenes e a suplementação mineral representam a base da dieta dos animais. O Brasil é um dos maiores produtores de carne do mundo, sendo que em 2016 o país foi responsável por mais de 15 % da produção mundial (9,28 milhões de toneladas). Estima-se que a produção de carne esteja aumentando em mais de 2 % ao ano na década atual, podendo a produção brasileira alcançar mais de 11 milhões de toneladas em 2023.

No entanto, o aumento da consciência sobre o uso da terra, o desmatamento de florestas nativas, e os debates em fóruns internacionais sobre produção de metano e outros impactos ambientais da produção animal, podem limitar a expansão das áreas para exploração pecuária, representando um obstáculo para a ampliação da produção animal. Várias estratégias têm sido estudadas com a finalidade de reduzir os impactos ambientais e aumentar a produtividade da pecuária de corte, incluindo a seleção de animais que apresentem fenótipo de maior eficiência alimentar. Especialistas no melhoramento genético de gado de corte têm propostas características fenotípicas que poderiam ser utilizados para esta finalidade. Tais características poderiam refletir no aumento da eficiência alimentar, ou seja, na redução do consumo, sem comprometer o desempenho, a eficiência reprodutiva ou a qualidade da carne dos animais.

A avaliação do consumo alimentar residual (CAR) é uma característica fenotípica que tem sido utilizada como parâmetro para estimar a eficiência alimentar independentemente do peso corporal e ganho de peso do animal. O CAR consiste na diferença entre o consumo de matéria seca observado e o consumo de matéria seca esperado, com base em algumas medidas de desenvolvimento do animal. Todavia, CAR é considerado de baixa a moderada herdabilidade em gado de corte, sugerindo que outros fatores (não genéticos) influenciam a eficiência alimentar.

A obtenção de energia pelos ruminantes depende tanto da habilidade da microbiota ruminal em fermentar os componentes da dieta e produzir ácidos orgânicos voláteis, quanto da capacidade de absorção e utilização desses nutrientes pelo animal (potencial genético). Os avanços nas técnicas para caracterização da composição de comunidades microbianas possibilitam o aprofundamento dos estudos sobre o papel

da microbiota ruminal na nutrição, crescimento e produtividade de bovinos. Para bovinos de corte, esta questão tem importância particular uma vez que praticamente toda proteína e gordura da carne são derivadas da atividade microbiana ruminal.

Dessa forma, a caracterização da microbiota ruminal de animais com alta e baixa eficiência alimentar pode fornecer informações importantes para o desenvolvimento de estratégias mais eficazes e específicas para a manipulação da microbiota visando aumentar a eficiência de conversão dos alimentos ingeridos pelo hospedeiro. No entanto, estudos que avaliam a relação entre o microbioma e a eficiência alimentar de bovinos de corte ainda são escassos. Para a raça Nelore, apenas um estudo reportou a caracterização do microbioma de Nelore, porém o estudo baseou-se na análise do trato gastrointestinal de apenas um indivíduo. Além disso, não existe na literatura trabalho onde foi investigada a associação da composição do microbioma do trato digestivo de animais da raça Nelore e fenótipos específicos do animal, a exemplo da eficiência alimentar.

Neste trabalho, foi investigada a composição da microbiota do rúmen, intestino, ceco e fezes e avaliados os parâmetros bioquímicos desses segmentos em bovinos Nelore de alta (12 animais com CAR negativo) e baixa (15 animais com CAR positivo) eficiência alimentar. No capítulo 2, avaliamos a hipótese de que existem diferenças na composição da comunidade de Bacteria e Archaea no rúmen de novilhos Nelore de alta e baixa eficiência alimentar. No Capítulo 3, o objetivo foi avaliar a composição da comunidade bacteriana no rúmen, intestino delgado, ceco e fezes dos 27 novilhos Nelore. Foi avaliada a hipótese de que novilhos Nelore com alta e baixa eficiência alimentar apresentam diferenças na composição bacteriana fecal.

Capítulo 1

Revisão de literatura

Associação entre composição do microbioma do trato gastrointestinal e eficiência alimentar de ruminantes: *An overview*

Introdução

Os ruminantes são mamíferos herbívoros e não sintetizam enzimas responsáveis pela degradação dos componentes fibrosos da dieta (celulose, hemicelulose e lignina). No entanto, estes são capazes de estabelecer relações simbióticas com microrganismos anaeróbios capazes de fermentar alimentos de diferentes origens, como carboidratos solúveis e insolúveis, proteínas e lipídeos (Jami and Mizrahi, 2012; Kamra, 2005). Com o desenvolvimento de técnicas refinadas de biologia molecular e disponibilidade de informações genômicas em bancos de dados públicos, a utilização de métodos independentes de cultivo e abordagens metagenômicas para caracterização de ecossistemas microbianos complexos têm sido cada vez mais frequente (Franzosa et al., 2014).

Estudos demonstraram que microrganismos residentes no trato gastrointestinal desempenham papel importante na fisiologia do hospedeiro (Arumugam et al., 2011; Turnbaugh and Gordon, 2009). Em camundongos e seres humanos foi demonstrada a relação entre a microbiota intestinal e características fisiológicas de obtenção de energia pelos hospedeiros (Ley et al., 2006; Turnbaugh e Gordon, 2009). Turnbaugh et al. (2009) demonstraram que a transferência da microbiota de um camundongo obeso para um magro resultou em mudanças fenotípicas significativas, como o aumento da deposição de tecido adiposo. Esse resultado evidenciou que a microbiota “obesa” possui maior capacidade de captura de energia dos componentes da dieta, fornecendo aporte extra de calorias para o animal. Considerando essas análises pioneiras, bem como o papel fundamental da microbiota ruminal para obtenção de energia pelos ruminantes, estudos têm buscado avaliar e explorar a possível ligação entre a microbiota do trato gastrointestinal e eficiência alimentar em ruminantes (Carberry et al., 2012; Guan et al., 2008; Hernandez-Sanabria et al., 2010, 2012; Jami et al., 2014; Jewell et al., 2015; Kern et al., 2016; McAllister et al., 2015; Myer et al., 2015a, 2016; Shabat et al., 2016).

Nessa revisão os resultados obtidos em diferentes estudos serão contrastados com o objetivo de traçar um paralelo entre a composição da microbiota e a eficiência alimentar em ruminantes. Tal tipo de comparação pode ser limitada uma vez que os estudos avaliam diferentes raças (potencial genético) e dietas, além de utilizarem diferentes metodologias de extração de DNA, técnicas moleculares para acesso da diversidade microbiana e *softwares* para análise dos dados, fatores descritos por influenciarem e/ou introduzirem *bias* na caracterização de comunidades microbianas (Henderson et al., 2013, 2015; Weimer, 2015). Apesar disso, buscaremos evidenciar a existência de marcadores microbianos específicos ou características da microbiota associados à eficiência alimentar.

Microbiota ruminal *versus* eficiência alimentar

Estudos pioneiros da relação entre a microbiota ruminal e o fenótipo de eficiência alimentar em bovinos

A técnica de eletroforese em gel com gradiente desnaturante (DGGE) tem sido amplamente utilizada para monitorar alterações na estrutura de comunidades microbianas (Bento et al., 2015; Carberry et al., 2012; Hernandez-Sanabria et al., 2010). A técnica se baseia na mobilidade eletroforética de fragmentos de DNA parcialmente desnaturados. Sequências de DNA amplificadas por PCR que apresentam o mesmo tamanho, no entanto possuem sequências de bases diferentes, migram de forma distinta em um gel de poliacrilamida que contém gradiente linear desnaturante, composto por uréia e formamida (Muyzer et al., 1993). Assim, é possível comparar o perfil de bandas (*fingerprint*) das comunidades microbianas submetidas a diferentes tratamentos.

A partir da utilização do DGGE, Guan et al. (2008) evidenciaram diferenças na composição bacteriana do rúmen, bem como na proporção dos produtos da fermentação ruminal de animais de alta (AE) e baixa eficiência alimentar (BE). Neste trabalho foram utilizadas novilhas de diferentes raças (mestiças, Angus e Charolês) e a eficiência alimentar (EA) foi mensurada a partir da avaliação do consumo alimentar residual (CAR) dos animais. O CAR consiste na diferença entre o consumo de matéria seca observado e o consumo de matéria seca esperado, com base em algumas medidas de desempenho animal (Koch et al, 1963). O consumo de matéria seca esperado é

estimado a partir da regressão linear do consumo alimentar mensurado *versus* medidas de desenvolvimento (como ganho médio diário de peso) para um grupo de animais na mesma dieta.

Mesmo considerando que foram utilizados animais de diferentes raças, Guan et al. (2008) observaram o agrupamento distinto dos animais de AE e BE a partir da construção de dendrogramas baseados no coeficiente de similaridade de Dice (D). Esse agrupamento também demonstrou maior similaridade entre os animais de AE (91 % de similaridade) em comparação com os animais de BE (71 % de similaridade), indicando que animais de AE compartilham mais grupos específicos de microrganismos que os de BE.

Guan et al. (2008) também demonstraram que animais de AE apresentaram proporções mais elevadas de ácido butírico no rúmen. Diferenças na concentração de ácidos orgânicos voláteis (AOV) podem indicar alterações da atividade microbiana ruminal, já que são os principais produtos da fermentação (Van Soest, 1994). As concentrações mais elevadas de ácido butírico podem indicar mudanças na população de bactérias que produzem ou metabolizam esse substrato, por exemplo. Todavia, a utilização de ferramentas que possibilitam a caracterização mais detalhada da microbiota ruminal seria necessária para afirmar esse tipo de associação. Visando a melhor caracterização da microbiota a partir do DGGE, estudos posteriores aliaram a essa técnica a excisão e sequenciamento do DNA das bandas (Hernandez-Sanabria et al., 2010) e a PCR quantitativa (qPCR) (Carberry et al., 2012; Hernandez-Sanabria et al., 2012; Zhou et al., 2009, 2010).

Hernandez-Sanabria et al. (2010) obtiveram o perfil de bandas da comunidade bacteriana do rúmen de 58 animais com diferentes eficiências alimentar (Hereford x Aberdeen Angus). Todas as bandas visualizadas foram excisadas do gel e sequenciadas (85 bandas), sendo que dessas, 74 foram identificadas e correlacionadas com a concentração de AOV ruminais. Bandas identificadas como *Prevotella* sp., *Prevotella oulorum*, *Clostridium* sp. e *Pelotomaculum thermopropionicum* apresentaram correlação com o perfil fermentativo. Não é surpreendente que, sendo a população predominante no rúmen (Stevenson e Weimer, 2007), *Prevotella* sp. esteja associada às diferenças no perfil de AOV. No entanto, a evidência de outras espécies envolvidas no processo indica que o balanço fermentativo seja reflexo das interações microbianas e não dependente de um grupo específico. Nesse estudo ainda se demonstrou que a

banda identificada como *Pelotomaculum thermopropionicum*, bactéria descrita como oxidante de ácido propiônico (Imachi et al., 2002), foi relacionada a menores concentrações desse ácido orgânico. Menores concentrações de ácido acético e butírico apresentaram associação com a banda identificada como *Moryella indoligenes*, bactéria que possui como produtos finais do metabolismo tais ácidos orgânicos (Carlier et al., 2007).

Independente do perfil de bandas, Hernandez-Sanabria et al. (2010) observaram que animais de AE apresentaram maior proporção de ácido isovalérico e menor proporção de ácido butírico do que os animais de BE. A menor proporção de ácido butírico em animais de AE contradiz as observações de Guan et al. (2008). Essa divergência pode ser explicada pela diferença entre as dietas dos animais utilizadas em cada trabalho. O estudo de Guan et al. (2008) avaliou animais sob dieta de alta densidade de energia (64,5 % de grãos de cevada, 20 % aveia, 9 % de feno de alfafa, 5 % de suplemento com 32 % de proteína), enquanto Hernandez-Sanabria et al. (2010) avaliaram dieta considerada de baixa densidade energética (74 % de aveia, 20 % de feno, 6 % de suplemento com 32 % de proteína). Estudos demonstram que a produção de ácido butírico é maior em animais alimentados com dieta de alta densidade de energia em comparação com dieta de baixa densidade energética (Cotta e Hespell, 1986; Russell e Hespell, 1981).

Considerando que ruminantes com maior eficiência alimentar produzam de 20 a 30 % menos metano (Hegarty et al., 2007), os animais utilizados para análise da comunidade bacteriana ruminal realizada por Hernandez-Sanabria et al. (2010), também foram utilizados para avaliação da composição de arqueias metanogênicas por Zhou, Hernandez-Sanabria e Guan (2009). Foi feito um *pool* do DNA microbiano extraído do líquido ruminal dos animais de AE (n=29) e outro dos animais dos animais de BE (n=29) para a construção de duas bibliotecas gênicas a partir da amplificação parcial do gene rRNA 16S (~800pb). Os produtos de PCR foram clonados e sequenciados. Os resultados indicaram que animais com BE apresentam maior riqueza e diversidade de espécies metanogênicas do que os animais com AE. Esses autores ainda realizaram a quantificação da população total de arqueias metanogênicas e de outras duas espécies, *Methanobrevibacter* sp. estirpe AbM4 e *Methanosphaera stadtmanae*. A escolha dessas duas espécies foi devido às suas distribuições uniformes entre as duas bibliotecas, além de *Methanobrevibacter* sp. estirpe AbM4 ter sido

identificada pela primeira vez no rúmen e *M. stadtmanae* ser estudada por ser capaz de produzir metano somente pela redução do metanol com H₂, diferentemente de *Methanobrevibacter* sp. estirpe AbM4, a qual é capaz de utilizar acetato como substrato (Miller e Lin, 2002). *M. stadtmanae* não possui as enzimas monóxido de carbono desidrogenase e o complexo acetil-coenzima A carboxilase necessários para utilização do acetato como substrato e síntese de acetil-coenzima A a partir de CO₂ e um grupo metil, respectivamente (Fricke et al., 2006). A população total de metanogênicas não variou entre os grupos de animais de AE e BE, porém os animais de BE apresentaram populações mais elevadas de *M. stadtmanae* e *Methanobrevibacter* sp. estirpe AbM4, sugerindo maior diversificação das vias de produção de metano nos animais menos eficientes (Zhou et al., 2009).

Em um segundo estudo, Hernandez-Sanabria et al. (2012) avaliaram o efeito da comunidade bacteriana ruminal sobre a EA de animais alimentados com duas dietas diferentes. Um rebanho de 108 bovinos (Hereford x Aberdeen Angus) foi mantido em dieta de baixa densidade energética (74 % de aveia, 20 % de feno, e 6 % suplemento com 32 % de proteína) durante 90 dias e, após uma semana de adaptação, foram transferidos para uma dieta de alta densidade energética (57 % de cevada, de 28 % aveia, 10 % alfafa, e 5 % suplemento com 32 % de proteína). Foram selecionados 60 animais com EA extremas (30 de AE e 30 de BE) dos quais foram coletadas amostras de líquido ruminal ao final de cada ciclo de alimentação. A comparação do perfil de bandas por DGGE e sequenciamento das mesmas demonstrou que para a dieta de baixa densidade energética *Prevotella* sp., *Lactobacillus* sp., *Succinivibrio ruminis* e *Moryella indoligenes* estavam associados aos animais de AE e *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Prevotella denticola*, *Bifidobacterium ruminantium* e *Robinsoniella peoriensis* estavam associados aos animais de BE. Enquanto para a dieta de alta densidade energética, *Prevotella maculosa*, *Prevotella ruminicola* e *Ruminococcus* sp. estavam associados aos animais de AE e *Clostridium indolis*, *Clostridium symbiosum*, *Succinomonas amylolytica* e *Selenomonas ruminantium* estavam associados aos animais de BE.

Hernandez-Sanabria et al. (2012) ainda realizaram a quantificação da população total de três espécies bacterianas, *Succinivibrio dextrinosolvens*, *Eubacterium rectale* e *Robinsoniella peoriensis*. As abundâncias de *Succinivibrio dextrinosolvens*, cujos principais produtos de fermentação são acetato e succinato (precursor de propionato)

(Russell e Hespell, 1981), e *Eubacterium rectale*, que é capaz de utilizar acetato e produzir ácido butírico (Flint et al., 2007), foram maiores em animais de AE quando alimentados com dieta de alta densidade energética. *Succinivibrio* sp. pode estar relacionado com o aumento da síntese de propionato, que é absorvido através do epitélio ruminal e direcionado para a gliconeogênese hepática (O'Herrin e Kenealy, 1993), melhorando a EA de animais em dieta com alta densidade energética. Enquanto *Eubacterium rectale*, pode estar associado com a redução da concentração de acetato que seria destinado para a metanogênese. O aumento da população de *Robinsoniella peoriensis* foi associado à BE. Esta bactéria foi identificada pela primeira vez no rúmen por Hernandez-Sanabria et al. (2010) e seus principais produtos de fermentação são acetato e succinato e, em menor quantidade, lactato e formato (Cotta et al., 2009). Os efeitos do aumento de *Robinsoniella* sp. em animais de BE podem estar relacionados com a maior disponibilização de ácido fórmico para a metanogênese.

Adicionalmente, Zhou, Hernandez-Sanabria e Guan (2010) avaliaram o perfil (DGGE e sequenciamento das bandas) e realizaram a quantificação total (qPCR) de arqueias metanogênicas sob as mesmas condições descritas para o trabalho de Hernandez-Sanabria et al. (2012). Não foi observada variação na proporção total de metanogênicas em nenhuma condição avaliada. Porém, foi demonstrado que o perfil de metanogênicas é afetado pela dieta, sendo que quando alimentados com dieta de baixa densidade energética os animais apresentaram predominantemente *Methanobrevibacter ruminantium* NT7 e sob dieta de alta densidade energética houve predominância de *Methanobrevibacter smithii*, *Methanobrevibacter* sp. AbM4 e/ou *M. ruminantium* NT7 entre os animais. Como discutido anteriormente, espécies diferentes de metanogênicas são capazes de produzir metano a partir de substratos e vias diferentes. Assim, a variação da predominância entre as duas dietas pode estar relacionada a diferenças na proporção dos substratos disponíveis para metanogênese em cada condição. Esses dados reforçam a ideia que não seja a comunidade total de metanogênicas que esteja relacionada à variação da produção de metano, e sim populações específicas e as vias metabólicas utilizadas para produção desse gás (Zhou et al., 2010).

A microbiota ruminal é composta não só por bactérias e arqueias, como também por fungos, os quais auxiliam na desconstrução da biomassa dietética a partir da ruptura física dos tecidos vegetais (Gordon e Phillips, 1998), e por protozoários, os

quais predam as bactérias ruminais e auxiliam na estabilização da fermentação ruminal (Santra e Karim, 2002). Considerando esses importantes papéis ecológicos, Carberry et al. (2012) quantificaram, por qPCR, a população total de protozoários e do gênero *Entodinium* e de fungos totais. Além disso, avaliaram o perfil da comunidade bacteriana e quantificaram as populações das espécies *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Prevotella brevis* e do gênero *Prevotella* em gado de corte (Limousin x Friesian). Os resultados não revelaram diferenças significativas nas populações totais de protozoários e de fungos, bem como na população de *Entodinium* spp., protozoário que é capaz de engolfar amido e bactérias amilolíticas, sendo relacionado à regulação do metabolismo desse carboidrato no rúmen (Santra e Karim, 2002). A proporção do gênero *Prevotella*, grupo capaz de metabolizar amido, proteína, peptídeos, hemicelulose e pectina e produzir ácido acético, succínico, e propiônico (Stevenson e Weimer, 2007), foi maior nos animais de BE.

Fibrobacter succinogenes, *R. albus* e *R. flavefaciens* são bactérias fibrolíticas que possuem maiores capacidades de degradação de celulose no rúmen (Kamra, 2005). Carberry et al. (2012) não observaram diferença na população de *F. succinogenes* entre os animais de AE e BE, enquanto a população de *R. albus* foi maior em animais de AE. A associação dessa espécie com o aumento da EA deve estar relacionada ao aumento da digestibilidade conferida pela maior abundância desse grupo. Já a população de *R. flavefaciens* foi menor em animais com AE, o que pode ser justificado pela associação negativa entre as abundâncias de *R. albus* e *R. flavefaciens* já relatada por Chen, Stevenson e Weimer (2004).

Os estudos utilizando as técnicas de DGGE e qPCR para avaliar diferenças na estrutura da comunidade microbiana ruminal em função da EA estabeleceram a base das hipóteses de estudos posteriores que utilizaram técnicas moleculares mais refinadas, como sequenciamento de nova geração com abordagens metagenômicas, para aprofundamento da caracterização da composição do microbioma ruminal (Jami et al., 2014; Jewell et al., 2015; McCann et al., 2014; Myer et al., 2015a; Rius et al., 2012; Shabat et al., 2016).

Utilização de sequenciamento de nova geração para análise da microbiota ruminal de bovinos com alta e baixa eficiência alimentar

O estudo da diversidade genética e composição da comunidade microbiana do rúmen por sequenciamento de nova geração têm demonstrado a presença de grupos microbianos que constituem o microbioma central (*core microbiome*), além de revelar diferenças, mesmo que discretas, na abundância de grupos específicos de microrganismos em função de determinados fatores, tais como eficiência alimentar (EA) (Henderson et al., 2015; Jami e Mizrahi, 2012; Shabat et al., 2016).

Rius et al. (2012) avaliaram a relação entre EA e microbiota ruminal de 16 vacas (Frísio-holandês), oito de alta (AE) e oito de baixa eficiência alimentar (BE), a partir do sequenciamento na plataforma 454 (Roche Diagnostics) dos genes 16S rRNA de bactérias e arqueias, 18S rRNA de protozoários ciliados e ITS-1 de fungos. As vacas de AE apresentaram maior digestibilidade de proteína (73 vs. 69 %) e matéria seca (75 vs. 70 %), indicando maior capacidade de degradação dos componentes da dieta pela microbiota ruminal desses animais. Por sua vez, as vacas de BE apresentaram maior concentração de amônia no rúmen, o que poderia indicar maior abundância de microrganismos proteolíticos e/ou desaminadores. Entretanto, apesar dessas diferenças em parâmetros que estão relacionados à atividade dos microrganismos ruminais, não foi observada diferenças na composição da microbiota entre os dois grupos. Deve-se ressaltar que neste trabalho não foi avaliada a interação dos grupos microbianos com os parâmetros bioquímicos, como foi realizado por Hernandez-Sanabria et al. (2010), o que poderia fornecer maiores evidências sobre a influência dos microrganismos sobre EA.

Para vacas lactantes, além do consumo alimentar residual (CAR), utilizado nos trabalhos listados anteriormente, a produção de leite (mensurada pela quantidade de proteína ou gordura) por unidade de massa seca consumida é amplamente utilizada como parâmetro de EA (National Research Council, 2001). Desta forma, Jami, White e Mizrahi (2014) avaliaram a relação entre a comunidade bacteriana ruminal (sequenciamento do gene 16S rRNA na plataforma 454), CAR e produção de leite de 15 vacas (Friesian-holandês). Assim como no estudo realizado por Rius et al. (2012), não foram observadas diferenças na composição da microbiota ruminal em função do CAR dos animais. Porém, foram observadas correlações significativas entre a composição do leite e microbiota ruminal. Houve grande variação na abundância dos

dois principais filos, Bacteroidetes e Firmicutes, sendo que o aumento da proporção Firmicutes/Bacteroidetes se mostrou associado ao aumento da produção de gordura do leite. Tal comportamento é semelhante ao reportado em estudos com humanos e camundongos, nos quais o aumento da razão Firmicutes/Bacteroidetes foi relacionado ao aumento de gordura no sangue e tecidos (Ley et al., 2006; Turnbaugh and Gordon, 2009). Jami, White e Mizrahi (2014) montaram uma matriz de correlação para avaliar a relação entre os parâmetros de eficiência e os gêneros que apresentaram pelo menos 0,1 % de abundância da microbiota e por essa razão foram considerados pelos autores mais relevantes para o ecossistema ruminal.

Prevotella, gênero mais abundante no ecossistema ruminal das vacas lactantes, apresentou correlação negativa significativa com a produção de gordura do leite. Esses resultados corroboram os resultados de Carberry et al. (2012), que também observaram o efeito negativo da abundância desse gênero sob a EA. Em contrapartida, já foi demonstrado que a inoculação de uma espécie específica desse gênero, *Prevotella bryantii*, está associada a atividades probióticas no rúmen, acarretando a redução da produção de lactato e o aumento do rendimento de gordura do leite (Chiquette et al., 2008). Jami, White e Mizrahi (2014) ainda demonstraram que a elevada abundância de *Prevotella* está relacionada à redução da abundância de alguns representantes do filo Firmicutes, tais como, ordem Clostridiales (gênero *Eubacterium*), família Lachnospiraceae, classe Negativicutes (gênero *Dialister*) e gênero *Lactobacillus*, os quais por sua vez apresentaram correlação positiva com o rendimento de gordura do leite. O efeito negativo do aumento da população de *Prevotella* sobre a abundância desses grupos deve estar relacionado ao aumento da competição por substrato, já que espécies de *Prevotella* são consideradas generalistas, ou seja, capazes de metabolizar grande parte dos nutrientes disponíveis no rúmen (Stevenson and Weimer, 2007). Logo, a influência negativa da abundância do gênero *Prevotella* sobre o rendimento de gordura do leite pode ser consequência da inibição por competição dos grupos que influenciariam positivamente esse parâmetro.

De forma semelhante ao trabalho de Jami, White e Mizrahi (2014), Jewell et al. (2015) avaliaram a EA tanto pelo CAR, quanto pela produção de leite de 14 vacas holandesas. Os autores caracterizaram a composição bacteriana ruminal tanto da fração líquida quanto da fração sólida por sequenciamento do gene 16S rRNA. Foi sugerido que 75 % dos microrganismos que compõem a microbiota ruminal estão

associados às partículas alimentares e respondem por aproximadamente 70 a 80 % da atividade celulolítica, proteolítica e amilolítica no rúmen (McAllister et al., 1994). Assim, a avaliação da porção sólida do conteúdo ruminal tem sido considerada relevante para caracterizar a associação entre a microbiota ruminal e o fenótipo de EA em ruminantes.

Diferente das observações feitas por Jami, White e Mizrahi (2014), os quais não identificaram diferenças na microbiota ruminal em função do CAR, Jewell et al. (2015) relataram que a composição da microbiota ruminal de vacas lactantes variou tanto em relação ao CAR quanto em relação à produção de leite. A abundância de *Prevotella* no líquido ruminal apresentou correlação negativa com a produção de leite, concordando com os resultados reportados por Jami, White e Mizrahi (2014). Para a fração sólida, foi observada alta correlação positiva entre a família Succinivibrionaceae e a produção de leite. Os membros desta família são capazes de fermentar glicose e outros carboidratos com produção de succinato e ácido acético e succínico, sendo que, em proporções menores, ácido fórmico e láctico podem ser produzidos (O'Herrin and Kenealy, 1993).

Em ambas as frações do conteúdo ruminal, Jewell et al. (2015) observaram o aumento da abundância de *Prevotella* nos animais com alto CAR, ou seja, considerados de BE. Nos animais com baixo CAR (AE) observou-se maior abundância da família Lachnospiraceae, a qual compreende espécies fibrolíticas e proteolíticas importantes para o balanço da fermentação ruminal (Meehan and Beiko, 2014). Adicionalmente, a abundância de membros desta família, como o gênero *Lachnospira*, tem sido relacionada a benefícios na saúde de humanos, tais como melhoria das respostas inflamatórias e até mesmo do humor (Li et al., 2016). Foi observada também sobreposição das diferenças na microbiota em função do CAR e da produção de leite, o que não é surpreendente uma vez que esses dois parâmetros são formas de medir a EA dos animais. A maior abundância dos gêneros *Anaerovibrio*, que está associado à produção de ácido acético e succínico, bem como hidrólise de lipídios (Privé et al., 2013), e *Butyrivibrio*, cujas espécies podem estar envolvidas tanto no metabolismo da fibra quanto de proteínas e produção de ácido butírico (Cotta e Hespell, 1986), foi observada nos animais de BE (alto CAR e baixa produção de leite). Enquanto a maior abundância de *Coprococcus*, gênero pertencente à família Lachnospiraceae, e da família Veillonellaceae foram relacionadas à AE (baixo CAR e alta produção de leite).

A família Veillonellaceae pertence à classe Negativicutes que já havia sido relatada por sua associação positiva com a produção de leite (Jami et al., 2014).

A avaliação da composição da microbiota ruminal, a partir de técnicas de sequenciamento de nova geração, em relação à EA de gado de corte (McCann et al., 2014; Myer et al., 2015a) revelou comportamentos similares aos observados para vacas lactantes. McCann et al. (2014) compararam a composição bacteriana ruminal de 16 touros da raça Brahman, oito de AE e oito de BE, por sequenciamento do gene rRNA 16S na plataforma 454. Não foram observadas diferenças significativas na diversidade e riqueza de espécies entre os animais de AE e BE, entretanto maiores abundâncias da família Prevotellaceae foram relacionadas a animais de BE (BE = 33,2 % e AE = 19,6 %).

Myer et al. (2015b) também avaliaram a composição da microbiota ruminal (sequenciamento do gene rRNA 16S na plataforma MiSeq, Illumina) de 32 novilhos de corte de diferentes raças, os quais foram agrupados em quatro grupos de acordo com o ganho de peso diário (GP) e consumo de massa seca (CMS): alto GP e alto CMS; alto GP e baixo CMS, baixo GP e baixo CMS, e baixo GP e alto CMS. Diferentemente dos trabalhos abordados anteriormente (Jami et al., 2014; Jewell et al., 2015; McCann et al., 2014), não foram observadas diferenças na proporção de *Prevotella* entre os grupos considerados de AE (alto GP e baixo CMS) e BE (baixo GP e alto CMS). As alterações da microbiota foram identificadas entre membros do filo Firmicutes, cuja abundância apresentou correlação positiva com o ganho de peso dos animais. Como demonstrado em trabalhos anteriores (Jami et al., 2014; Jewell et al., 2015) o aumento da abundância de representantes da classe Negativicutes, famílias Veillonellaceae (gênero *Dialister*) e Acidaminococcaceae (*Acidaminococcus*), e da família Lachnospiraceae foi associada aos animais de AE (alto GP e baixo CMS). Enquanto maiores abundâncias dos gêneros *Anaerovibrio* (também observado por Jewell et al., 2015) e *Succiniclasicum* foram associados à BE (baixo GP e alto CMS). Espécies de *Succiniclasicum* estão geralmente relacionadas com a fermentação de succinato e produção de propionato (Van Gylswyk, 1995). Os resultados de Myer et al. (2015b) ainda indicaram que as maiores abundâncias do filo Lentisphaerae estavam associadas com os animais de BE (baixo GP e alto CMS). O aumento da abundância deste filo já havia sido relatado em animais durante acidose ruminal subaguda (Mao et

al., 2013), sugerindo que o aumento desse grupo microbiano pode ser negativo para o hospedeiro.

Apesar da caracterização da microbiota ruminal evidenciar importantes diferenças entre os animais de AE e BE, a comparação do metagenoma, a partir do sequenciamento de todo o conteúdo gênico, bem como do metaboloma, a partir da identificação dos metabólitos produzidos, pode fornecer maiores informações sobre o papel dos microrganismos na EA. Visando essa caracterização global, Shabat et al. (2016) avaliaram, além da composição da microbiota ruminal (sequenciamento do gene rRNA 16S na plataforma MiSeq, Illumina), o metagenoma (plataforma HiSeq2500, Illumina) e o metaboloma (cromatografia gasosa acoplada à espectrômetro de massa) de 78 vacas Friesian-holandesas. Para obter melhor cobertura e facilitar a montagem dos metagenomas, foi realizado um *pool* com os *reads* obtidos para os animais de AE e outro para os de BE.

De forma geral, Shabat et al. (2016) observaram diferenças na abundância de 18 grupos bacterianos, muitos dos quais já foram relatados em trabalhos anteriores (Carberry et al., 2012; Jami et al., 2014; Jewell et al., 2015; Myer et al., 2015a), e de 34166 genes entre vacas de AE e BE. Menor diversidade (índice de Shannon) e maior dominância de espécies e genes foram observadas em vacas de AE. A análise do fluxo metabólico na *Kyoto Encyclopedia of Genes and Genomes* (KEGG) evidenciou que vacas de BE apresentavam maior número de vias metabólicas, bem como de enzimas associadas à degradação e absorção de proteínas, biossíntese de aminoácidos e produção de metano. A partir desses resultados, pode-se sugerir que a maior diversidade de espécies, genes e vias metabólicas dos microrganismos ruminantes afetam de forma negativa o processo de obtenção de energia pelos hospedeiros. Ou seja, microbiomas mais eficientes tendem a ser menos complexos e mais especializados para suprir os requerimentos de energia do hospedeiro (Shabat et al., 2016).

Shabat et al. (2016) também observaram que as vacas de AE possuíam maiores concentrações de ácido propiônico, butírico, valérico e isovalérico e, conseqüentemente, maior concentração total de ácidos orgânicos voláteis (AOV) no rúmen. Essas diferenças devem estar relacionadas com o aumento da produtividade do hospedeiro, já que aproximadamente 70 % da energia dos ruminantes é obtida a partir da absorção dos AOV (Seymour et al., 2005). Foi observada ainda redução da produção de metano pelas vacas de AE, o que pode estar associado com o desvio de

H₂ para produção de ácido propiônico e butírico, que representa um mecanismo dissipador de elétrons no rúmen (Ungerfeld, 2015). Essa hipótese pode ser ainda reafirmada pela dominância do gênero *Megasphaera* e espécie *Coprococcus catus* em animais de AE, as quais possuem capacidade de utilizar ácido láctico como fonte de energia e produzir ácido propiônico como produto da fermentação (Prabhu et al., 2012; Ungerfeld, 2015). A redução da produção de metano se mostrou associada ao aumento da razão propionato/acetato, comportamento que já havia sido relatado para animais com maior retenção de energia (Russell, 1998).

Dessa forma, a compreensão da dinâmica e potencialidades funcionais da comunidade microbiana do rúmen em função da eficiência alimentar representa um passo importante para o direcionamento de estratégias de manipulação ruminal eficazes e desenvolvimento de novas tecnologias de gestão da pecuária. Além disso, a análise das comunidades microbianas de outras porções do trato gastrointestinal, como intestino delgado e grosso, seria importante para o melhor entendimento do reflexo da microbiota sobre a eficiência do hospedeiro (Myer et al., 2015a).

Microbiota intestinal *versus* eficiência alimentar

Em ruminantes, apesar da maior parte da digestão dos componentes da dieta ocorrer a partir da fermentação ruminal por microrganismos, a microbiota do trato gastrointestinal (TGI) distal (intestino delgado e grosso) tem importante papel no bem estar e, em menor grau, na obtenção de energia pelo hospedeiro (Myer et al., 2015c; Oliveira et al., 2013). Sendo assim, além de avaliarem o efeito da microbiota ruminal sobre a eficiência alimentar de bovinos, Myer et al. (2015a) também avaliaram a composição da comunidade microbiana do jejuno, cólon e ceco dos animais divididos em quatro grupos de acordo com o ganho de peso (GP) e consumo de matéria seca (CMS) (Myer et al., 2015b, 2015c, 2016).

A abundância e diversidade da microbiota do jejuno foram consideravelmente menores que o observado nas outras porções avaliadas (Myer et al., 2015a, 2015b, 2015c, 2016). Essa redução era esperada em função das características e secreção enzimática pelo hospedeiro no intestino delgado (Oliveira et al., 2013). A comparação da beta diversidade (a qual possibilita a avaliação da divergência entre os animais) da microbiota do jejuno não possibilitou o agrupamento dos animais de acordo com a EA. Porém, foi demonstrado o aumento da abundância de três gêneros específicos no

jejuno de animais de alta EA (alto GP e baixo CMS): *Acidaminococcus*, *Ammoniphilus* e *Butyrivibrio*.

Acidaminococcus são bactérias fermentadoras de aminoácidos (Chang et al., 2010) cujo aumento da abundância também foi observado no rúmen dos animais de AE (Myer et al., 2015a). O aumento deste gênero no jejuno dos animais mais eficientes pode ser indicativo de maior atividade proteolítica no intestino. *Ammoniphilus* são bactérias aeróbias, dependentes de amônio e oxalotróficas (utilizam oxalato como fonte de carbono) (Zaitsev et al., 1998). A degradação de oxalato no TGI é comum e preventiva para a formação de cálculo nos rins em humanos (Allison et al., 1985). O gênero *Butyrivibrio* pertence à família Lachnospiraceae, que também apresentou maior abundância no rúmen dos animais mais eficientes (Jami et al., 2014; Jewell et al., 2015; Myer et al., 2015a; Shabat et al., 2016), e está relacionado com a digestão de proteína e fibra (atividade hemicelulolítica) no rúmen (Cotta and Hespell, 1986). Assim, a maior abundância desse gênero no jejuno pode representar um incremento para obtenção de energia pela digestão de componentes fibrosos que escapam da degradação ruminal. *Butyrivibrio* ainda está relacionado com a produção de ácido butírico, que representa uma das principais fontes de energia para os enterócitos (Wächtershäuser and Stein, 2000). Dentre outros efeitos que o ácido butírico exerce sobre as células epiteliais gastrointestinais, tais como estímulo para proliferação e diferenciação celular (Guilloteau et al., 2010), foi descrito que esse ácido orgânico tem efeito sobre a expressão de leptina nos adipócitos de bovinos, afetando assim a regulação da ingestão de alimentos e do gasto energético (Soliman et al., 2007).

O intestino grosso é um sítio potencial para degradação pós-ruminal de celulose e amido uma vez que apresenta grande diversidade de microrganismos, maior até mesmo que o observado no rúmen (Myer et al., 2015b, 2015c; Oliveira et al., 2013). Adicionalmente, em função da associação do ceco e cólon ao sistema linfático, a microbiota dessas porções está relacionada com a constante estimulação do sistema imune e competição com bactérias patogênicas, exercendo papel fundamental na defesa do hospedeiro (Moreto e Perez-Bosque, 2009).

Considerado a comparação da beta diversidade entre as comunidades microbianas do ceco e do colón, Myer et al. (2015a, 2015c) também não identificaram agrupamento dos animais em relação à EA. Como observado para o rúmen (Jami et al., 2014), a razão Firmicutes/Bacteroidetes no cólon foi maior nos animais mais

eficientes (alto GP e baixo CMS) (Myer et al., 2015c). Maiores abundâncias da família Ruminococcaceae, a qual compreende bactérias celulolíticas, foram observadas no ceco e cólon de animais com maior GP (Myer et al., 2015b, 2015c). Desse modo, esse grupo pode estar relacionado à maior degradação intestinal das fibras residuais da fermentação ruminal. O gênero *Blautia*, que é comumente encontrado no TGI de bovinos e caprinos (Eren et al., 2015), também apresentou maior proporção no intestino de animais com maior GP (Myer et al., 2015b, 2015c). Esse gênero pertence à família Lachnospiraceae e também pode estar relacionado com o aumento do suprimento de energia para o hospedeiro pela degradação de polissacarídeos remanescentes (Biddle et al., 2013).

Os animais com menores GP apresentaram maiores abundâncias de *Prevotella*, que foi previamente descrita pela predominância ruminal em animais de BE (Jami et al., 2014; Jewell et al., 2015) e *Oscillospira* no intestino grosso (Myer et al., 2015b, 2015c). O aumento da abundância do gênero *Oscillospira* no rúmen é reportado para animais em dieta com elevado teor de amido (Kim et al., 2011). Assim, a maior abundância desse gênero no cólon e ceco pode estar relacionada com a maior concentração de amido no intestino grosso, ou seja, maior escape do rúmen em animais de BE.

Diante do exposto, pode-se concluir que animais com alta e baixa EA possuem diferenças na abundância de grupos microbianos relevantes para a degradação dos componentes da dieta. Todavia, é necessária a ampliação dos estudos que avaliem não só a composição como também a interação e funcionalidade dos grupos microbianos, a fim de caracterizar melhor a influência dos microrganismos sobre a EA (Li and Guan, 2017), e apesar do crescente número de estudos, a avaliação da composição do microbioma do TGI de bovinos de corte ainda são escassos (Myer et al., 2015a). Além disso, estudos com o objetivo de comparar a microbiota das fezes de ruminantes com diferentes EA podem ser interessantes, uma vez que amostras fecais representam um método não invasivo para acessar a composição da comunidade microbiana do TGI (Tapio et al., 2016).

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Capítulo 2

Differences in rumen bacterial and archaeal community of Nelore steers with positive and negative residual feed intake

Introduction

Ruminants (cattle, goats, sheep, etc.) have great economical and social relevance, due to their role in the production of meat and milk, which represent the main sources of protein in human diets. Brazil harbor the biggest commercial cattle herd in the world, with 226 million animals (FAO, 2016). About 80 % of this total is composed of zebu breeds (*Bos indicus*), which are considered well adapted to the environmental conditions and production systems found in tropicals countries.

The Brazilian herd is predominantly composed of the Nelore breed, which is considered well adapted to the environmental conditions and tropical production systems, in which perennial tropical grasses and mineral supplementation are the basis of the diet (Millen et al., 2011). In extensive production systems, ruminants consume about 30 % of the crop cultivated on Earth and occupy another 30 % of the planet's surface (Shabat et al., 2016; Thornton, 2010). In addition to the environmental impacts related to farmland occupation, cattle production has been associated with a significant portion of the world's greenhouse gas emissions due to methane production (Hristov et al., 2015). The reduction of methane emission by ruminants is not only desirable from the environmental viewpoint, but also from the economical aspect, given that it represents a loss of up to 11 % of the dietary energy consumed by the animal (Tapio et al., 2017).

Several strategies have been studied aiming to reduce methane emission in beef and dairy farms (Cottle and Van Der Werf, 2017; Eckard et al., 2010). Among these, strategies that increase the efficiency of feed utilization have the potential to reduce production costs and the environmental footprint of livestock production while maintaining the levels of productivity. With the projected growth of the world population in the next three decades, it is expected that meat production will have to double to meet the global demand for animal protein sources (FAO, 2016). However, expanding herd size and land use to meet this demand is undesirable, due to the environmental impacts associated with livestock production (Thornton, 2010).

In this context, there is an increasing interest in the use of feed efficiency indexes/markers to select animals that produce more meat or milk consuming less feed. Previous studies indicated that some phenotypic characteristics (e.g. energy corrected milk, residual feed intake) might be related with feed efficiency (FE) in both dairy and beef cattle (Connor et al., 2013; Herd et al., 2003). In beef cattle, residual feed intake (RFI), which consists of the difference between the observed dry matter intake and the expected dry matter intake based on some animal development measures (Koch et al., 1963), has been widely used to calculate the feed efficiency of growing cattle. The expected dry matter intake is determined from linear regression of measured feed intake versus developmental measures (as mean daily weight gain) for a group of animals on the same diet. However, previous studies demonstrated that RFI has low to moderate heritability in beef cattle ($h^2 = 0.25$, Korver et al., 1991, $h^2 = 0.39$, Arthur et al., 2001), which suggests that feed efficiency is also affected by non-genetic factors.

Ruminants are herbivorous mammals that do not synthesize enzymes responsible for fiber degradation (cellulose, hemicellulose and lignin), but they are able to establish symbiotic relationships with anaerobic microorganisms capable of fermenting substrates of different origins, such as soluble and insoluble carbohydrates, proteins and lipids (Jami et al., 2013; Kamra, 2005). Therefore, feed conversion of ruminants depends both on the ability of the ruminal microbiota to ferment the components of the diet and produce volatile organic acids, and the ability of these animals to absorb and utilize these nutrients (genetic potential). Because of the fundamental role of ruminal microorganisms providing energy sources to their hosts, as well as the advances in the techniques to characterize the composition of microbial communities, studies have sought to demonstrate and explore the relationship between the ruminal microbiota and FE (Carberry et al., 2012; Jami et al., 2014; Jewell et al., 2015; Kern et al., 2016; Myer et al., 2015a; Shabat et al., 2016).

Despite the fact that studies demonstrated association between microbiome composition and FE in dairy cows (Jami et al., 2014; Jewell et al., 2015; Rius et al., 2012; Shabat et al., 2016), the relationship between RFI and composition of the rumen microbiota is not as clear for beef cattle. Moreover, there is a lack of studies addressing feed efficiency and rumen microbiome composition in Nelore cattle, which represents more than 90 % of the Brazilian beef cattle and one of the most abundant sources of meat in the world (FAO, 2016; ABIEC, 2017).

In this study, we examined the hypothesis that Nelore cattle of high and low FE (negative and positive RFI, respectively) have differences in the composition of their ruminal microbiome. For this, we sampled rumen contents from 27 Nelore steers (12 with negative RFI and 15 with positive RFI) and evaluated biochemical parameters and the composition of the ruminal microbiota in the solid and liquid fractions by deep sequencing of the 16S rRNA gene.

Materials and Methods

Animal selection, diets and sampling collection

The experimental procedures used in this study were approved by the Ethics Committee on Animal Use of the *Instituto de Zootecnia/CEUA-IZ*, Nova Odessa, SP, Brazil (protocol 213-15), in accordance with guidelines of State Law No. 11.977 of the State of São Paulo, Brazil, and by Ethics Committee on Production Animal Use of the *Universidade Federal de Viçosa/CEUAP-UFV*, Viçosa, MG, Brazil (protocol 026/2015).

A contemporary group of 129 young Nelore bulls (7 months of age and 239 ± 30.1 kg of initial body weight - BW) from the Instituto de Zootecnia – IZ were subjected to a growth period of 98 days receiving a diet formulated to meet the requirements for 1 kg/d of BW gain. The diet was composed of 615 g/kg corn silage, 33 g/kg Brachiaria hay, 167 g/kg dry ground corn, 163 g/kg soybean meal, 3.6 g/kg urea, 0.4 g/kg ammonium sulfate, and 18 g/kg mineral mixture (on a dry matter basis). Before the beginning of the test period, all bulls were weighed, vaccinated, dewormed, and received individual numbered tags.

Cattle were fed using a GrowSafe® automated feeding system (GrowSafe Systems Ltd, Airdrie, Canada). The RFI (kg/day) was calculated during the growth period as the error term of the equation: $DMI = \beta_0 + \beta_P * BW_{0.75} + \beta_G * ADG + \varepsilon(RFI)$, where DMI is the dry matter intake observed during the test, β_0 is the intercept of the equation, $BW_{0.75}$ is the mid-test metabolic live weight, ADG is the average daily weight gain during the test, and β_P and β_G are the regression coefficients of $BW_{0.75}$ and ADG, respectively. Average daily gain was estimated by the linear regression coefficient of live weight as a function of days in test. Mid-test metabolic live weight was calculated by the equation $BW_{0.75} = [\alpha + \beta * (DIT/2)]_{0.75}$, where α is the intercept of the regression equation corresponding to the initial live weight, β is the linear

regression coefficient corresponding to the average daily gain, and DIT are the days in test (Fidelis et al., 2017).

From the 129 animals used in the growth period, a total of 27 steers were randomly selected (12 with negative RFI and 15 with positive RFI) to the finishing period. The RFI values were significantly different ($P < 0.05$) between negatives (-0.93 ± 0.17) and positives (0.87 ± 0.14) and the steers were separated on two feed efficiency groups: negative-RFI (n-RFI, more efficient) and positive-RFI (p-RFI, less efficient), respectively.

The 27 animals, averaging 22.5 ± 0.8 mo of age and 401 ± 42 kg of BW, were confined in individual pens measuring 4 m \times 2 m and equipped with GrowSafe® automated feeding systems (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) with free access to diet and water. Cattle were adapted to the diets, facilities, and management for 22 d and fed the finishing diet for a 103-d period. The finishing diet was composed of 333 g/kg corn silage, 17 g/kg Brachiaria hay, 465 g/kg dry ground corn, 163 g/kg soybean meal, 6 g/kg urea, 4 g/kg ammonium sulfate, and 13 g/kg mineral mixture (dry matter basis), formulated to meet the requirements of 1.3 kg of daily gain with a target finish weight of at least 550 kg.

After that, steers were transported to a commercial abattoir for slaughter. Handling was conducted in accordance with good animal welfare practices, and slaughtering procedures followed strict guidelines established and regulated by the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2017). After slaughter, the ruminal contents were collected and filtered through four layers of cheese cloth to separate the liquid and solid fractions. The pH was measured (pH meter HI9124, Hanna Instruments, Woonsocket, Rhode Island, EUA), and each fraction was stored in plastic containers at -20°C for further analyses.

Concentration of ammonia and volatile fatty acids

Ammonia concentration was determined by the colorimetric method of Chaney and Marbach (1962). Absorbance was measured at 630 nm in a spectrophotometer Spectronic 20D (Thermo Fisher Scientific, Madison, WI, USA) and ammonium chloride (NH_4Cl , concentrations varying from 20.38 to 203.83 mg/l) was used as the standard.

Organic acids were determined in a Dionex Ultimate 3000 Dual detector HPLC apparatus (Dionex Corporation, Sunnyvale, CA, USA) equipped with a refractive

index (RI) detector Shodex RI-101 maintained at 40 °C. The organic acids were separated on a Phenomenex Rezex ROA ion exclusion column (300 × 7.8 mm) (Phenomenex Inc. Torrance, CA, USA) maintained at 45°C. Analyses were performed isocratically under the following conditions: mobile phase H₂SO₄ 5 mmol l⁻¹, flow rate 0.7 ml min⁻¹, column temperature 40 °C, injection volume 20 µl. Rumen fluid samples (2.0 ml) were centrifuged (12 000 × g, 10 min) and the cell-free supernatants were treated as described by Siegfried et al. (1984). Stock solutions of the standards were prepared using the following organic acids: acetic, succinic, propionic, valeric, isovaleric, isobutyric and butyric acid. All organic acids were prepared with a final concentration of 10 mmol/l, except isovaleric acid (5 mmol/l) and acetic acid (20 mmol/l). Stock solutions were diluted 2-, 4-, 8- and 16-fold in 5 mmol/l H₂SO₄ to be used as standards in the HPLC analysis.

DNA extraction and sequencing

Total genomic DNA was extracted separately from the ruminal solids and liquids following a mechanical disruption and phenol/chloroform extraction protocol described by Stevenson and Weimer (2007), which has been shown to generate high-quality, high-abundance DNA representative of the ruminal bacterial community (Henderson et al., 2013) Genomic DNA extracted was quantified using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) and sequenced at the University of Wisconsin (Madison, USA).

The V4 hypervariable region of the bacterial and V6-V8 of the archaeal 16S rRNA gene were amplified using primers described by Kozich et al., (2013) and Kittelmann et al., (2013), respectively. For Bacteria, PCR reactions consisted of 50 ng template DNA, 0.4 µM of each primer, 1 X Kapa Hifi HotStart ReadyMix (KAPA Biosystems), and water to 25 µL. For archaea, DNA was increased to 100 ng and primers to 1.6 µM each. PCR was performed at 95°C for 3 min, 95°C for 30 s, 55°C for 30 s, 72°C for 30 s (25 cycles for bacteria and 35 cycles for archaea) and a final extension step at 72°C for 5 min. PCR products were purified by PureLink® Pro 96 PCR Purification Kit (Invitrogen) and a second PCR was performed on products to attach Illumina sequencing adapters and unique dual indices. PCR reactions were similar to those for V4 (bacteria), except that 5 µL of non-quantified PCR product was used as template DNA and 8 cycles were performed. PCR products were recovered by gel extraction in AquaPör LM low-melt agarose (National Diagnostics, Atlanta, GA)

using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA). Purified DNA was quantified by Qubit® Fluorometer (Invitrogen) and equimolar pooled to create a single sample at 1×10^9 ng per μL . Sequencing was performed using the v2 kit for Bacteria (2×250 bp) and v3 for Archaea (2×300 pb) with paired-end method on an Illumina MiSeq following manufacturer's guidelines (Illumina, Inc., San Diego, CA, USA).

Sequence analysis

Bacteria and Archaea sequences were processed separately using Mothur (v1.39.5) (Schloss et al., 2009). Paired-end reads were joined using default parameters in `make.contigs` and sequences with a length shorter than 200 bp or longer than 500 bp containing ambiguous characters or exhibiting a homopolymer greater than 8 bp were removed. Archaeal and bacterial sequences were aligned using the SILVA 16S rRNA gene reference database (Quast et al., 2012) and the sequences that did not align to the correct location were removed. Identical sequences were grouped in `unique.seqs` command and sequences that were two or fewer base pairs different were considered the same and grouped in `pre.cluster` to keep computational needs down as well as to account for sequencing error. Chimeric sequences were detected using Uchime algorithm (Edgar et al., 2011) and removed. The singletons (sequences that occur only once in the entire dataset) were also removed because they provide little usable data and are often the result of error. The bacterial and archaeal sequences were taxonomy assigned using SILVA 16S rRNA gene reference database (Quast et al., 2012) with a bootstrap cut-off of 80. All sequences were grouped into operational taxonomic units (OTUs) by uncorrected pairwise distances clustered by the furthest neighbor method with a similarity cutoff of 97 %. Before proceeding with the analysis the coverage of all samples was assessed by Good's coverage (Bacteria > 97 % and Archaea > 99 %). Due to different sequencing depths, OTU tables were normalized to equal sequence counts (established by the sample that presented the lowest number of sequences), with 10,001 bacterial and 1,166 archaeal sequences per sample. The normalized OTU tables were used to determine the alpha diversity (Chao1, Shannon and Simpson) indexes and the relative abundance (reads/total reads in a sample) of OTUs.

Statistical analysis

The differences in bacterial and archaeal alpha-diversity indices and taxa level, as well as the concentration of ammonia and organic acids of rumen samples according to each feed efficiency group were assessed by t-test performed in MiniTab[®] 17.1.0 (Minitab, Inc., Quality Plaza, 1829 Pine Hall Road, State College, Pennsylvania 16801, USA). *P*-values below 0.05 were considered significant.

To evaluate the clustering of the steers using the OTU composition of rumen contents, a Principal Coordinate Analysis (PCoA) of the Bray-Curtis dissimilarity metric (beta diversity index), and non-parametric analysis of similarities (ANOSIM, number of permutation = 1000) were performed using the Past software (Hammer et al., 2001).

Initially, the Kolmogorov-Smirnov, Shapiro-Wilk, D'AlgoStino and Pearson tests were performed using GraphPad Prism v. 5.00 for Windows (GraphPad Software, San Diego California USA) to check if the relative abundances of bacterial and archaeal OTUs followed a Gaussian distribution. Because most OTUs in our samples did not follow normal distribution ($P > 0.05$), differences in relative abundances according to feed efficiency group were assessed by White's non-parametric t-test using the Statistical Analysis of Taxonomic and Functional Profiles Statistical Analysis of Taxonomic and Functional Profiles – STAMP v 2.1.3 software (Parks et al., 2014). Differences were considered significant if the *P*-values were less than 0.05.

Analysis of OTUs that were associated with animals showing negative or positive RFIs were based on sequences that were detected in at least 50 % of the steers classified in each feed efficiency group (7 steers to p-RFI and 6 steers to n-RFI). Venn diagrams were built in jvenn: an interactive Venn diagram viewer (Bardou et al., 2014), which provides both the number and the identification of shared and exclusive OTUs in the two feed efficiency groups. The shared OTUs were plotted in a volcano plot using Graphpad (Prism, version 5.01) to visualize differences on the relative abundances between the more efficient group (n-RFI) and the less efficient group (p-RFI). For this, we calculated the fold change (ratio between n-RFI and p-RFI relative abundance of each OTU) and use the *p*-values obtained on White's non-parametric t-test.

Spearman's rank correlation was estimated using R (v 3.4.1, corr package) to assess the relationship of ammonia concentration, proportions of VFAs (acetic,

succinic, propionic, valeric, isovaleric, isobutyric and butyric acid), VFA total concentration and acetate-to-propionate ratio versus relative abundance of bacterial out. Significant correlations ($P < 0.05$) were visualized on a network generated in Cytoscape v 3.2.1 (Shannon et al., 2003). The networks were built using sheets with shared and exclusives correlations of each feed efficiency group.

Results

Sequencing

We generated 3,201,058 bacterial raw sequences with a maximum length of 500 pb, average length of 253 pb and minimum length of 245 pb across all samples. To Archaea, we generated 1,202,160 raw sequences with a maximum length of 600 pb, average length of 446 pb and minimum length of 35 pb across all samples. After trimming, quality filtering and removal of chimeras, 1,727,202 high-quality bacterial sequences and 251,013 archaeal sequences were obtained. Samples pooled by liquid and solid ruminal fractions yielded a mean of $31,985 \pm \text{SD } 11,119$ bacterial and $4,827 \pm 2,106$ archaeal sequences, respectively.

The Good's coverage of the bacterial community was $> 97 \%$ and for the archaeal community was $> 99 \%$, indicating sufficient coverage for comparisons of sequence abundance between samples and analysis of community shifts. The summary sequence counts and OTUs that passed the steps of filtering, clean up and normalization are shown in Table 1. Only reads and OTUs that were identified in at least 50 % of all the steers in a specific RFI group (7 animals to p-RFI and 6 animals to n-RFI) were considered for the analysis of the feed efficiency phenotype in the Nelore steers (cut-off* in Table 1). This criteria was based on previous observations indicating that species of ruminal bacteria that exhibit a heritable component show high presence ($\geq 50 \%$) across animals (Sasson et al., 2017).

Table 1. Summary of sequencing data (Bacteria and Archaea) derived from ruminal contents of Nelore steers according to ruminal phase and RFI group.

		Steers (n)	Good's coverage	After filtering and clean-up		After normalization		After cut-off*	
				Reads	OTUs	Reads	OTUs	Reads	OTUs
BACTERIA									
Liquid	p-RFI	15	0.991 ± 0.003	38836 ± 14279	1441 ± 188	9973 ± 72	1068 ± 126	8627 ± 417	706 ± 66
	n-RFI	12	0.989 ± 0.003	31429 ± 8034	1436 ± 118	9926 ± 106	1079 ± 84	8760 ± 451	713 ± 37
Solid	p-RFI	15	0.989 ± 0.003	28964 ± 8102	1231 ± 173	9923 ± 98	936 ± 153	8597 ± 474	624 ± 74
	n-RFI	12	0.987 ± 0.006	27754 ± 9581	1245 ± 201	10000 ± 120	995 ± 118	8845 ± 500	666 ± 51
ARCHAEA									
Liquid	p-RFI	14	0.998 ± 0.001	3898 ± 1728	38 ± 9	1164 ± 4	29 ± 4	1144 ± 30	22 ± 2
	n-RFI	12	0.998 ± 0.002	4575 ± 3116	37 ± 9	1164 ± 4	28 ± 5	1152 ± 9	22 ± 3
Solid	p-RFI	14	0.998 ± 0.001	5379 ± 1646	41 ± 7	1163 ± 2	27 ± 4	1145 ± 20	21 ± 2
	n-RFI	12	0.999 ± 0.001	5519 ± 1428	44 ± 7	1164 ± 2	31 ± 4	1153 ± 9	24 ± 2

Values represent mean and standard deviation. *Reads and OTUs that were detected in at least half of the steers in each feed efficiency group (at least 7 animals to p-RFI and 6 animals to n-RFI).

Specific bacterial OTUs are associated with the p-RFI and n-RFI phenotype

Alpha diversity was measured using Chao1 richness, Simpson's and Shannon's diversity indexes and results demonstrated that the bacterial community of Nelore steers did not vary between RFI groups (T-test $P > 0.05$), regardless of the ruminal phase (Table 2).

Table 2. Alpha-diversity metrics of bacterial and archaeal communities in the rumen of Nelore steers according to RFI group.

	Liquid			Solid		
	p-RFI	n-RFI	<i>P</i> -value	p-RFI	n-RFI	<i>P</i> -value
BACTERIA						
Chao	1730 ± 301	1750 ± 281	0.863	1442 ± 388	1564 ± 379	0.417
Shannon	5.35 ± 0.34	5.44 ± 0.21	0.404	5.28 ± 0.27	5.32 ± 0.20	0.690
Simpson	0.018 ± 0.008	0.015 ± 0.007	0.345	0.017 ± 0.006	0.016 ± 0.006	0.903
ARCHAEA						
Chao	37.57 ± 9.36	48.11 ± 12.93	< 0.05	34.87 ± 6.87	42.26 ± 9.27	< 0.05
Shannon	1.86 ± 0.31	1.72 ± 0.34	0.292	1.81 ± 0.24	1.78 ± 0.33	0.898
Simpson	0.26 ± 0.12	0.3 ± 0.15	0.488	0.27 ± 0.09	0.28 ± 0.13	0.980

Values represent mean and standard deviation.

Beta diversity analysis showed that the Bray-Curtis dissimilarities of the bacterial communities differed according to the ruminal phase (solid or liquid fraction) (Anosim, $P < 0.001$), whereas the dissimilarities between bacterial communities from steers showing p-RFI or n-RFI did not vary significantly in the ruminal contents (Figure 1).

Taxonomic analysis of the ruminal bacterial community in the liquid fraction revealed 3672 unique OTUs (mean 1073 ± SD 108 per sample after normalization) that were assigned to 20 phyla, 38 classes, 67 orders, 110 families and 237 genera. Nearly 2.92 % (± 0.44 %), 3.96 % (± 0.43 %), 4.65% (± 0.51 %), 7.43 % (± 0.76 %) and 22.37 % (± 0.91 %) of the OTUs could not be assigned to any phylum, class, order, family or genus, respectively. The RL bacterial community was dominated by phyla Firmicutes (55.74 ± 2.17 %), Bacteroidetes (20.53 ± 1.5 %), Tenericutes (5.59 ± 0.91 %), Verrucomicrobia (3.25 ± 0.43 %), Spirochaetae (3.1 ± 0.72 %), Actinobacteria (2.62 ± 0.59 %) and Proteobacteria (2.2 ± 0.41 %) (Figure 2). The most abundant families included the Ruminococcaceae (21.23 ± 1.02 %), Lachnospiraceae (15.87 ± 1.6 %), Prevotellaceae (10.16 ± 1.26 %) and Christensenellaceae (5.81 ± 1.03 %) (Figure 3). In the RS bacterial community, 3342 unique OTUs (mean of 962 ± 132

OTUs per sample after normalization) were assigned to 18 phyla, 32 classes, 62 orders, 97 families and 209 genera. Nearly 2.16 (± 0.5 %), 3.19 (± 0.7 %), 3.69 (± 0.67 %), 6.04 (± 0.9 %) and 21.85 (± 1.27 %) of OTUs could not be assigned to any phylum, class, order, family or genus, respectively. The RS bacterial community was dominated by phyla Firmicutes (59.17 ± 1.65 %), Bacteroidetes (20.58 ± 1.60 %), Tenericutes (4.57 ± 1.08 %), Spirochaetae (3.27 ± 0.44 %), Actinobacteria (2.83 ± 0.53 %), Verrucomicrobia (1.94 ± 0.35 %) and Proteobacteria (1.62 ± 0.34 %) (Figure 2). Abundant families included the Ruminococcaceae (20.57 ± 1.91 %), Lachnospiraceae (20.18 ± 1.30 %), Prevotellaceae (10.17 ± 1.20 %) and Christensenellaceae (6.65 ± 0.94 %) (Figure 3).

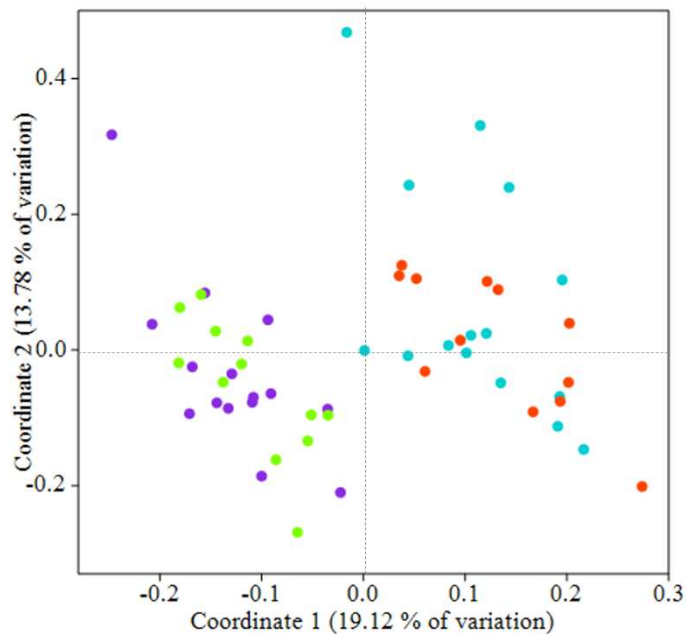


Figure 1. Principal Coordinate Analysis (PCoA) using Bray-Curtis dissimilarity metric for bacterial communities from rumen of steers. Individual points represent a rumen sample and colors represent ruminal fractions and RFI groups. Blue and orange dots represent samples from rumen liquid fraction of p-RFI and n-RFI steers, respectively. Purple and green dots represent samples from rumen solids obtained from p-RFI and n-RFI steers, respectively.

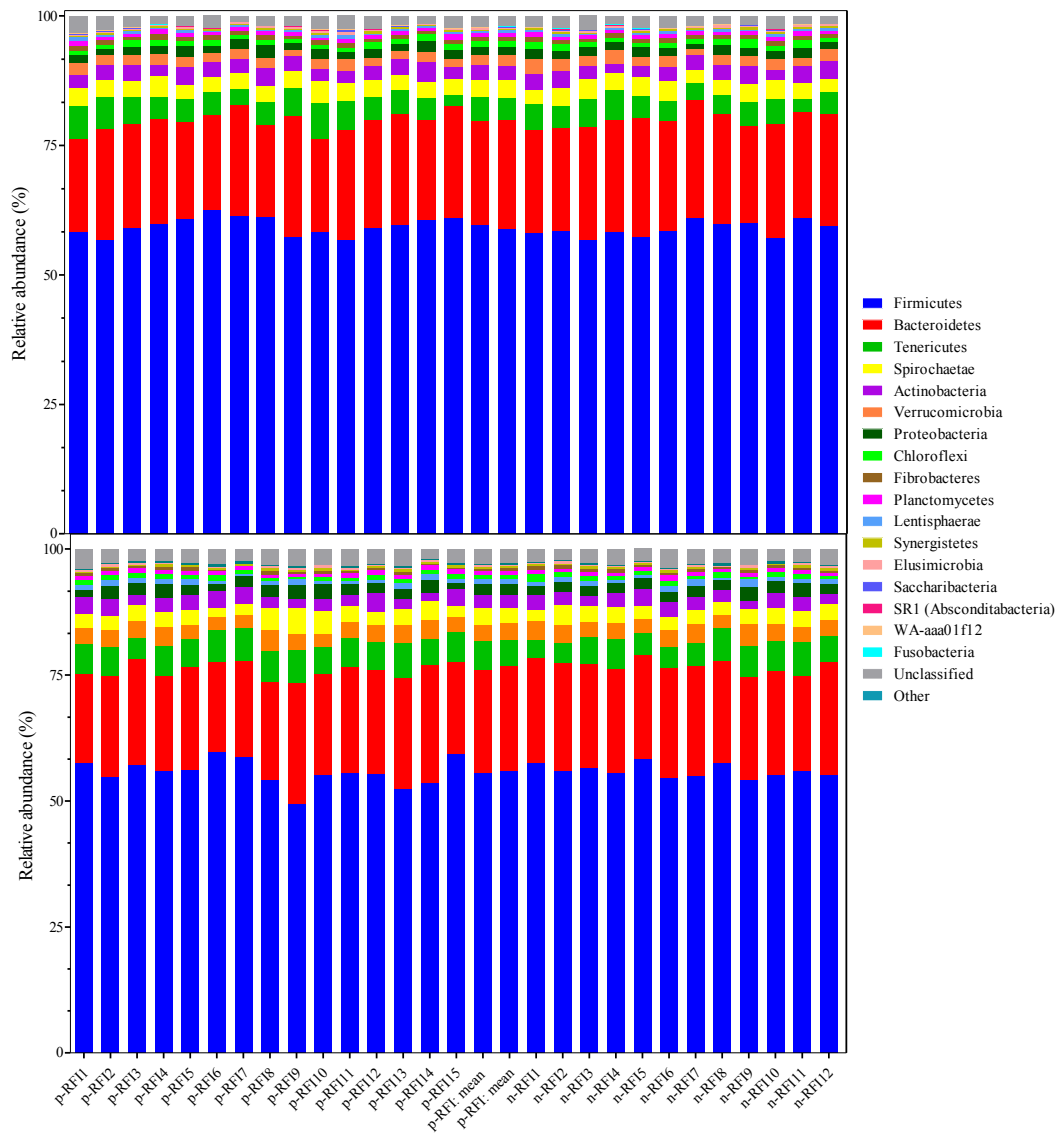


Figure 2. Ruminal bacterial composition at phylum-level according to the RFI group. Each bar represents a different steer and the mean composition of the p-RFI and n-RFI bacterial community is shown in the center of the figure. The composition of rumen liquids is represented at the top chart and samples derived from rumen solids are represented in the bottom chart. “Other” corresponds to the sum of phyla that showed relative abundance < 0.1 %.

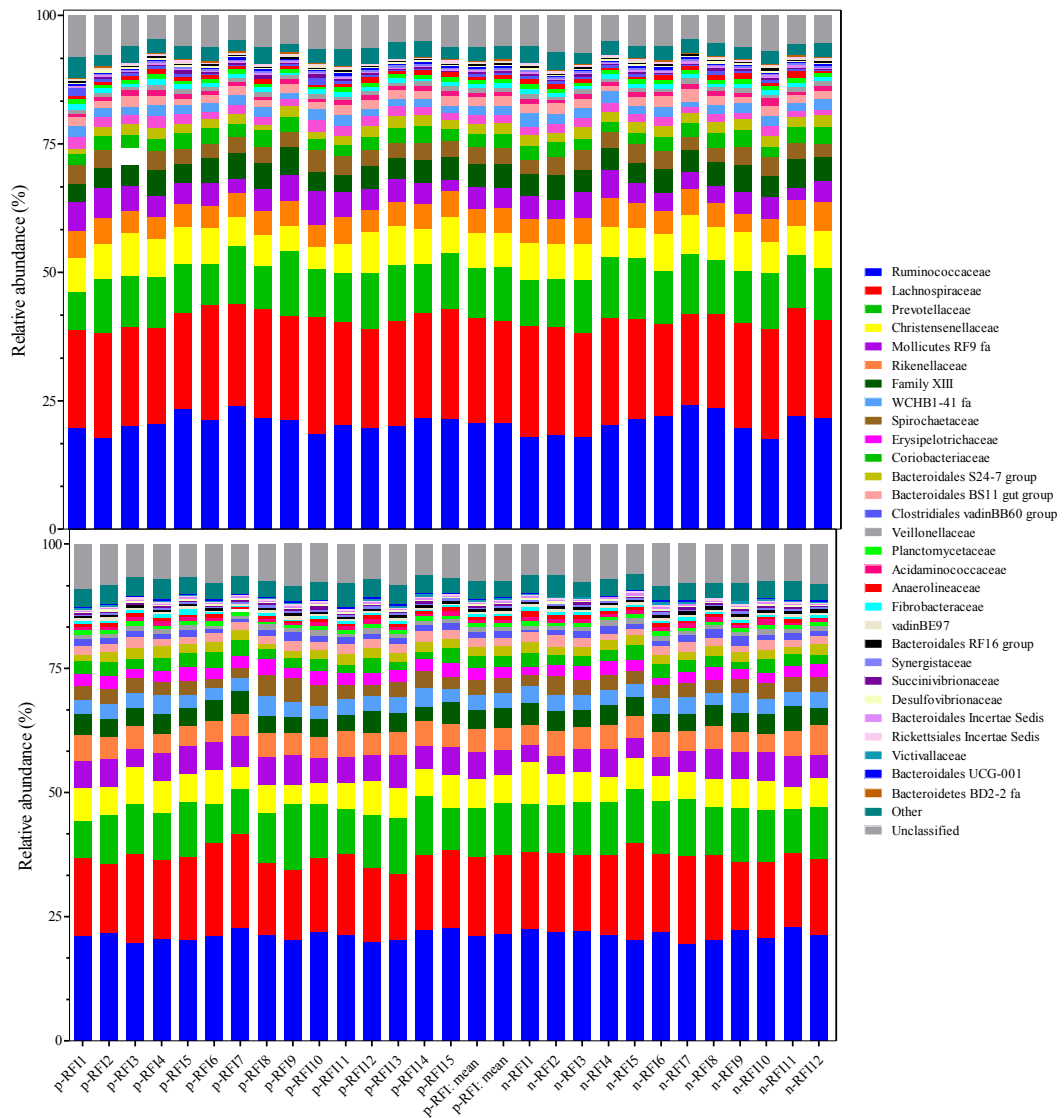


Figure 3. Ruminal bacterial composition at family-level according to the RFI group. Each bar represents a different steer and the mean composition of the p-RFI and n-RFI bacterial community is shown in the center of the figure. The composition of rumen liquids is represented at the top chart and samples derived from rumen solids are represented in the bottom chart. “Other” corresponds to the sum of phyla that showed relative abundance < 0.2 %.

Although the composition of the bacterial communities did not vary between steers with different feed efficiency phenotype, the relative abundance of some taxonomic groups (t-test, $P < 0.05$) were different according to the ruminal phase (solid or liquid fraction) (Figure 4). Among these, Anaeroplasmatales and Lineage I order, ODP1230B8-23, Lachnospiraceae NK3A20 group, Bacteroidales RF16 group, Lachnospiraceae ND3007 group and Bacteroidetes BD2-2 ge genus in rumen liquids

and *Desulfovibrio*, horsej-a03 and Prevotellaceae UCG-001 genus in rumen solids showed higher abundance on more efficient steers (n-RFI).

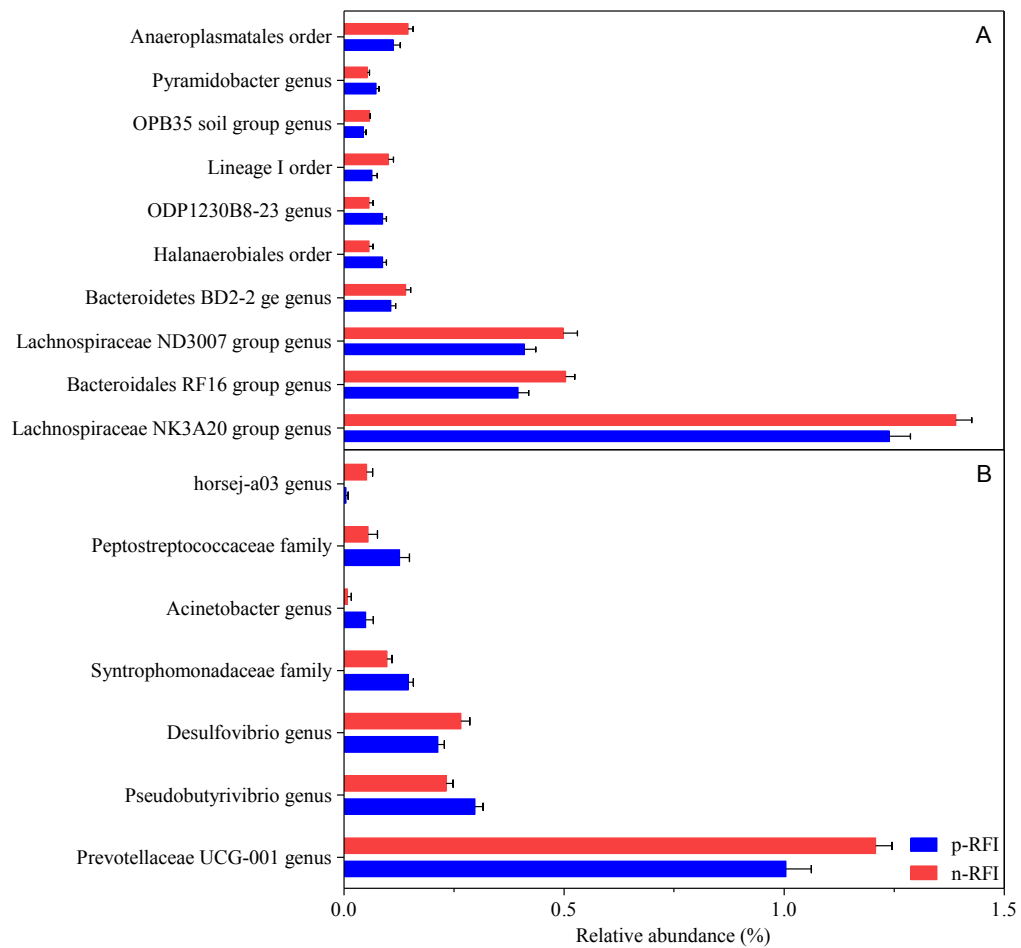


Figure 4. Taxonomic groups showing significant differences (t-test, $P < 0.05$) in relative abundance in liquid (A) and solid (B) ruminal fractions between p-RFI and n-RFI steers. The blue bars represent the p-RFI group and the red bars represent n-RFI group. Data are expressed as mean relative abundance with the standard error.

Moreover, the Firmicutes to Bacteroidetes (F/B) ratio, an index previously reported as being associated with dysbiosis or differences in phenotypes in humans, rodents and ruminants, was significantly higher (t-test, $P < 0.05$) in p-RFI steers than in n-RFI steers (Figure 5). In the liquid ruminal fraction, the average F/B ratio was 2.90 ± 0.07 to the p-RFI steers and 2.67 ± 0.04 to the n-RFI steers. In the RS fraction, the average ratio was 2.99 ± 0.08 to p-RFI and 2.75 ± 0.05 to the n-RFI.

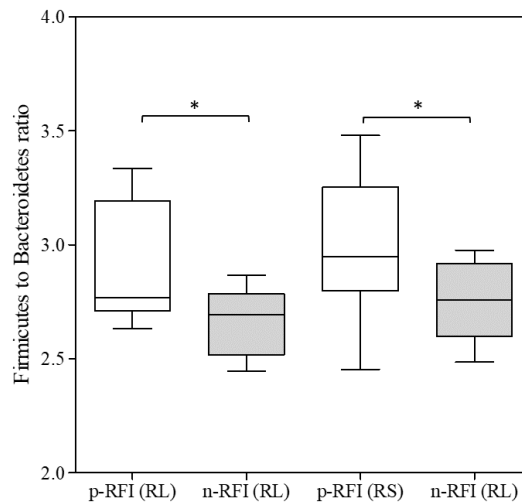


Figure 5. The Firmicutes to Bacteroidetes ratio in liquid (RL) and solid (RS) ruminal phase, according to the RFI group. White bars represent the p-RFI steers and grey bars represent the n-RFI steers. (*) Significantly different ($P < 0.05$) by the t-test.

Our Venn diagrams showed that out of 1132 bacterial OTUs identified in the liquid fraction of at least 50 % of the steers from each feed efficiency group, 182 OTUs were only found in the p-RFI steers (corresponding to 3.43 % of relative abundance), while the n-RFI steers showed 149 exclusive OTUS (2.84 % of relative abundance) (Figure 6). In the solid ruminal phase, 955 bacterial OTUs were identified in at least half of the animals with p-RFI or n-RFI. From these, 105 OTUs were unique to the p-RFI steers (2.57 % of relative abundance), while 126 OTUs were only identified in n-RFI steers (3.54 % of relative abundance) (Figure 6).

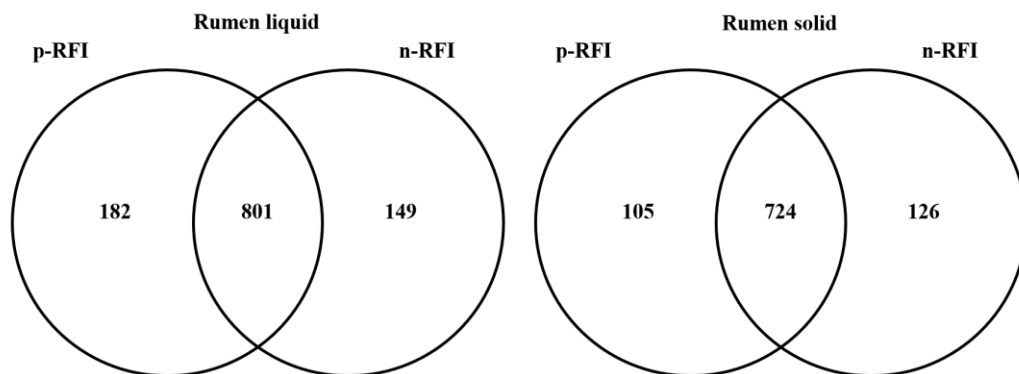


Figure 6. Venn diagram showing the number of bacterial OTUs, from liquid and solid ruminal fractions, shared between p-RFI and n-RFI steers. Only bacterial OTUs that were identified in at least 50 % of the steers from each feed efficiency group (at least 7 animals to p-RFI and 6 animals to n-RFI) are represented.

Our data revealed that the most abundant bacterial OTUs in the rumen contents of p-RFI and n-RFI steers accounted for less than 2 % of the total OTUs and were represented by members of the Christensenellaceae and Lachnospiraceae families or were classified within the genus *Prevotella* (Table 3).

Table 3. Most abundant exclusive bacterial OTUs identified in rumen contents (liquid and solid phases) of Nelore steers showing low (p-RFI) or high (n-RFI) feed efficiency.

Rumen liquid	Taxonomy*	Relative abundance (%)	SEM	
p-RFI	Otu00210	Bacteroidales BS11 gut group ge	0.168	0.138
	Otu00232	<i>Prevotella</i> 1	0.133	0.067
	Otu00204	<i>Prevotella</i> 1	0.129	0.085
	Otu00123	Bacteroidales BS11 gut group ge	0.125	0.063
	Otu00389	Bacteroidales BS11 gut group ge	0.080	0.059
	Otu00343	Firmicutes unclassified	0.077	0.064
	Otu00440	Christensenellaceae R-7 group	0.076	0.042
	Otu00509	Prevotellaceae UCG-003	0.068	0.051
	Otu00145	Lachnospiraceae unclassified	0.063	0.030
	Otu00554	<i>Sphaerochaeta</i>	0.063	0.046
n-RFI	Otu00153	<i>Saccharofermentans</i>	0.178	0.114
	Otu00167	Rikenellaceae RC9 gut group	0.122	0.035
	Otu00163	Lachnospiraceae probable genus 10	0.101	0.050
	Otu00094	Lachnospiraceae AC2044 group	0.080	0.034
	Otu00256	Prevotellaceae NK3B31 group	0.077	0.037
	Otu00653	Prevotellaceae UCG-003	0.076	0.037
	Otu00182	<i>Succiniclasticum</i>	0.070	0.026
	Otu00654	Bacteroidales BS11 gut group ge	0.052	0.022
	Otu00649	Lachnospiraceae ND3007 group	0.050	0.021
	Otu00380	Christensenellaceae R-7 group	0.048	0.023
Rumen solids	Taxonomy*	Relative abundance (%)	SEM	
p-RFI	Otu00021	Christensenellaceae R-7 group	0.682	0.336
	Otu00123	Bacteroidales BS11 gut group ge	0.200	0.105
	Otu00253	Lachnospiraceae unclassified	0.110	0.036
	Otu00368	<i>Saccharofermentans</i>	0.085	0.030
	Otu00210	Bacteroidales BS11 gut group ge	0.077	0.059
	Otu00311	Bacteroidales BS11 gut group ge	0.070	0.038
	Otu00464	<i>Fibrobacter</i>	0.069	0.021
	Otu00493	<i>Butyrivibrio</i> 2	0.053	0.027
	Otu00343	Firmicutes unclassified	0.050	0.038
	Otu00528	Bacteroidales S24-7 group ge	0.050	0.018
n-RFI	Otu00056	Bacteroidales BS11 gut group ge	0.407	0.263
	Otu00045	<i>Prevotella</i> 1	0.302	0.220

Otu00163	Lachnospiraceae probable genus 10	0.289	0.136
Otu00094	Lachnospiraceae AC2044 group	0.199	0.077
Otu00247	Bacteroidales UCG-001 ge	0.195	0.092
Otu00256	Prevotellaceae NK3B31 group	0.171	0.081
Otu00153	<i>Saccharofermentans</i>	0.142	0.067
Otu00365	<i>Prevotella</i> 1	0.096	0.081
Otu00116	Lachnospiraceae ND3007 group	0.076	0.029
Otu00348	Bacteroidales BS11 gut group ge	0.063	0.042

(*) Taxonomy for each OTU is given at the highest classifiable level. SEM, standard error of mean.

Additionally, volcano plot analysis was used to visualize the differences in relative abundance of bacterial OTUs that were shared between the rumen contents of p-RFI and n-RFI steers (Figure 7). Out of 801 shared OTUs found in the liquid ruminal fraction, 54.1 % was enriched in n-RFI steers, and 14 OTUs were statistically significant (White's non-parametric t-test, $P < 0.05$), whereas 45.9 % of the OTUs was enriched in p-RFI, and only four OTUs were statistically significant (White's non-parametric t-test, $P < 0.05$) (Table 4). In the solid ruminal fraction, out of 724 OTUs, approximately 54 % was enriched in the n-RFI steers and 46 % was enriched in the animals with p-RFI. However, only nine OTUs were statistically higher in n-RFI and 17 were statistically more abundant in the p-RFI steers (Table 4). As observed for the exclusive OTUs identified in both rumen portions, different strains belonging to Christensenellaceae, Lachnospiraceae, Prevotellaceae, as well as OTUs belonging to the Ruminococcaceae family were enriched in p-RFI or n-RFI steers (Table 4).

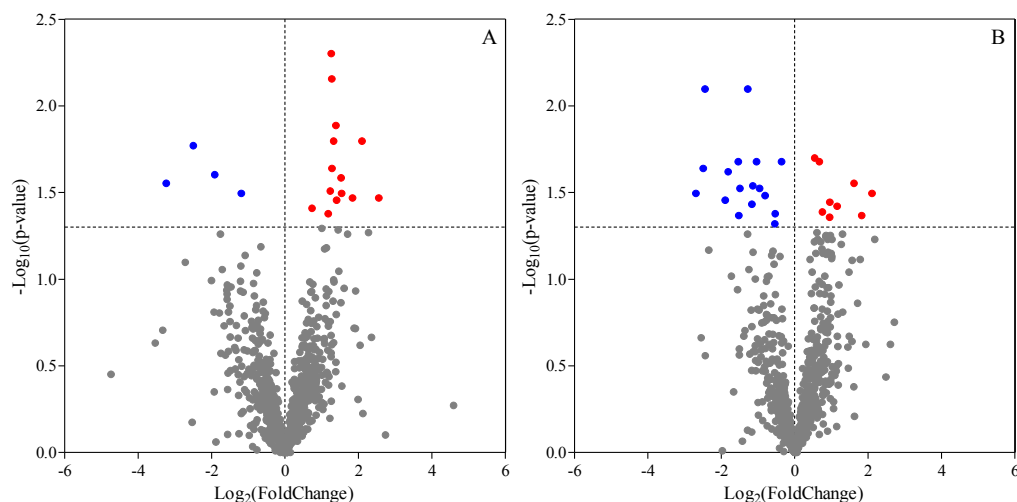


Figure 6. Differences in relative abundance of shared OTUs from liquid (A) and solid (B) ruminal communities. Each point represents an OTU and points that showed $\text{Log}_2(\text{FoldChange}) > 0$ were OTUs enriched in the n-RFI steers, while points that showed $\text{Log}_2(\text{FoldChange}) < 0$ were OTUs enriched in the p-RFI steers. Red points

are OTUs statistically greater in n-RFI and blue points were statistically greater in p-RFI (White's non-parametric t-test, $P < 0.05$).

Table 4. Bacterial OTUs that were statistically more abundant (White's non-parametric t-test, $P < 0.05$) in n-RFI (grey lines) or p-RFI (white lines) steers.

	p-RFI	SEM	n-RFI	SEM	Taxonomy*
Rumen liquid					
Otu00040	0.457	0.071	1.093	0.212	Bacteroidales RF16 group ge
Otu00118	0.146	0.028	0.342	0.078	Prevotellaceae UCG-003
Otu00212	0.046	0.015	0.268	0.117	Bacteroidales BS11 gut group ge
Otu00353	0.045	0.012	0.130	0.032	<i>Ruminococcus</i> 2
Otu00149	0.047	0.007	0.113	0.021	Lachnospiraceae XPB1014 group
Otu00301	0.010	0.003	0.043	0.012	Lachnospiraceae probable genus 10
Otu00442	0.021	0.003	0.034	0.005	Lachnospiraceae NK3A20 group
Otu00694	0.012	0.003	0.032	0.010	Ruminococcaceae UCG-014
Otu00563	0.008	0.003	0.029	0.009	Lachnospiraceae NK3A20 group
Otu00897	0.010	0.003	0.024	0.005	Lachnospiraceae NK3A20 group
Otu00969	0.010	0.002	0.023	0.005	Ruminococcaceae UCG-010
Otu00834	0.008	0.003	0.021	0.004	Christensenellaceae R-7 group
Otu00779	0.007	0.002	0.019	0.005	<i>Senegalimassilia</i>
Otu00685	0.006	0.002	0.015	0.003	Ruminococcaceae unclassified
Otu00103	0.144	0.031	0.063	0.014	Ruminococcaceae NK4A214 group
Otu00404	0.079	0.024	0.021	0.008	Halanaerobiales ODP1230B8.23ge
Otu00121	0.182	0.105	0.019	0.008	Ruminococcaceae NK4A214 group
Otu00454	0.066	0.023	0.012	0.004	Erysipelotrichaceae UCG-004
Rumen solid					
Otu00003	1.752	0.328	3.404	0.639	Rikenellaceae RC9 gut group
Otu00149	0.167	0.031	0.282	0.037	Lachnospiraceae XPB1014 group
Otu00144	0.110	0.012	0.161	0.016	<i>Desulfovibrio</i>
Otu00118	0.081	0.009	0.130	0.017	Prevotellaceae UCG-003
Otu00212	0.039	0.015	0.120	0.028	Bacteroidales BS11 gut group ge
Otu00369	0.025	0.007	0.088	0.029	Bacteroidales S24-7 group ge
Otu00563	0.029	0.005	0.056	0.012	Lachnospiraceae NK3A20 group
Otu00807	0.013	0.003	0.030	0.007	Lachnospiraceae unclassified
Otu00478	0.007	0.002	0.029	0.009	<i>Ruminobacter</i>
Otu00017	0.654	0.086	0.449	0.042	Clostridiales unclassified
Otu00087	0.394	0.049	0.273	0.032	Lachnospiraceae XPB1014 group
Otu00159	0.136	0.008	0.106	0.009	Ruminococcaceae NK4A214 group
Otu00121	0.223	0.082	0.063	0.017	Ruminococcaceae NK4A214 group
Otu00266	0.056	0.010	0.028	0.006	Ruminococcaceae UCG-010
Otu00496	0.052	0.008	0.027	0.006	Christensenellaceae R-7 group
Otu00142	0.052	0.011	0.022	0.005	<i>Prevotella</i> 1
Otu00233	0.104	0.031	0.019	0.009	<i>Prevotella</i> 1

Otu00519	0.049	0.013	0.017	0.006	ClostridialesFamily XIII AD3011 group
Otu00431	0.089	0.042	0.016	0.006	Lachnospiraceae XPB1014 group
Otu00680	0.026	0.005	0.015	0.003	Lachnospiraceae UCG-008
Otu00632	0.036	0.010	0.013	0.004	Ruminococcaceae unclassified
Otu00404	0.040	0.014	0.011	0.003	HalanaerobialesODP1230B8.23 ge
Otu00809	0.020	0.004	0.009	0.002	<i>Atopobium</i>
Otu00452	0.054	0.035	0.008	0.002	Spirochaetaceae unclassified
Otu00735	0.022	0.006	0.008	0.002	Christensenellaceae R-7 group
Otu00843	0.017	0.004	0.007	0.002	Ruminococcaceae UCG-010

The values represent relative abundance and standard error of mean (SEM). (*) Taxonomy for each OTU is given at the highest classifiable level.

Richness and specific OTUs of archaeal community are associated with the p-RFI and n-RFI phenotype

The alpha diversity analysis of RL and RS archaeal communities demonstrated that Simpson's and Shannon's diversity did not vary significantly in response to the RFI group (t-test $P > 0.05$), although the Chao1 richness metric of the rumen samples derived from n-RFI steers (45.18 ± 11.1) was significantly greater than the p-RFI steers (36.22 ± 8.12) (Table 2).

Beta diversity analysis, summarized on a PCoA with Bray-Curtis dissimilarities, showed difference between the RL and RS communities given that samples clustered separately (Anosim, $P < 0.001$). In response to the efficiency group, no differences were observed given that p-RFI and n-RFI cluster are overlapped to both RL and RS communities (Figure 7).

Our analyses of taxonomic composition of the archaeal community revealed an average of 78 unique OTUs in the rumen contents (liquid and solid phase) of Nelore steers. All OTUs were assigned to the Euryarchaeota phyla and around 82.26 ± 0.47 % were classified in the Methanobacteria class, Methanobacteriales order and Methanobacteriaceae family; 15.63 ± 0.53 % of the archaeal OTUs were assigned to the Thermoplasmata class, Thermoplasmatales order and Thermoplasmatales Incertae Sedis family. Less abundant groups of ruminal archaea accounted for 1.89 ± 0.01 % of the sequences and were classified in the Methanomicrobia class, Methanosarcinales order and Methanosarcinaceae family (Figure 8).

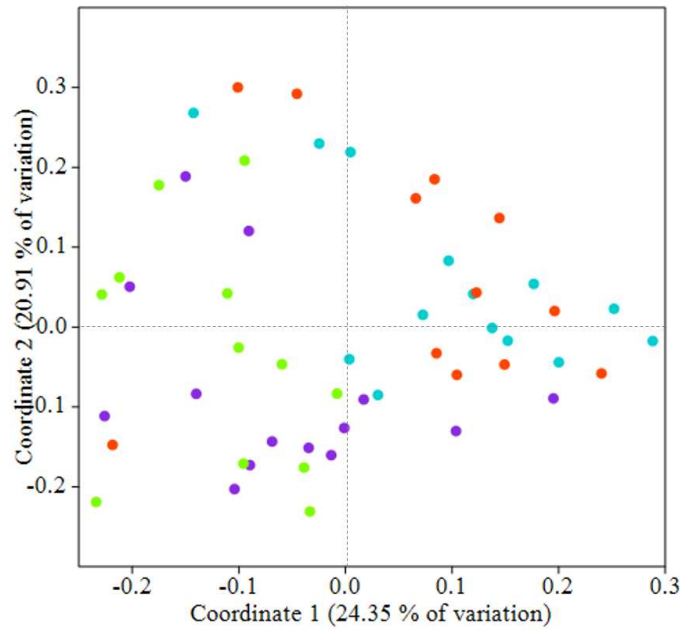


Figure 7. Principal Coordinate Analysis (PCoA) using Bray-Curtis dissimilarity metric for archaeal communities from rumen of steers. Individual points represent a rumen sample and colors represent ruminal fractions and RFI groups. Blue and orange dots represent samples from rumen liquid fraction of p-RFI and n-RFI steers, respectively. Purple and green dots represent samples from rumen solids obtained from p-RFI and n-RFI steers, respectively.

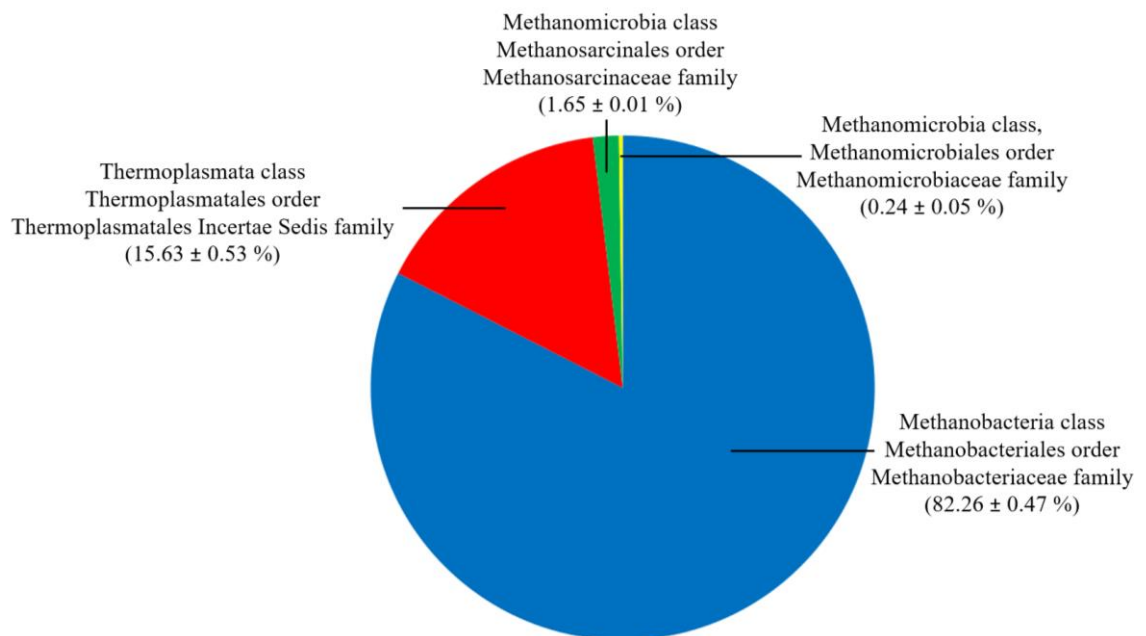


Figure 8. Composition of the ruminal archaea at class, order and family level.

Nearly 17.43 (± 5.82 %) of the sequences in ruminal liquids and 16.27 (± 4.41 %) in ruminal solids of OTUs could not be assigned at genus level (Figure 9). The archaeal community in ruminal contents was dominated by members of the genus *Methanobrevibacter* (77.23 ± 6 % in RS), followed by *Methanosphaera* (3.21 ± 1.67 %) and *Methanimicrococcus* (1.65 ± 1.27 %) (Figure 9). When considering the feed efficiency group, the relative abundance of *Methanimicrococcus* was, on average, two fold higher in the n-RFI steers than in the p-RFI steers (t-test, $P < 0.05$) (Figure 10).

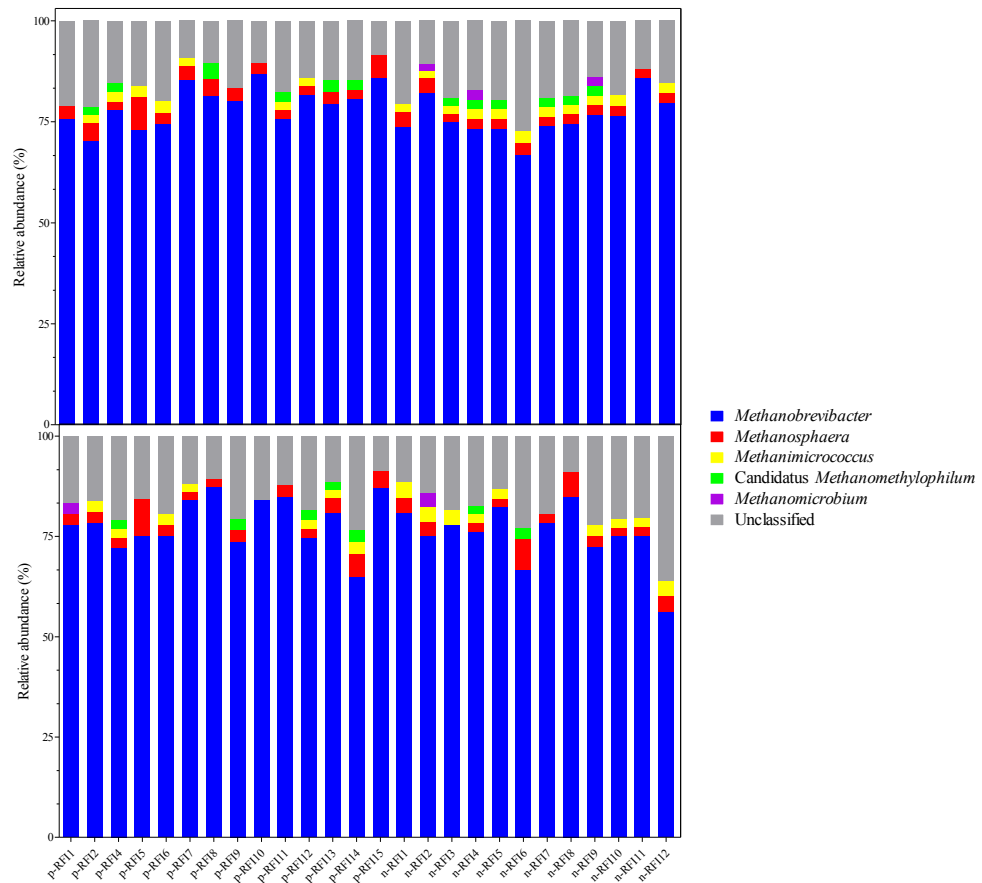


Figure 9. Ruminal archaeal composition at genus-level according to the RFI group. Each bar represents a different steer and the composition of rumen liquids is represented at the top chart and samples derived from rumen solids are represented in the bottom chart.

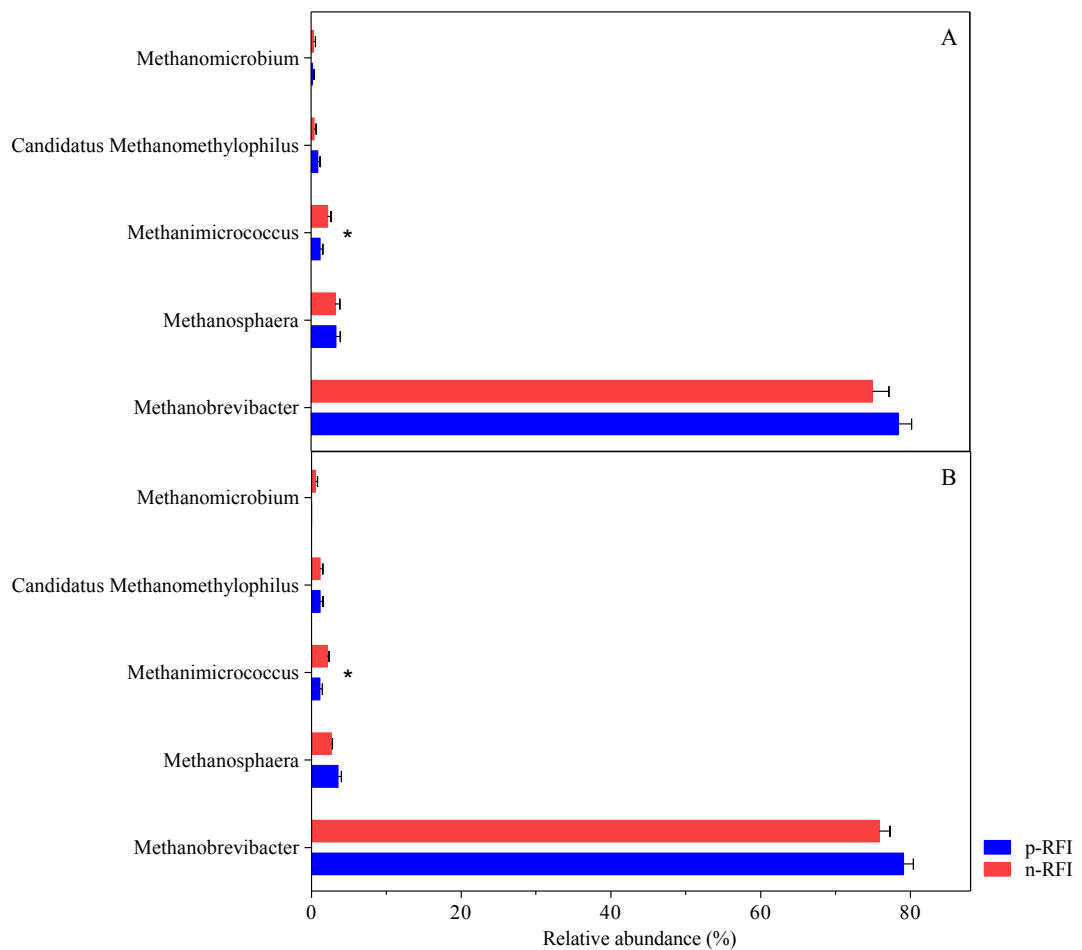


Figure 10. Archaeal composition at genus-level in liquid (A) and solid (B) ruminal fractions according to the RFI group. The blue bars represent the p-RFI group and the red bars represent n-RFI group. Data are expressed as mean relative abundance with the standard error.

Our Venn diagrams showed that out of 32 archaeal OTUs identified in the liquid fraction of at least 50 % of the steers from each feed efficiency group, four (all assigned as *Methanobrevibacter*) were only found in the p-RFI steers (corresponding to 3.36 % of relative abundance), while the n-RFI steers showed five exclusive OTUS (two *Methanobrevibacter*, one *Methanimicrococcus*, one Methanobacteriaceae and one Thermoplasmatales Incertae Sedis, corresponding to 0.6 % of relative abundance). In the solid ruminal phase, 31 archaeal OTUs were identified in at least half of the steers with p-RFI or n-RFI. From these, two OTUs (both assigned as *Methanobrevibacter*) were unique to the p-RFI steers (0.81 % of relative abundance), while five OTUs (three *Methanobrevibacter*, one *Methanimicrococcus* and one

Methanobacteriaceae unclassified) were only identified in n-RFI steers (about 1 % of relative abundance) (Figure 11).

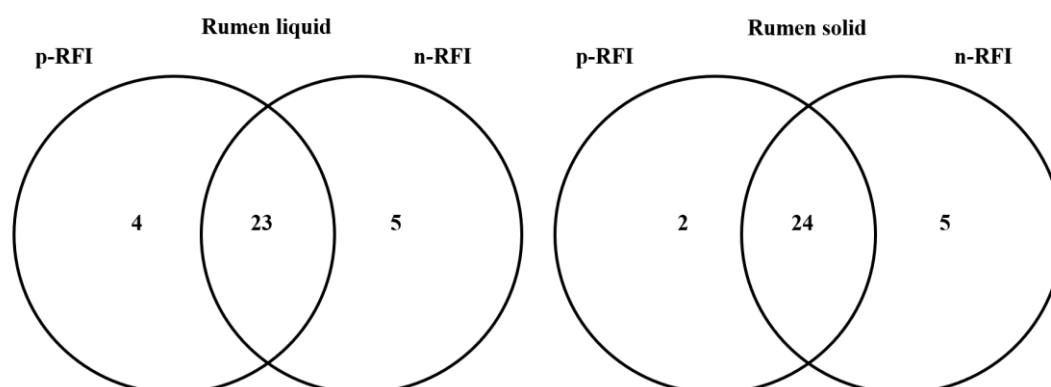


Figure 11. Venn diagram showing the number of archaeal OTUs, from liquid and solid ruminal fractions, shared between p-RFI and n-RFI steers. Only archaeal OTUs that were identified in at least 50 % of the steers from each feed efficiency group (at least 7 animals to p-RFI and 6 animals to n-RFI) are represented.

Considering the OTUs shared in the liquid ruminal phase, 60.9 % was enriched in the p-RFI steers, with OTU0016 (*Methanobrevibacter*) being almost 2.5 fold higher compared to the n-RFI steers (White’s non-parametric t-test, $P < 0.05$) (Table 5). In contrast, most shared OTUs (62.5 %) identified in the solid ruminal phase showed higher abundance in the n-RFI steers, although OTU0001 (*Methanobrevibacter*), one of the most abundant archaeal sequences, was approximately two fold higher in the p-RFI steers compared to the n-RFI steers (Table 5).

Table 5. Relative abundance of shared OTUs in n-RFI steers (grey lines) or p-RFI steers (white lines).

	p-RFI	SEM	n-RFI	SEM	P-value	Taxonomy*
Rumen liquid						
Otu0003	16.096	4.723	19.021	5.390	0.692	<i>Methanobrevibacter</i>
Otu0002	10.816	1.917	11.629	5.393	0.944	<i>Methanobrevibacter</i>
Otu0007	5.531	1.579	10.045	2.754	0.187	<i>Methanobrevibacter</i>
Otu0004	2.733	0.635	4.728	1.692	0.368	<i>Methanobrevibacter</i>
Otu0010	1.098	0.494	1.132	0.373	0.950	<i>Methanobrevibacter</i>
Otu0011	0.971	0.362	1.058	0.754	0.959	<i>Methanobrevibacter</i>
Otu0008	0.959	0.323	1.513	0.682	0.521	<i>Methanobrevibacter</i>
Otu0021	0.178	0.049	0.186	0.053	0.879	Thermoplasmatales Incertae Sedis uncultured
Otu0025	0.135	0.022	0.136	0.042	0.955	<i>Methanobrevibacter</i>
Otu0001	33.641	4.770	29.597	5.956	0.618	<i>Methanobrevibacter</i>

Otu0006	11.323	1.817	9.266	2.223	0.466	<i>Methanobrevibacter</i>
Otu0005	7.712	1.320	7.273	1.758	0.849	<i>Methanobrevibacter</i>
Otu0009	1.246	0.354	0.896	0.176	0.448	<i>Methanosphaera</i>
Otu0018	0.392	0.117	0.386	0.129	0.975	<i>Methanobrevibacter</i>
Otu0016	0.375	0.072	0.157	0.047	0.028	<i>Methanobrevibacter</i>
Otu0017	0.331	0.149	0.315	0.087	0.937	Thermoplasmatales Incertae Sedis uncultured
Otu0019	0.252	0.071	0.186	0.058	0.498	Thermoplasmatales Incertae Sedis uncultured
Otu0023	0.246	0.047	0.129	0.047	0.113	<i>Methanobrevibacter</i>
Otu0022	0.221	0.023	0.179	0.029	0.257	<i>Methanobrevibacter</i>
Otu0024	0.197	0.046	0.100	0.028	0.086	<i>Methanobrevibacter</i>
Otu0028	0.178	0.039	0.158	0.069	0.829	Thermoplasmatales Incertae Sedis uncultured
Otu0026	0.172	0.048	0.165	0.059	0.884	Thermoplasmatales Incertae Sedis uncultured
Otu0032	0.129	0.050	0.079	0.019	0.399	<i>Methanobrevibacter</i>

Rumen solid

Otu0004	14.044	4.360	19.658	4.964	0.425	<i>Methanobrevibacter</i>
Otu0003	12.475	3.971	18.040	4.716	0.400	<i>Methanobrevibacter</i>
Otu0005	5.084	0.904	5.600	1.064	0.710	<i>Methanobrevibacter</i>
Otu0008	3.410	0.600	8.224	4.314	0.398	<i>Methanobrevibacter</i>
Otu0007	1.296	0.348	2.544	0.916	0.249	<i>Methanobrevibacter</i>
Otu0016	0.350	0.091	0.394	0.104	0.766	<i>Methanobrevibacter</i>
Otu0020	0.264	0.034	0.322	0.057	0.422	<i>Methanobrevibacter</i>
Otu0017	0.221	0.053	0.286	0.057	0.409	Thermoplasmatales Incertae Sedis uncultured
Otu0019	0.178	0.062	0.200	0.050	0.789	Thermoplasmatales Incertae Sedis uncultured
Otu0021	0.166	0.052	0.208	0.055	0.600	Thermoplasmatales Incertae Sedis uncultured
Otu0025	0.160	0.029	0.222	0.045	0.265	<i>Methanobrevibacter</i>
Otu0026	0.111	0.035	0.136	0.031	0.625	Thermoplasmatales Incertae Sedis uncultured
Otu0018	0.111	0.044	0.136	0.056	0.798	<i>Methanobrevibacter</i>
Otu0028	0.086	0.027	0.115	0.021	0.386	Thermoplasmatales Incertae Sedis uncultured
Otu0030	0.049	0.014	0.122	0.034	0.093	<i>Methanobrevibacter</i>
Otu0002	29.377	3.823	24.994	5.477	0.528	<i>Methanobrevibacter</i>
Otu0001	19.077	4.415	8.696	2.327	0.043	<i>Methanobrevibacter</i>
Otu0006	5.850	1.226	4.457	1.337	0.483	<i>Methanobrevibacter</i>
Otu0009	2.898	0.358	2.384	0.440	0.389	<i>Methanosphaera</i>
Otu0010	1.484	0.826	0.609	0.258	0.448	<i>Methanobrevibacter</i>
Otu0011	0.393	0.147	0.294	0.154	0.651	<i>Methanobrevibacter</i>
Otu0024	0.215	0.059	0.150	0.044	0.404	<i>Methanobrevibacter</i>
Otu0022	0.172	0.040	0.079	0.024	0.068	<i>Methanobrevibacter</i>
Otu0023	0.166	0.045	0.150	0.029	0.781	<i>Methanobrevibacter</i>

The values represent relative abundance and standard error of mean (SEM). Bold *P*-values are < 0.05 (White's non-parametric t-test). (*) Taxonomy for each OTU is given at the highest classifiable level.

Correlations between microbial community and ruminal fermentation parameters

The molar proportion of acetic, propionic, succinic, butyric, isobutyric, valeric and isovaleric acids, acetate-to-propionate ratio, total concentration of short-chain volatile fatty acids (SCFA), ammonia concentration and ruminal pH did not vary between the p-RFI and n-RFI steers (t-test, $P > 0.05$). On the other hand, Spearman's rank correlation analysis indicated significant associations between ruminal fermentation parameters and 638 and 544 OTUs identified in the liquid and solid ruminal phases, respectively.

We performed microbial-metabolite network analysis to visualize correlations (represented by nodes connected by an edge) between the ruminal bacterial community of p-RFI and n-RFI steers with fermentation products (Figure 12). In the liquid ruminal phase, 193 OTUs from p-RFI steers, 183 OTUs from n-RFI steers and 44 OTUs shared by both phenotypic groups were positively associated with at least one ruminal fermentation parameter. The p-RFI steers had nearly two times more OTUs correlating with the concentration of acetic acid, isobutyric acid and with ruminal pH than the n-RFI steers. However, the opposite was observed for ammonia concentration and total VFA in the n-RFI steers, where the number of OTUs correlating with these parameters was higher compared to the OTUs in the liquid ruminal phase of the p-RFI steers (Figure 12). In the solid ruminal phase 183 and 180 bacterial OTUs from the p-RFI and n-RFI steers, respectively, and 57 OTUs shared by both phenotypic groups were positively associated with at least one biochemistry parameter. Isobutyric acid and pH were more correlated with OTUs from the p-RFI steers than the n-RFI ones, while ammonia concentration was more correlated with the OTUs from steers with the n-RFI phenotype (Figure 12).

Analysis of the negative correlations between the ruminal microbiota and the fermentation parameters revealed 198 and 200 OTUs from p-RFI, and n-RFI steers, respectively, and 39 OTUs shared by both phenotypic groups that were negatively associated with at least one biochemical parameter. Propionic acid and total VFA were correlated with more OTUs from the p-RFI than the n-RFI steers, while isovaleric acid and pH were more correlated with the microbiota of n-RFI steers than the p-RFI animals (Figure 12). Also, 148 and 163 OTUs from the p-RFI and n-RFI steers and 35 from both groups were associated with at least one fermentation parameter in the solid phase. Total VFA was negatively correlated with more OTUs of the p-RFI steers than

the n-RFI group, while pH was more negatively correlated with the OTUs from the n-RFI animals (Figure 12).

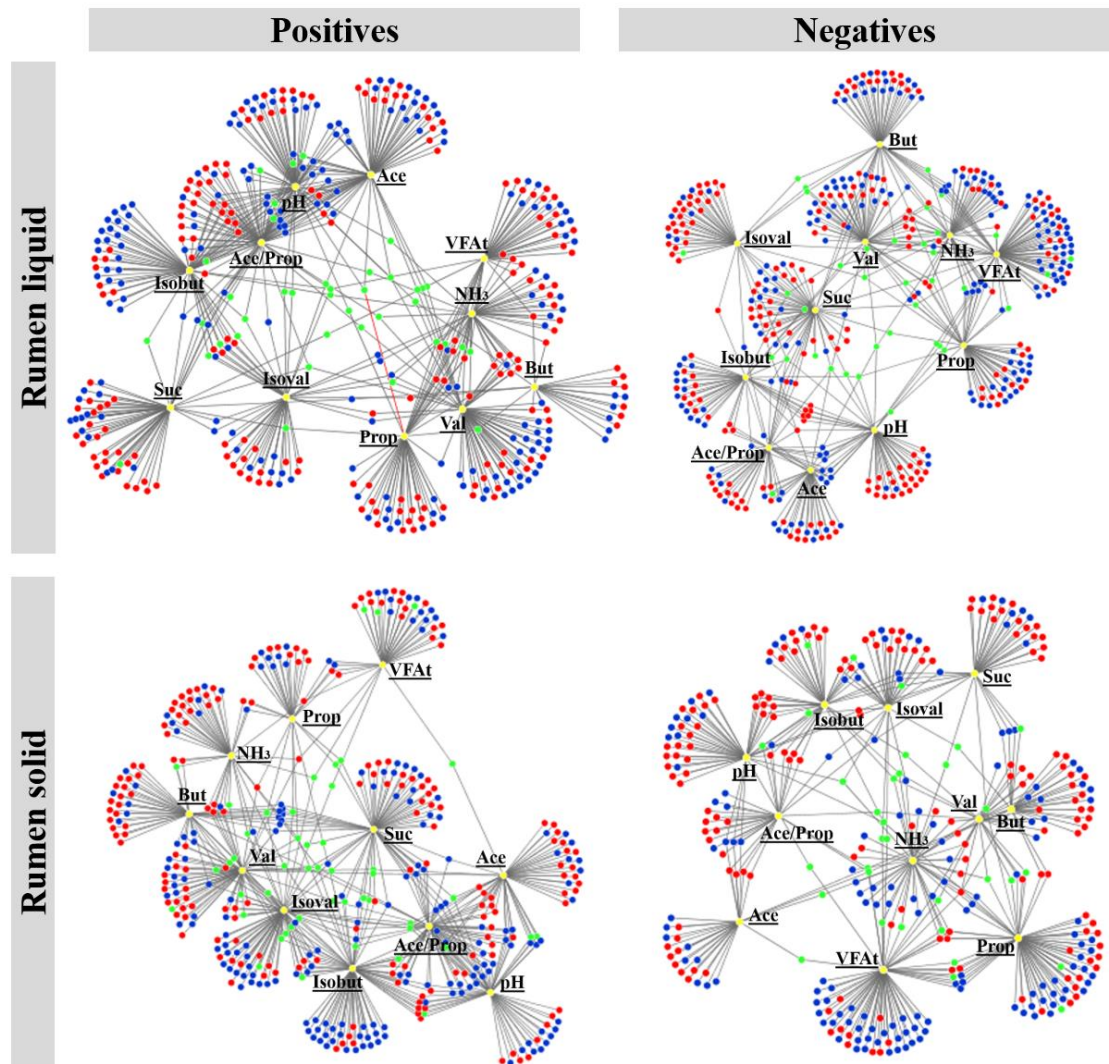


Figure 12. Correlation network between liquid and solid ruminal OTUs and fermentation parameters according to each RFI group. The edges represent significant associations, detected by Spearman's rank correlation ($P < 0.05$), red points represent OTUs correlated only with n-RFI steers, blue points represent OTUs correlated only with p-RFI steers, green points represent OTUs correlated with both RFI groups and yellow points represent the biochemistry parameters (molar proportion of acetic, Ace; propionic, Prop; succinic, Suc; butyric, But; isobutyric, Isobut; valeric, Val; and isovaleric acids, Isoval; acetate-to-propionate ratio, Ace/Prop; total concentration of these volatile fatty acids, VFAT; ammonia concentration, NH_3 ; and ruminal pH). These analyses were performed using OTUs that were shared by at least 50% of the steers in each feed efficiency group.

Among the most abundant OTUs showing positive or negative correlations with the fermentation parameters, we observed a predominance of OTUs from

different genera of the Prevotellaceae, Ruminococcaceae, Rikenellaceae, Lachnospiraceae and Christensenellaceae families, in the bacterial communities of both the solid and liquid ruminal phase (Tables S1 to S4, supplementary material). In addition to the differences at OTU level, taxonomic differences were also observed between the most abundant OTUs correlated with steers in the p-RFI or n-RFI groups. For example, the most abundant OTUs that were positively correlated with total VFAs were assigned as *Papillibacter*, Bacteroidales BS11 gut group ge, Ruminococcaceae ge, Prevotellaceae UCG-004, Lachnospiraceae unclassified, Bacteroidales BS11 gut group ge and *Treponema 2* in the p-RFI steers, while in the n-RFI steers the most abundant OTUs were assigned as Rikenellaceae RC9 gut group, *Prevotella 1*, *Phocaeicola*, *Anaerotruncus*, Bacteroidales BS11 gut group ge, Bacteroidales S24-7 group ge, Anaerolineaceae unclassified, Christensenellaceae R-7 group and Lachnospiraceae XPB1014 group (Table S1).

Discussion

The production of grazing cattle under tropical conditions presents peculiarities associated with the fact that the nutritional composition of the forage vary considerably according to the season (dry and rainy season), being necessary to implement protein supplementation programs to improve animal performance and the overall production efficiency (Detmann et al., 2014). Therefore, selecting animals with improved feed efficiency could lower the costs of protein supplementation with potential benefits to farmers. Here, we investigated the composition of ruminal microbial communities of beef cattle (Nelore breed) raised under tropical conditions and with high and low feed efficiency phenotypes.

The steers were classified in different feed efficiency groups based on their residual feed intake (RFI), a measure that is widely used to estimate feed efficiency in beef cattle and has the advantage of being independent of the growth and weight of the animal (Basarab et al., 2003; Herd et al., 2003). However, the evaluation of the RFI has limitations since it depends on the confinement of the herd for up to 90 days, daily record of the quantities of the feed supplied and the refusals, and weight assessments of the animals. In addition, if animals are housed in groups, individual feed intake can only be verified using specialized equipment (eg: Grow-Safe). In Brazil, limitations are more accentuated by the fact that beef cattle are raised predominantly on pasture

in extensive systems and very few farmers have access to the infrastructure needed to measure RFI on a commercial scale. In this study, we evaluated the ruminal microbiota of 12 Nelore steers with negative RFI (high feed efficiency) and 15 with positive RFI (low feed efficiency). These 27 steers were selected from a contemporary herd of 129 animals, whose RFI were individually measured.

We generate high quality sequencing data (coverage > 97 %), allowing a deep and reliable characterizing the ruminal microbiota composition (Bacteria and Archaea). Our results confirmed previous observations demonstrating that microbial communities in the solid and liquid phases are distinct (Jami and Mizrahi, 2012; Jewell et al., 2015; Michelland et al., 2011) (Figure 1 and 7), which reinforces the relevance of a separate analysis to evaluate the contributions of feed-attached and planktonic ruminal microbiota to the feed efficiency phenotype.

Our study also demonstrated that lower feed efficiency Nelore steers harbor a ruminal microbiota with increased Firmicutes/Bacteroidetes ratio (Figure 5). Changes in the overall composition of the gut microbiota reflecting in an increased Firmicutes/Bacteroidetes ratio has been related to certain animal phenotypes such as higher fat deposition in blood and tissues of humans and mice (Ley et al., 2006; Turnbaugh and Gordon, 2009) and increased milk fat in dairy cows (Jami et al., 2014). We also identified a higher relative abundance (0.13 %) of the Peptostreptococcaceae family (belonging to the phylum Firmicutes) in the rumen of low efficient animals (p-RFI steers) (Figure 4). This bacterial family comprises the hyperammonia-producing bacteria (HAB), such as *Peptostreptococcus anaerobius* (Paster et al., 1993), which are known to have rates of amino acid deamination 10-20 times higher than the mixed ruminal bacteria. Ruminal ammonia production from dietary components (rumen degradable protein, peptides and amino acids) is essential for microbial protein synthesis in the rumen, which supplies up to 90 % of the nitrogen demand of the host. However, excessive ammonia production represents losses of nitrogen through the urine, contributing to environmental contamination and resulting in energy losses to the animal (Rychlik and Russell, 2000). This is probably not the case in tropical production systems, where supplementation of grazing animals is a common practice to adjust the nutritional imbalance of the pastures and improve animal performance (Bento et al., 2016; Detmann et al., 2014), but inefficient nitrogen utilization could represent metabolic and economical losses.

Our results revealed specific OTUs belonging to the Lachnospiraceae and Ruminococcaceae families and the *Prevotella* genus associated with Nelore steers showing n-RFI (more efficient) or p-RFI (less efficient) (Table 3 and 4). Our data supports previous observations indicating that these microbial groups have a role in the feed efficiency phenotype of beef and dairy cattle (Carberry et al., 2012; Jewell et al., 2015; McCann et al., 2014; Myer et al., 2015a; Shabat et al., 2016). Lachnospiraceae family comprises fibrolytic and proteolytic species while members of the Ruminococcaceae family are mainly represented by the ruminal cellulolytic bacteria. The genus *Prevotella* is one of the most abundant taxa in the rumen of cattle and comprise bacterial species that are metabolic versatile, being able to grow on starch, protein, peptides, hemicellulose and pectin (Meehan and Beiko, 2014; Stevenson and Weimer, 2007). These findings indicate that OTUs with greater abundance in the negative RFI animals may contribute more efficiently to the metabolism and energy utilization of dietary components. Hypothetically, some strains of *Prevotella* with great metabolic versatility may have positive effects on rumen fermentation in high-efficiency steers, while some strains that occupy specific niches in the rumen of low efficiency animals could have a negative influence on fermentation by inhibiting (through competition or production of antimicrobials) other microbial populations that were more efficient in the degradation of certain substrates. For example, the production of bacteriocins by species of *Prevotella* has been demonstrated in isolates obtained from human gingival fibroblasts (Kaewsrichan et al., 2005) and production of antimicrobial peptides appears to be a common trait among several species of ruminal bacteria. The controversial effects of *Prevotella* on the ruminal ecosystem have been reported in different studies. In 2008, Chiquette et al., demonstrated that *Prevotella* inoculation is associated with probiotic activities in the rumen, leading to decreased lactate production and increased milk fat yield. Nonetheless, Jami et al., (2014) evidenced a strong and significant negative correlation between the abundance of *Prevotella* and fat production in milk.

Our analysis also evidenced that, to each feed efficiency group, different OTUs were correlated with the fermentation products (Figure 12) and most of these OTUs belonged to the same taxonomic groups (Tables S1 to S4, supplementary material). These findings reinforce the idea that feed efficiency phenotype is associated with specific rumen bacteria, which may contribute differently to rumen metabolism and

host physiology. The need to further understand the metabolic potential and ecological interactions of rumen microorganisms has been largely recognized (Creevey et al., 2014). Recent studies based on culture-dependent and culture-independent (as RNAseq) methods have sought to improve the characterization of functional groups involved in ruminal fermentation, expanding our understanding of their role in this ecosystem (Bento et al., 2015; Boonsaen et al., 2018; Zhang et al., 2018). For example, Bento et al. (2015) found that some HAB isolated from the rumen of Nelore steers fed with tropical forages were able to metabolize sugars as a source of carbon, which challenged the previous idea that this group of bacteria were composed only by obligate amino acid fermenters (Krause and Russell, 1996). These findings suggested that carbohydrate-metabolizing HAB have a potentially competitive advantage for the exploration and colonization of ecological niches in the rumen.

In addition to the complex bacterial community that are responsible to hydrolyze nonstructural and structural carbohydrates, proteins, peptides, amino amides, and lipids into VFAs, ammonia, hydrogen and CO₂, the archaeal community represents an important group to the balance of ruminal fermentation and could also play a role in the energy retention balance in ruminants. The methanogenic archaea consume H₂, preventing the accumulation of reducing equivalents that would limit the fermentation of dietary components (McAllister and Newbold, 2008; Morgavi et al., 2010). Our work evidenced that negative RFI Nelore steers exhibit greater archaeal richness in the rumen (Table 1). The contribution of increased archaeal richness to feed efficiency is not obvious but may be related to the more efficient H₂ sink, allowing better ruminal fermentation and greater digestibility of feed particles (McAllister and Newbold, 2008). We also observed that *Methanobrevibacter* was the most abundant genus of archaea in the rumen of Nelore steers, followed by *Methanosphaera* and *Methanimicrococcus* (Figure 9). These methanogenic archaea use the hydrogenotrophic pathway for methane production oxidizing H₂ or formic acid as electron donors (Morgavi et al., 2010). Although some ruminal archaea (e.g. members of the Methanosarcinales order and the *Methanosphaera* genus) that use the methylotrophic or the acetoclastic pathways were also found the rumen of Nelore steers, we could not speculate about the relationship between different methane production pathways and the feed efficiency phenotype. Our observed differences (exclusive or more abundant OTUs identified in each feed efficiency group) were

assigned to OTUs classified, mainly, in the *Methanobrevibacter* genus, which uses primarily the hydrogenotrophic pathway for methane production (Table 5).

Despite the results of microbial composition suggesting functional differences associated with animal phenotypes, the characterization of the microbiome genetic potential (evaluated through metagenomic analysis), gene expression and metabolomics will be relevant to clarify the relationship between rumen function and feed efficiency. Shabat et al., (2016) evaluated the taxonomic composition and genetic potential (metagenomic sequencing) of the ruminal microbiota of Hostein Friesian cows. The authors observed a lower diversity (Shannon index) and greater species and gene dominance in cows that were more efficient in milk production. The metabolic flux analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed that less efficient cows had a greater number of metabolic pathways and enzymes associated with protein degradation and absorption, amino acid biosynthesis and methane production. The authors suggested that the greater diversity of species, genes and metabolic pathways of the ruminal microorganisms negatively affect the process of energy obtaining by their hosts. That is, more efficient microbiomes tend to be less complex and more specialized to meet the energy requirements of the host (Shabat et al., 2016).

Although the current study is the first to evaluate the ruminal microbiome of Nelore steers varying in feed efficiency phenotype, other studies focused on the analysis of carcass and meat quality (Fidelis et al., 2017), as well the expression of genes in the ruminal epithelium of these animals (Benedeti et al., 2018). Fidelis et al. (2017) observed that the low RFI steers consumed 0.479 kg/day less feed than the high RFI animals, which consumed 0.483 kg/day more feed than expected. No differences were observed in terms of edible portion of carcass, amount of bone, trimmings and meat quality. These findings demonstrate that, despite consuming less feed, the carcass traits and meat of more efficient animals were similar to those of the animals that consumed more food, i.e., lower feed consumption did not compromise production characteristics or body carcass performance and meat quality. Benedeti et al., (2018) evaluated the expression of genes involved in metabolism and transport in the rumen epithelium of Nelore steers. These results revealed a decrease in the expression of genes encoding major proteins involved in the process of oxidative phosphorylation in rumen epithelium of low RFI compared to high RFI animals. However, no differences

were observed in the expression of genes related to thermogenesis, pumping of ions and turnover of ruminal epithelial proteins. These results may indicate a higher production and energy expenditure in the rumen epithelium of high RFI animals, contributing to their lower efficiency.

Therefore, host features, as capacity of absorption and metabolism of the volatile fatty acids to meat production, could also be related with the feed efficiency phenotype in Nelore steers. In addition, our findings indicate that Nelore steers with high and low feed efficiency show specific differences in bacterial and archaeal ruminal communities. These differences involve important functional groups involved in the ruminal fermentation of dietary compounds. Future studies including the analysis of the genetic and functional capabilities of rumen microorganisms, using metagenomics, metatranscriptomics and metabolomics approaches can provide greater understanding about the relationship between ruminal microbiome and feed efficiency in Nelore steers. Additionally, further efforts to isolate, characterize, and quantify ruminal strains that vary in abundance and diversity between n-RFI and p-RFI Nelore steers will be of value to understand the metabolic attributes and ecological functions of microorganisms with potential causal roles in the feed efficiency phenotype of beef cattle.

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Supplementary material

Table S1. Relative abundance of the most abundant OTUs showing positive correlation with ruminal fermentation parameters in the p-RFI steers

Parameter	Liquid				Solid			
	OTU	Taxonomy*	Relative abundance (%)	SEM	OTU	Taxonomy*	Relative abundance (%)	SEM
Ace	Otu00064	<i>Prevotella</i> 1	0.468	0.198	Otu00028	Rikenellaceae RC9 gut group	0.735	0.126
	Otu00057	Bacteroidales BS11 gut group ge	0.461	0.181	Otu00031	<i>Prevotella</i> 1	0.619	0.307
	Otu00031	<i>Prevotella</i> 1	0.449	0.135	Otu00016	<i>Prevotella</i> 1	0.455	0.108
	Otu00197	Rikenellaceae RC9 gut group	0.170	0.079	Otu00086	Lachnospiraceae AC2044 group	0.455	0.054
	Otu00138	Prevotellaceae UCG-001	0.139	0.047	Otu00095	Rikenellaceae RC9 gut group	0.407	0.071
Prop	Otu00003	Rikenellaceae RC9 gut group	1.951	0.399	Otu00003	Rikenellaceae RC9 gut group	1.752	0.328
	Otu00044	Christensenellaceae R-7 group	0.629	0.550	Otu00078	Lachnospiraceae ge	0.331	0.065
	Otu00048	Bacteroidales BS11 gut group ge	0.448	0.068	Otu00066	<i>Phocaeicola</i>	0.293	0.101
	Otu00198	Planctomycetaceae p-1088-a5 gut group	0.097	0.026	Otu00177	<i>Fibrobacter</i>	0.176	0.043
	Otu00214	<i>Saccharofermentans</i>	0.094	0.026	Otu00258	Prevotellaceae UCG-004	0.104	0.045
But	Otu00172	<i>Succiniclasicum</i>	0.105	0.037	Otu00187	<i>Acetitomaculum</i>	0.150	0.053
	Otu00187	<i>Acetitomaculum</i>	0.059	0.019	Otu00124	Ruminococcaceae NK4A214 group	0.133	0.025
	Otu00386	Bifidobacteriaceae uncultured	0.054	0.025	Otu00181	Clostridiales Family XIII unclassified	0.132	0.021
	Otu00312	Christensenellaceae R-7 group	0.051	0.009	Otu00071	Verrucomicrobia WCHB1-41 ge	0.126	0.018
	Otu00376	Rikenellaceae RC9 gut group	0.034	0.011	Otu00172	<i>Succiniclasicum</i>	0.093	0.037
Isobut	Otu00006	<i>Butyrivibrio</i> 2	0.588	0.137	Otu00015	Rikenellaceae RC9 gut group	1.026	0.299
	Otu00015	Rikenellaceae RC9 gut group	0.464	0.151	Otu00023	<i>Papillibacter</i>	0.955	0.062
	Otu00057	Bacteroidales BS11 gut group ge	0.461	0.181	Otu00021	Christensenellaceae R-7 group	0.682	0.336
	Otu00013	<i>Pseudobutyrvibrio</i>	0.450	0.064	Otu00096	<i>Prevotella</i> 1	0.309	0.131
	Otu00096	<i>Prevotella</i> 1	0.288	0.121	Otu00072	Ruminococcaceae ge	0.192	0.044
Suc	Otu00011	<i>Prevotella</i> 1	1.826	0.626	Otu00011	<i>Prevotella</i> 1	0.506	0.094
	Otu00112	Bacteroidales UCG-001 ge	0.198	0.034	Otu00082	<i>Moryella</i>	0.288	0.026
	Otu00150	Ruminococcaceae UCG-005	0.162	0.028	Otu00187	<i>Acetitomaculum</i>	0.150	0.053
	Otu00180	Ruminococcaceae UCG-002	0.130	0.021	Otu00124	Ruminococcaceae NK4A214 group	0.133	0.025
	Otu00245	Christensenellaceae R-7 group	0.087	0.023	Otu00320	Bacteroidetes BD2-2 ge	0.112	0.071
Va	Otu00047	Ruminococcaceae NK4A214 group	0.442	0.124	Otu00047	Ruminococcaceae NK4A214 group	0.426	0.083

Isoval	Otu00035	<i>Mogibacterium</i>	0.415	0.152	Otu00081	Lachnospiraceae NK3A20 group	0.281	0.039
	Otu00116	Lachnospiraceae ND3007 group	0.189	0.061	Otu00183	<i>Ruminococcus</i> 1	0.144	0.021
	Otu00181	Clostridiales Family XIII unclassified	0.143	0.034	Otu00181	Clostridiales Family XIII unclassified	0.132	0.021
	Otu00065	Rikenellaceae RC9 gut group	0.128	0.043	Otu00342	Chloroflexi unclassified	0.079	0.015
	Otu00015	Rikenellaceae RC9 gut group	0.464	0.151	Otu00015	Rikenellaceae RC9 gut group	1.026	0.299
	Otu00098	Prevotellaceae UCG-003	0.147	0.031	Otu00036	Rikenellaceae U29-B03	0.511	0.175
	Otu00164	Veillonellaceae UCG-001	0.135	0.030	Otu00103	Ruminococcaceae NK4A214 group	0.295	0.037
	Otu00245	Christensenellaceae R-7 group	0.087	0.023	Otu00184	Lachnospiraceae XPB1014 group	0.174	0.051
	Otu00274	Prevotellaceae UCG-003	0.083	0.022	Otu00183	<i>Ruminococcus</i> 1	0.144	0.021
Ace/Prop	Otu00064	<i>Prevotella</i> 1	0.468	0.198	Otu00015	Rikenellaceae RC9 gut group	1.026	0.299
	Otu00015	Rikenellaceae RC9 gut group	0.464	0.151	Otu00011	<i>Prevotella</i> 1	0.506	0.094
	Otu00057	Bacteroidales BS11 gut group ge	0.461	0.181	Otu00095	Rikenellaceae RC9 gut group	0.407	0.071
	Otu00197	Rikenellaceae RC9 gut group	0.170	0.079	Otu00074	Lachnospiraceae XPB1014 group	0.358	0.068
	Otu00207	<i>Spirochaeta</i> 2	0.114	0.019	Otu00064	<i>Prevotella</i> 1	0.350	0.107
VFAT	Otu00038	<i>Papillibacter</i>	0.328	0.047	Otu00025	Bacteroidales BS11 gut group ge	0.136	0.039
	Otu00210	Bacteroidales BS11 gut group ge	0.168	0.138	Otu00210	Bacteroidales BS11 gut group ge	0.077	0.059
	Otu00234	Ruminococcaceae ge	0.089	0.023	Otu00327	<i>Treponema</i> 2	0.076	0.020
	Otu00258	Prevotellaceae UCG-004	0.082	0.043	Otu00234	Ruminococcaceae ge	0.069	0.019
	Otu00253	Lachnospiraceae unclassified	0.058	0.023	Otu00220	Bacteroidales BS11 gut group ge	0.061	0.035
pH	Otu00064	<i>Prevotella</i> 1	0.468	0.198	Otu00028	Rikenellaceae RC9 gut group	0.735	0.126
	Otu00023	<i>Papillibacter</i>	0.372	0.030	Otu00011	<i>Prevotella</i> 1	0.506	0.094
	Otu00197	Rikenellaceae RC9 gut group	0.170	0.079	Otu00095	Rikenellaceae RC9 gut group	0.407	0.071
	Otu00190	Rikenellaceae RC9 gut group	0.120	0.046	Otu00074	Lachnospiraceae XPB1014 group	0.358	0.068
	Otu00236	Victivallales vadinBE97 ge	0.112	0.017	Otu00072	Ruminococcaceae ge	0.192	0.044
NH	Otu00048	Bacteroidales BS11 gut group ge	0.448	0.068	Otu00003	Rikenellaceae RC9 gut group	1.752	0.328
	Otu00191	Ruminococcaceae NK4A214 group	0.117	0.030	Otu00005	<i>Succiniclasticum</i>	1.541	0.175
	Otu00239	Ruminococcaceae ge	0.081	0.014	Otu00019	Prevotellaceae NK3B31 group	1.023	0.805
	Otu00316	Anaerolineaceae unclassified	0.041	0.009	Otu00041	<i>Saccharofermentans</i>	0.677	0.268
	Otu00302	Ruminococcaceae UCG-010	0.033	0.008	Otu00052	Clostridiales unclassified	0.394	0.108

The values represent relative abundance and standard error of mean (SEM). (*) Taxonomy for each OTU is given at the highest classifiable level. Biochemistry parameters: molar proportion of acetic, Ace; propionic, Prop; succinic, Suc; butyric, But; isobutyric, Isobut; valeric, val; and isovaleric acids, Isoval; acetate-to-propionate ratio, Ace/Prop; total concentration of these volatile fats acids, VFAT; ammonia concentration, NH₃; and pH.

Table S2. Relative abundance of the most abundant OTUs showing positive correlation with ruminal fermentation parameters in the n-RFI steers.

Parameter	Liquid				Solid			
	OTU	Taxonomy*	Relative abundance (%)	SEM	OTU	Taxonomy*	Relative abundance (%)	SEM
Ace	Otu00156	Ruminococcaceae UCG-005	0.204	0.086	Otu00088	<i>Fibrobacter</i>	0.344	0.141
	Otu00154	Rikenellaceae RC9 gut group	0.130	0.033	Otu00101	Veillonellaceae UCG-001	0.192	0.025
	Otu00120	<i>Butyrivibrio</i> 2	0.096	0.035	Otu00131	Christensenellaceae R-7 group	0.167	0.080
	Otu00269	<i>Selenomonas</i> 1	0.070	0.012	Otu00276	<i>Lachnoclostridium</i> 10	0.155	0.107
	Otu00280	<i>Treponema</i> 2	0.060	0.014	Otu00251	Bacteroidetes BD2-2 ge	0.118	0.023
Prop	Otu00028	Rikenellaceae RC9 gut group	0.380	0.082	Otu00184	Lachnospiraceae XPB1014 group	0.208	0.047
	Otu00326	Prevotellaceae UCG-003	0.182	0.094	Otu00109	Prevotellaceae UCG-004	0.168	0.071
	Otu00205	Verrucomicrobia WCHB1-41 ge	0.148	0.063	Otu00310	Clostridiales Family XIII AD3011 group	0.093	0.037
	Otu00109	Prevotellaceae UCG-004	0.137	0.056	Otu00375	Lachnospiraceae unclassified	0.075	0.016
	Otu00159	Ruminococcaceae NK4A214 group	0.110	0.017	Otu00347	Ruminococcaceae UCG-002	0.068	0.018
But	Otu00195	Ruminococcaceae UCG-014	0.121	0.040	Otu00026	Lachnospiraceae NK3A20 group	0.694	0.183
	Otu00220	Bacteroidales BS11 gut group ge	0.084	0.031	Otu00091	Ruminococcaceae NK4A214 group	0.277	0.048
	Otu00341	Ruminococcaceae UCG-010	0.076	0.017	Otu00183	<i>Ruminococcus</i> 1	0.221	0.102
	Otu00238	Bacteroidales BS11 gut group ge	0.052	0.026	Otu00097	Clostridiales Family XIII AD3011 group	0.212	0.023
	Otu01121	Bacteroidales RF16 group ge	0.024	0.006	Otu00226	<i>Prevotella</i> 1	0.147	0.019
Isobut	Otu00043	Christensenellaceae R-7 group	0.373	0.171	Otu00012	<i>Prevotella</i> 1	0.928	0.188
	Otu00162	Bacteroidales BS11 gut group ge	0.277	0.223	Otu00033	Lachnospiraceae unclassified	0.656	0.166
	Otu00033	Lachnospiraceae unclassified	0.245	0.081	Otu00163	Lachnospiraceae probable genus 10	0.289	0.136
	Otu00063	<i>Prevotella</i> 1	0.147	0.037	Otu00155	<i>Ruminococcus</i> 1	0.232	0.125
	Otu00119	Anaerolineaceae uncultured	0.120	0.022	Otu00072	Ruminococcaceae ge	0.161	0.042
Suc	Otu00025	Bacteroidales BS11 gut group ge	1.132	0.569	Otu00022	Lachnospiraceae XPB1014 group	0.810	0.251
	Otu00040	Bacteroidales RF16 group ge	1.093	0.212	Otu00045	<i>Prevotella</i> 1	0.302	0.220
	Otu00069	<i>Prevotella</i> 1	0.596	0.233	Otu00145	Lachnospiraceae unclassified	0.258	0.133
	Otu00180	Ruminococcaceae UCG-002	0.274	0.073	Otu00124	Ruminococcaceae NK4A214 group	0.133	0.036
	Otu00242	Bacteroidales S24-7 group ge	0.085	0.043	Otu00157	<i>Anaerotruncus</i>	0.130	0.016
Val	Otu00098	Prevotellaceae UCG-003	0.276	0.094	Otu00026	Lachnospiraceae NK3A20 group	0.694	0.183
	Otu00209	Rikenellaceae RC9 gut group	0.228	0.075	Otu00098	Prevotellaceae UCG-003	0.396	0.191
	Otu00053	<i>Treponema</i> 2	0.197	0.032	Otu00081	Lachnospiraceae NK3A20 group	0.299	0.057

Isoval	Otu00093	<i>Prevotella</i> 1	0.162	0.060	Otu00139	Ruminococcaceae NK4A214 group	0.249	0.141
	Otu00236	Victivallales vadinBE97 ge	0.133	0.017	Otu00134	Clostridiales Family XIII AD3011 group	0.145	0.042
	Otu00021	Christensenellaceae R-7 group	0.616	0.311	Otu00075	Christensenellaceae R-7 group	0.604	0.452
	Otu00067	<i>Mogibacterium</i>	0.391	0.138	Otu00050	<i>Prevotella</i> 1	0.358	0.106
	Otu00188	Clostridiales Family XIII AD3011 group	0.208	0.079	Otu00048	Bacteroidales BS11 gut group ge	0.293	0.043
	Otu00201	Verrucomicrobia WCHB1-41 ge	0.134	0.023	Otu00211	Ruminococcaceae unclassified	0.139	0.034
	Otu00102	Rikenellaceae RC9 gut group	0.132	0.030	Otu00405	Ruminococcaceae unclassified	0.066	0.014
Ace/Prop	Otu00156	Ruminococcaceae UCG-005	0.204	0.086	Otu00070	Ruminococcaceae NK4A214 group	0.306	0.058
	Otu00150	Ruminococcaceae UCG-005	0.181	0.033	Otu00447	Bacteroidales S24-7 group ge	0.060	0.023
	Otu00119	Anaerolineaceae uncultured	0.120	0.022	Otu00371	<i>Treponema</i> 2	0.058	0.039
	Otu00274	Prevotellaceae UCG-003	0.084	0.014	Otu00342	Chloroflexi unclassified	0.057	0.018
	Otu00426	Rikenellaceae RC9 gut group	0.068	0.025	Otu00319	Ruminococcaceae UCG-010	0.055	0.016
VFAt	Otu00015	Rikenellaceae RC9 gut group	0.615	0.147	Otu00203	Bacteroidales S24-7 group ge	0.083	0.027
	Otu00048	Bacteroidales BS11 gut group ge	0.416	0.065	Otu00316	Anaerolineaceae unclassified	0.077	0.010
	Otu00018	<i>Prevotella</i> 1	0.269	0.123	Otu00295	<i>Prevotella</i> 1	0.073	0.028
	Otu00066	<i>Phocaeicola</i>	0.240	0.043	Otu00284	Christensenellaceae R-7 group	0.068	0.011
	Otu00157	<i>Anaerotruncus</i>	0.129	0.015	Otu00379	Lachnospiraceae XPB1014 group	0.066	0.013
pH	Otu00057	Bacteroidales BS11 gut group ge	0.357	0.155	Otu00012	<i>Prevotella</i> 1	0.928	0.188
	Otu00033	Lachnospiraceae unclassified	0.245	0.081	Otu00033	Lachnospiraceae unclassified	0.656	0.166
	Otu00153	<i>Saccharofermentans</i>	0.178	0.114	Otu00095	Rikenellaceae RC9 gut group	0.392	0.049
	Otu00197	Rikenellaceae RC9 gut group	0.119	0.054	Otu00163	Lachnospiraceae probable genus 10	0.289	0.136
	Otu00099	<i>Prevotella</i> 1	0.088	0.023	Otu00155	<i>Ruminococcus</i> 1	0.232	0.125
NH	Otu00036	Rikenellaceae U29-B03	0.574	0.259	Otu00024	<i>Succiniclasticum</i>	0.754	0.128
	Otu00140	Rikenellaceae RC9 gut group	0.291	0.099	Otu00098	Prevotellaceae UCG-003	0.396	0.191
	Otu00098	Prevotellaceae UCG-003	0.276	0.094	Otu00018	<i>Prevotella</i> 1	0.340	0.140
	Otu00018	<i>Prevotella</i> 1	0.269	0.123	Otu00139	Ruminococcaceae NK4A214 group	0.249	0.141
	Otu00053	<i>Treponema</i> 2	0.197	0.032	Otu00125	Ruminococcaceae NK4A214 group	0.134	0.014

The values represent relative abundance and standard error of mean (SEM). (*) Taxonomy for each OTU is given at the highest classifiable level. Biochemistry parameters: molar proportion of acetic, Ace; propionic, Prop; succinic, Suc; butyric, But; isobutyric, Isobut; valeric, val; and isovaleric acids, Isoval; acetate-to-propionate ratio, Ace/Prop; total concentration of these volatile fats acids, VFAt; ammonia concentration, NH₃; and pH.

Table S3. Relative abundance of the most abundant OTUs showing negative correlation with ruminal fermentation parameters in the p-RFI steers.

Parameter	Liquid				Solid			
	OTU	Taxonomy*	Relative abundance (%)	SEM	OTU	Taxonomy*	Relative abundance (%)	SEM
Ace	Otu00044	Christensenellaceae R-7 group	0,629	0,550	Otu00019	Prevotellaceae NK3B31 group	1,023	0,805
	Otu00019	Prevotellaceae NK3B31 group	0,504	0,383	Otu00234	Ruminococcaceae ge	0,069	0,019
	Otu00048	Bacteroidales BS11 gut group ge	0,448	0,068	Otu00220	Bacteroidales BS11 gut group ge	0,061	0,035
	Otu00035	<i>Mogibacterium</i>	0,415	0,152	Otu00529	Clostridiales unclassified	0,054	0,013
	Otu00181	Clostridiales Family XIII unclassified	0,143	0,034	Otu00519	Clostridiales Family XIII AD3011 group	0,049	0,013
Prop	Otu00032	Rikenellaceae RC9 gut group	0,868	0,235	Otu00015	Rikenellaceae RC9 gut group	1,026	0,299
	Otu00064	<i>Prevotella</i> 1	0,468	0,198	Otu00011	<i>Prevotella</i> 1	0,506	0,094
	Otu00015	Rikenellaceae RC9 gut group	0,464	0,151	Otu00095	Rikenellaceae RC9 gut group	0,407	0,071
	Otu00207	<i>Spirochaeta</i> 2	0,114	0,019	Otu00064	<i>Prevotella</i> 1	0,350	0,107
	Otu00105	Lachnospiraceae FCS020 group	0,083	0,022	Otu00072	Ruminococcaceae ge	0,192	0,044
But	Otu00057	Bacteroidales BS11 gut group ge	0,461	0,181	Otu00130	Prevotellaceae YAB2003 group	0,307	0,045
	Otu00148	Bacteroidales S24-7 group ge	0,118	0,050	Otu00099	<i>Prevotella</i> 1	0,275	0,100
	Otu00170	Rikenellaceae RC9 gut group	0,100	0,017	Otu00133	<i>Saccharofermentans</i>	0,214	0,042
	Otu00241	Bacteroidales S24-7 group ge	0,078	0,018	Otu00231	<i>Papillibacter</i>	0,163	0,062
	Otu00362	Verrucomicrobia WCHB1-41 ge	0,068	0,018	Otu00106	Bacteroidales BS11 gut group ge	0,128	0,039
Isobut	Otu00156	Ruminococcaceae UCG-005	0,148	0,049	Otu00033	Lachnospiraceae unclassified	0,753	0,143
	Otu00173	Ruminococcaceae ge	0,121	0,043	Otu00087	Lachnospiraceae XPB1014 group	0,394	0,049
	Otu00198	Planctomycetaceae p-1088-a5 gut group	0,097	0,026	Otu00210	Bacteroidales BS11 gut group ge	0,077	0,059
	Otu00400	Clostridiales vadinBB60 group ge	0,082	0,019	Otu00327	<i>Treponema</i> 2	0,076	0,020
	Otu00258	Prevotellaceae UCG-004	0,082	0,043	Otu00414	<i>Treponema</i> 2	0,049	0,007
Suc	Otu00012	<i>Prevotella</i> 1	1,024	0,256	Otu00030	<i>Prevotella</i> 1	0,697	0,267
	Otu00061	Bacteroidales S24-7 group ge	0,464	0,255	Otu00012	<i>Prevotella</i> 1	0,679	0,121
	Otu00050	<i>Prevotella</i> 1	0,383	0,106	Otu00055	Christensenellaceae R-7 group	0,411	0,076
	Otu00055	Christensenellaceae R-7 group	0,337	0,046	Otu00271	<i>Ruminococcus</i> 1	0,069	0,012
	Otu00261	Lachnospiraceae UCG-008	0,104	0,024	Otu00422	Bacteroidales UCG-001 ge	0,048	0,016
Val	Otu00058	Ruminococcaceae UCG-005	0,366	0,076	Otu00014	<i>Saccharofermentans</i>	1,499	0,177
	Otu00232	<i>Prevotella</i> 1	0,133	0,067	Otu00137	Lachnospiraceae UCG-009	0,286	0,032
	Otu00236	Victivallales vadinBE97 ge	0,112	0,017	Otu00107	Prevotellaceae NK3B31 group	0,211	0,182

Isoval	Otu00389	Bacteroidales BS11 gut group ge	0,080	0,059	Otu00405	Ruminococcaceae unclassified	0,077	0,010
	Otu00126	<i>Ruminococcus</i> 1	0,077	0,015	Otu00295	<i>Prevotella</i> 1	0,063	0,011
	Otu00156	Ruminococcaceae UCG-005	0,148	0,049	Otu00053	<i>Treponema</i> 2	0,509	0,109
	Otu00263	<i>Ruminococcus</i> 1	0,096	0,044	Otu00087	Lachnospiraceae XPB1014 group	0,394	0,049
	Otu00635	<i>Howardella</i>	0,038	0,007	Otu00068	Lachnospiraceae ge	0,360	0,059
	Otu00617	Spirochaetaceae unclassified	0,021	0,010	Otu00114	Lachnospiraceae unclassified	0,216	0,016
Ace/Prop	Otu00612	Prevotellaceae UCG-001	0,020	0,009	Otu00177	<i>Fibrobacter</i>	0,176	0,043
	Otu00003	Rikenellaceae RC9 gut group	1,951	0,399	Otu00003	Rikenellaceae RC9 gut group	1,752	0,328
	Otu00044	Christensenellaceae R-7 group	0,629	0,550	Otu00008	<i>Saccharofermentans</i>	1,528	0,259
	Otu00048	Bacteroidales BS11 gut group ge	0,448	0,068	Otu00018	<i>Prevotella</i> 1	0,767	0,530
	Otu00198	Planctomycetaceae p-1088-a5 gut group	0,097	0,026	Otu00078	Lachnospiraceae ge	0,331	0,065
VFAt	Otu00214	<i>Saccharofermentans</i>	0,094	0,026	Otu00066	<i>Phocaeicola</i>	0,293	0,101
	Otu00059	Ruminococcaceae UCG-005	0,325	0,092	Otu00015	Rikenellaceae RC9 gut group	1,026	0,299
	Otu00096	<i>Prevotella</i> 1	0,288	0,121	Otu00023	<i>Papillibacter</i>	0,955	0,062
	Otu00143	Prevotellaceae UCG-001	0,230	0,056	Otu00031	<i>Prevotella</i> 1	0,619	0,307
	Otu00091	Ruminococcaceae NK4A214 group	0,185	0,072	Otu00042	<i>Prevotella</i> 1	0,334	0,158
pH	Otu00192	Ruminococcaceae ge	0,162	0,047	Otu00096	<i>Prevotella</i> 1	0,309	0,131
	Otu00018	<i>Prevotella</i> 1	0,795	0,611	Otu00003	Rikenellaceae RC9 gut group	1,752	0,328
	Otu00044	Christensenellaceae R-7 group	0,629	0,550	Otu00052	Clostridiales unclassified	0,394	0,108
	Otu00048	Bacteroidales BS11 gut group ge	0,448	0,068	Otu00048	Bacteroidales BS11 gut group ge	0,350	0,061
	Otu00035	<i>Mogibacterium</i>	0,415	0,152	Otu00258	Prevotellaceae UCG-004	0,104	0,045
NH	Otu00234	Ruminococcaceae ge	0,089	0,023	Otu00234	Ruminococcaceae ge	0,069	0,019
	Otu00072	Ruminococcaceae ge	0,343	0,084	Otu00028	Rikenellaceae RC9 gut group	0,735	0,126
	Otu00201	Verrucomicrobia WCHB1-41 ge	0,114	0,011	Otu00074	Lachnospiraceae XPB1014 group	0,358	0,068
	Otu00381	Ruminococcaceae UCG-001	0,077	0,042	Otu00114	Lachnospiraceae unclassified	0,216	0,016
	Otu00504	Rikenellaceae RC9 gut group	0,037	0,016	Otu00072	Ruminococcaceae ge	0,192	0,044
	Otu00413	Mollicutes RF9 ge	0,032	0,010	Otu00149	Lachnospiraceae XPB1014 group	0,167	0,031

The values represent relative abundance and standard error of mean (SEM). (*) Taxonomy for each OTU is given at the highest classifiable level. Biochemistry parameters: molar proportion of acetic, Ace; propionic, Prop; succinic, Suc; butyric, But; isobutyric, Isobut; valeric, val; and isovaleric acids, Isoval; acetate-to-propionate ratio, Ace/Prop; total concentration of these volatile fats acids, VFAt; ammonia concentration, NH₃; and pH.

Table S4. Relative abundance of the most abundant OTUs showing negative correlation with ruminal fermentation parameters in the n-RFI steers.

Parameter	Liquid				Solid			
	OTU	Taxonomy*	Relative abundance (%)	SEM	OTU	Taxonomy*	Relative abundance (%)	SEM
Ace	Otu00036	Rikenellaceae U29-B03	0,574	0,259	Otu00026	Lachnospiraceae NK3A20 group	0,694	0,183
	Otu00111	<i>Prevotella</i> 1	0,298	0,113	Otu00226	<i>Prevotella</i> 1	0,147	0,019
	Otu00190	Rikenellaceae RC9 gut group	0,117	0,029	Otu00118	Prevotellaceae UCG-003	0,130	0,017
	Otu00220	Bacteroidales BS11 gut group ge	0,084	0,031	Otu00412	Anaerolineaceae uncultured	0,082	0,058
	Otu00699	Verrucomicrobia WCHB1-41 ge	0,040	0,015	Otu00354	Clostridiales Family XIII ge	0,053	0,016
Prop	Otu00119	Anaerolineaceae uncultured	0,120	0,022	Otu00063	<i>Prevotella</i> 1	0,072	0,018
	Otu00367	Chloroflexi unclassified	0,105	0,050	Otu00359	<i>Fretibacterium</i>	0,070	0,013
	Otu00274	Prevotellaceae UCG-003	0,084	0,014	Otu00319	Ruminococcaceae UCG-010	0,055	0,016
	Otu00426	Rikenellaceae RC9 gut group	0,068	0,025	Otu00407	Anaerolineaceae unclassified	0,050	0,014
	Otu00575	Ruminococcaceae unclassified	0,062	0,027	Otu00040	Bacteroidales RF16 group ge	0,050	0,008
But	Otu00078	Lachnospiraceae ge	0,178	0,031	Otu00129	Lachnospiraceae unclassified	0,279	0,121
	Otu00122	Rikenellaceae RC9 gut group	0,165	0,039	Otu00227	Prevotellaceae unclassified	0,173	0,033
	Otu00129	Lachnospiraceae unclassified	0,154	0,060	Otu00256	Prevotellaceae NK3B31 group	0,171	0,081
	Otu00165	Lachnospiraceae XPB1014 group	0,091	0,017	Otu00165	Lachnospiraceae XPB1014 group	0,160	0,033
	Otu00088	<i>Fibrobacter</i>	0,052	0,032	Otu00162	Bacteroidales BS11 gut group ge	0,111	0,075
Isobut	Otu00098	Prevotellaceae UCG-003	0,276	0,094	Otu00098	Prevotellaceae UCG-003	0,396	0,191
	Otu00053	<i>Treponema</i> 2	0,197	0,032	Otu00018	<i>Prevotella</i> 1	0,340	0,140
	Otu00109	Prevotellaceae UCG-004	0,137	0,056	Otu00082	<i>Moryella</i>	0,324	0,048
	Otu00152	<i>Anaerovorax</i>	0,136	0,021	Otu00068	Lachnospiraceae ge	0,254	0,037
	Otu00167	Rikenellaceae RC9 gut group	0,122	0,035	Otu00187	<i>Acetitomaculum</i>	0,194	0,064
Suc	Otu00084	Prevotellaceae UCG-001	0,345	0,098	Otu00110	Lachnospiraceae NK4A136 group	0,438	0,199
	Otu00116	Lachnospiraceae ND3007 group	0,316	0,109	Otu00020	<i>Prevotella</i> 1	0,373	0,114
	Otu00018	<i>Prevotella</i> 1	0,269	0,123	Otu00149	Lachnospiraceae XPB1014 group	0,282	0,037
	Otu00020	<i>Prevotella</i> 1	0,267	0,095	Otu00104	Ruminococcaceae NK4A214 group	0,164	0,042
	Otu00179	Christensenellaceae R-7 group	0,120	0,079	Otu00208	Lachnospiraceae unclassified	0,140	0,025
Val	Otu00001	Christensenellaceae R-7 group	5,419	0,980	Otu00033	Lachnospiraceae unclassified	0,656	0,166
	Otu00007	Ruminococcaceae NK4A214 group	1,647	0,214	Otu00095	Rikenellaceae RC9 gut group	0,392	0,049
	Otu00035	<i>Mogibacterium</i>	0,356	0,079	Otu00122	Rikenellaceae RC9 gut group	0,172	0,042

Isoval	Otu00156	Ruminococcaceae UCG-005	0,204	0,086	Otu00240	<i>Fibrobacter</i>	0,123	0,065
	Otu00124	Ruminococcaceae NK4A214 group	0,186	0,067	Otu00330	<i>Treponema 2</i>	0,064	0,015
	Otu00059	Ruminococcaceae UCG-005	0,615	0,237	Otu00099	<i>Prevotella 1</i>	0,228	0,066
	Otu00255	Clostridiales Family XIII ge	0,207	0,078	Otu00183	<i>Ruminococcus 1</i>	0,221	0,102
	Otu00314	Bacteroidales S24-7 group ge	0,139	0,054	Otu00166	Peptococcaceae uncultured	0,191	0,045
	Otu00469	Ruminococcaceae unclassified	0,062	0,021	Otu00096	<i>Prevotella 1</i>	0,118	0,053
	Otu00134	Clostridiales Family XIII AD3011 group	0,061	0,018	Otu00293	<i>Succiniclasticum</i>	0,092	0,026
Ace/Prop	Otu00326	Prevotellaceae UCG-003	0,182	0,094	Otu00109	Prevotellaceae UCG-004	0,168	0,071
	Otu00317	Rikenellaceae RC9 gut group	0,083	0,027	Otu00215	Lachnospiraceae UCG-002	0,113	0,016
	Otu00297	Prevotellaceae UCG-001	0,063	0,016	Otu00310	Clostridiales Family XIII AD3011 group	0,093	0,037
	Otu00347	Ruminococcaceae UCG-002	0,058	0,010	Otu00375	Lachnospiraceae unclassified	0,075	0,016
	Otu00160	<i>Ruminococcus 1</i>	0,056	0,019	Otu00347	Ruminococcaceae UCG-002	0,068	0,018
VFAt	Otu00101	Veillonellaceae UCG-001	0,202	0,017	Otu00011	<i>Prevotella 1</i>	0,311	0,051
	Otu00107	Prevotellaceae NK3B31 group	0,150	0,044	Otu00072	Ruminococcaceae ge	0,161	0,042
	Otu00310	Clostridiales Family XIII AD3011 group	0,079	0,016	Otu00153	<i>Saccharofermentans</i>	0,142	0,067
	Otu00345	<i>Senegalimassilia</i>	0,047	0,014	Otu00135	Bacteroidales S24-7 group ge	0,141	0,075
	Otu00374	<i>Senegalimassilia</i>	0,039	0,007	Otu00221	Lachnospiraceae XPB1014 group	0,086	0,027
pH	Otu00140	Rikenellaceae RC9 gut group	0,291	0,099	Otu00018	<i>Prevotella 1</i>	0,340	0,140
	Otu00098	Prevotellaceae UCG-003	0,276	0,094	Otu00068	Lachnospiraceae ge	0,254	0,037
	Otu00053	<i>Treponema 2</i>	0,197	0,032	Otu00139	Ruminococcaceae NK4A214 group	0,249	0,141
	Otu00152	<i>Anaerovorax</i>	0,136	0,021	Otu00114	Lachnospiraceae unclassified	0,211	0,023
	Otu00167	Rikenellaceae RC9 gut group	0,122	0,035	Otu00179	Christensenellaceae R-7 group	0,187	0,140
NH	Otu00001	Christensenellaceae R-7 group	5,419	0,980	Otu00033	Lachnospiraceae unclassified	0,656	0,166
	Otu00035	<i>Mogibacterium</i>	0,356	0,079	Otu00042	<i>Prevotella 1</i>	0,405	0,145
	Otu00042	<i>Prevotella 1</i>	0,334	0,091	Otu00095	Rikenellaceae RC9 gut group	0,392	0,049
	Otu00101	Veillonellaceae UCG-001	0,202	0,017	Otu00155	<i>Ruminococcus 1</i>	0,232	0,125
	Otu00119	Anaerolineaceae uncultured	0,120	0,022	Otu00251	Bacteroidetes BD2-2 ge	0,118	0,023

The values represent relative abundance (%) and standard error of mean (SEM). (*) Taxonomy for each OTU is given at the highest classifiable level. Biochemistry parameters: molar proportion of acetic, Ace; propionic, Prop; succinic, Suc; butyric, But; isobutyric, Isobut; valeric, val; and isovaleric acids, Isoval; acetate-to-propionate ratio, Ace/Prop; total concentration of these volatile fats acids, VFAt; ammonia concentration, NH₃; and pH.

Capítulo 3

Differences in bacterial community composition across the gastrointestinal tract and in fecal samples of Nelore steers with positive and negative residual feed intake

Introduction

The gastrointestinal tract (GIT) microbiome is known to play a key role in the physiology of the mammalian host, such as the stimulation of the immune system, the production of vitamins and inhibition of pathogenic bacteria (Cho and Blaser, 2012; Ley et al., 2006; Turnbaugh and Gordon, 2009). This association is even more relevant in ruminants, herbivorous animals that are dependent on a symbiotic association with anaerobic microorganisms to digest plant polysaccharides in the rumen (Krause and Russell, 1996).

The rumen represents the main site for converting the ingested dietary components into energy to the host, while the microbiota colonizing the distal GIT (small and large intestine) are considered crucial for the animal welfare and, in a lesser extent, to the energy obtaining functions of the host (Mao et al., 2015; Myer et al., 2015c). The volatile organic acids are the main end-products of microbial fermentation and these metabolites are used by the ruminant as major energy sources, contributing significantly to the efficiency and productivity of the animal (Russell and Hespell, 1981). Several studies have reported that ruminants showing a high or low feed efficiency phenotype show differences in the composition of their rumen microbiomes (Jami et al., 2014; Jewell et al., 2015; Li and Guan, 2017; Myer et al., 2015a). Residual feed intake (RFI), defined as the difference between the actual dry matter intake and the expected dry matter intake of each animal (Koch et al., 1963), is a measure often used to evaluate feed efficiency of growing beef cattle. Selection of cattle for reduced RFI could result in animals that consume less food without affecting their level of production. Nonetheless, in order to obtain the RFI data long term feeding trials are required, which is considered laborious and expensive to the farmers.

Besides making the production more profitable by decreasing feeding costs, the selection of cattle with low RFI has also social and environmental benefits. More efficient animals are better suited to the challenges of meeting the demand for protein

sources to sustain global population growth, decrease the enteric emissions of greenhouse gases (e.g. methane), and reduce the pressure to increase the area of arable land diverted to livestock production (Morgavi et al., 2010; Thornton, 2010). Ruminants (cattle, goats, sheep, etc.) are responsible for most of the milk and meat produced worldwide, and these foods represent the main sources of protein in the human diet. Brazil is one of the main producers of meat in the world, holding about 15 % of the global meat exports (FAO, 2016). More than 90 % of the Brazilian commercial cattle herds are of animals belonging to the Nelore breed, a variety of Zebu (*Bos indicus*), which are well adapted to the environmental conditions and tropical production systems (ABIEC, 2017).

Considering the global economic importance of Zebu cattle for meat production, understanding the composition and function of the Nelore GIT microbiome associated with specific phenotypic traits could be useful for developing new management strategies to help improve animal productivity and reduce the environmental impact of cattle production. Additionally, understanding the structure of the microbial community during animal development could be important to identify opportunities to manipulate the microbiota of young animals aiming greater overall productivity in the adult life.

Previously, Oliveira et al., (2013) described the composition of the bacterial community in the GIT of a Nelore steer, however the characterization was performed using a single animal. To gain more in-depth knowledge of the Nelore microbiome, the present study investigated the bacterial composition of the rumen, small intestine, cecum and feces of 27 Nelore steers showing differences in feed efficiency (determined by RFI measurements). Since most of the studies associating ruminant feed efficiency to microbial communities focus on analysis of rumen samples (whose collection depends on invasive methods or sacrifice of animals), we also examined the hypothesis that Nelore cattle with high and low feed efficiency (negative and positive RFI, respectively) have differences in the composition of their fecal microbiome.

Materials and Methods

Animal selection, diets and sampling collection

The experimental procedures used in this study were approved by the Ethics Committee on Animal Use of the *Instituto de Zootecnia/CEUA-IZ*, Nova Odessa, SP,

Brazil (protocol 213-15), in accordance with guidelines of São Paulo State Law No. 11.977, Brazil, and by Ethics Committee on Production Animal Use of the *Universidade Federal de Viçosa/CEUAP-UFV*, Viçosa, MG, Brazil (protocol 026/2015).

A contemporary group of 129 young Nelore bulls (7 month of age and 239 ± 30.1 kg of initial body weight - BW) from the Instituto de Zootecnia – IZ were subjected to a growth period of 98 days receiving the same diet formulated to meet the requirements for 1 kg/d of BW gain. The growth period diet was composed of 615 g/kg corn silage, 33 g/kg *Brachiaria* hay, 167 g/kg dry ground corn, 163 g/kg soybean meal, 3.6 g/kg urea, 0.4 g/kg ammonium sulfate, and 18 g/kg mineral mixture (dry matter basis). Before the test, all bulls were weighed, vaccinated, dewormed, and received individual numbered tags.

Cattle were fed using a GrowSafe® automated feeding system (GrowSafe Systems Ltd, Airdrie, Canada). The RFI (kg/day) was calculated during the growth period as the error term of the equation: $DMI = \beta_0 + \beta_P * BW_{0.75} + \beta_G * ADG + \epsilon(RFI)$, where DMI is the dry matter intake observed during the test, β_0 is the intercept of the equation, $BW_{0.75}$ is the mid-test metabolic live weight, ADG is the average daily weight gain during the test, and β_P and β_G are the regression coefficients of $BW_{0.75}$ and ADG, respectively. Average daily gain was estimated by the linear regression coefficient of live weight as a function of days in test. Mid-test metabolic live weight was calculated by the equation $BW_{0.75} = [\alpha + \beta * (DIT/2)]^{0.75}$, where α is the intercept of the regression equation corresponding to the initial live weight, β is the linear regression coefficient corresponding to the average daily gain, and DIT are the days in test (Fidelis et al., 2017).

From the 129 animals used in the growth period, a total of 27 steers were randomly selected (12 negatives and 15 positives RFI animals) to finishing period. The RFI values were significantly different ($P < 0.05$) between negatives (-0.93 ± 0.17) and positives (0.87 ± 0.14), so the steers were separated on two feed efficiency groups: Low-RFI (more efficient) and High-RFI (less efficient), respectively.

The 27 animals, averaging 22.5 ± 0.8 mo of age and 401 ± 42 kg of BW, were confined in individual pens measuring 4×2 m and equipped with GrowSafe® automated feeding systems (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) with free access to diet and water. Cattle were adapted to the diets, facilities, and

management for 22 d and fed the finishing diet for a 103-d period. The finishing diet was composed of 333 g/kg corn silage, 17 g/kg brachiaria hay, 465 g/kg dry ground corn, 163 g/kg soybean meal, 6 g/kg urea, 4 g/kg ammonium sulfate, and 13 g/kg mineral mixture (dry matter basis), formulated to meet the requirements of 1.3 kg of daily gain with a target finish weight of at least 550 kg.

After that, steers were transported to a commercial abattoir for slaughter. The fecal samples were collected a day before the slaughter and stored in plastic containers at -20°C. Handling was conducted in accordance with good animal welfare practices, and slaughtering procedures followed strict guidelines established and regulated by the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2017). After slaughter, the ruminal, intestinal (small intestine) and cecal contents were collected in plastic containers and stored at -20°C for further analyses. The rumen samples were filtered through four layers of cheese cloth to separate the liquids and solids fraction.

Concentration of volatile fatty acids

Organic acids were determined by HPLC in a Dionex Ultimate 3000 Dual detector HPLC (Dionex Corporation, Sunnyvale, CA, USA) coupled to a refractive index (RI) Shodex RI-101 maintained at 40°C using an ion exclusion column Phenomenex Rezex ROA, 300 × 7.8 mm maintained at 45°C. Mobile phase was prepared with 5 mmol/l sulfuric acid (H₂SO₄) and the flow was 0.7 ml/min. The samples (2.0 ml) were centrifuged (12,000 × g, 10 min) and the cell-free supernatants were treated as described by Siegfried et al. (1984). Stock solutions of the standards were prepared using the following organic acids: acetic, succinic, propionic, valeric, isovaleric, isobutyric and butyric acid. All organic acids were prepared with a final concentration of 10 mmol/l, except isovaleric acid (5 mmol/l) and acetic acid (20 mmol/l). Stock solutions were diluted 2-, 4-, 8- and 16-fold in 5 mmol/l H₂SO₄ to be used as standards in the HPLC analysis.

DNA extraction and sequencing

Total genomic DNA from each sample was extracted following a mechanical disruption and phenol/chloroform extraction protocol described by Stevenson and Weimer (2007), which has been shown to generate high-quality, high-abundance DNA representative of complex bacterial community (Henderson et al., 2013). Genomic

DNA extracted was quantified using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) and sequenced at the University of Wisconsin-Madison (USA).

The V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primers described by Kozich et al., (2013). PCR reactions consisted of 50 ng template DNA, 0.4 μ M of each primer, 1X Kapa Hifi HotStart ReadyMix (KAPA Biosystems), and water to 25 μ L. PCR was performed at 95°C for 3 min, 95°C for 30 s, 55°C for 30 s, 72°C for 30 s (25 cycles) and a final extension step at 72°C for 5 min. PCR products were purified by PureLink® Pro 96 PCR Purification Kit (Invitrogen) and a second PCR was performed on products to attach Illumina sequencing adapters and unique dual indices. PCR reactions were similar to those for V4 except that 5 μ L of non-quantified PCR products were used as template DNA and 8 cycles were performed. PCR products were recovered by gel extraction in AquaPör LM low-melt agarose (National Diagnostics, Atlanta, GA) using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA). Purified DNA was quantified by Qubit® Fluorometer (Invitrogen) and equimolar amounts were pooled to create a single sample at 1×10^9 ng per μ L. Sequencing was performed using the v2 kit (2×250 bp) with paired-end method on an Illumina MiSeq following manufacturer's guidelines (Illumina, Inc., San Diego, CA, USA).

Sequence analysis

Bacteria sequences were processed using Mothur (v1.39.5) (Schloss et al., 2009). Paired-end reads were joined using default parameters in make.contigs and sequences with a length shorter than 200 bp or longer than 500 bp containing ambiguous characters or exhibiting a homopolymer greater than 8 bp were removed. The sequences were aligned using the SILVA 16S rRNA gene reference database (Quast et al., 2012) and sequences that did not align to the correct location were removed. Identical sequences were grouped using unique.seqs command and sequences that were two or fewer base pairs different were considered the same and grouped in pre.cluster to keep computational needs down as well as to account for sequencing error. Chimeric sequences were detected using Uchime algorithm (Edgar et al., 2011) and removed. The singletons (sequences that occur only once in the entire dataset) were also removed because they provide little usable data and are often the

result of error. The sequences were taxonomy assigned using SILVA 16S rRNA gene reference database (Quast et al., 2012) with a bootstrap cut-off of 80. All sequences were grouped into operational taxonomic units (OTUs) by uncorrected pairwise distances clustered by the furthest neighbor method with a similarity cut-off of 97 %. Before proceeding with analysis, the coverage of all samples was assessed by Good's coverage (all > 97 %). Due to different sequencing depths, OTU tables were normalized to equal sequence counts (5122 sequences, established by the sample that presented the lowest number of sequences). The normalized OTU tables were used to determine the alpha diversity (Chao1, Shannon and Simpson) indexes and the relative abundance (reads/total reads in a sample) of OTUs.

Statistical analysis

The differences in bacterial alpha-diversity indices and VFA concentration in portions of gastrointestinal tract were assessed by ANOVA, followed to Tukey test, performed in MiniTab[®] 17.1.0 (Minitab, Inc., Quality Plaza, 1829 Pine Hall Road, State College, Pennsylvania 16801, USA). *P*-values below 0.05 were considered significant.

To evaluate the clustering of steers using the OTU composition from each portion of the gastrointestinal tract, a Principal Coordinate Analysis (PCoA), using Bray-Curtis dissimilarity index (beta diversity index), and non-parametric analysis of similarities (ANOSIM, number of permutation = 1000) were performed using the Past software (Hammer et al., 2001).

Venn diagrams were built in jvenn (Bardou et al., 2014) to visualize shared and exclusives OTUs of the gastrointestinal tract portions. This analysis was performed using OTUs that were detected in at least 50 % of all steers analyzed in this study (13 steers). The Kolmogorov-Smirnov, Shapiro-Wilk, D'AlgoStino and Pearson tests were performed using GraphPad Prism v. 5.00 for Windows (GraphPad Software, San Diego California USA) to check if the relative abundance of bacterial OTUs from each portion of gastrointestinal tract followed a Gaussian distribution. Since most of OTUs did not follow this distribution ($P > 0.05$), the differences in relative abundances according to feed efficiency group were assessed by White's non-parametric t-test using the software Statistical analysis of taxonomic and functional profiles – STAMP v 2.1.3 (Parks et al., 2014). *P*-values below 0.05 were considered significant.

To compare the fecal bacterial composition of p-RFI and n-RFI steers, Venn diagrams were built using the OTUs that were identified in at least a 50 % of the steers belonging to each feed efficiency group (7 animals to p-RFI and 6 animals to n-RFI). The shared OTUs were plotted on a volcano plot to visualize the differences in relative abundances of OTUs identified in the n-RFI steers versus the p-RFI steers. For this, we calculated the fold change (ratio between n-RFI and p-RFI relative abundance of each OTU) and used the p-values obtained on White's non-parametric t-test to visualize the data. In addition, the differences in alpha-diversity indices and VFA concentration of fecal samples according to each feed efficiency group were assessed by t-test performed in MiniTab[®] 17.1.0 (Minitab, Inc., Quality Plaza, 1829 Pine Hall Road, State College, Pennsylvania 16801, USA). *P*-values below 0.05 were considered significant.

Results

Sequencing

We generated 10,000,278 bacterial raw sequences with a maximum length of 500 pb, average length of 253 pb and minimum length of 243 pb across all samples. After trimming, quality filtering and removal of chimeras, 5,697,607 (mean 42,204 ± SD 45,615 to samples) high-quality bacterial sequences were obtained. The Good's coverage was > 97 % indicating that our sequencing effort sufficiently covered the diversity of bacterial communities in rumen liquids (RL) and solids (RS), small intestine (SI), cecum and feces of the Nelore steers. Considering each animal and portion of gastrointestinal tract (GIT), the minimum and maximum number of different OTUs before normalization were 413 in SI and 1745 in RL. The summary of sequence counts and OTUs that passed the steps of filtering, clean up and normalization are shown in Table 1. Only reads and OTUs that were identified in at least 50 % of all the steers in a specific RFI group (7 animals to p-RFI and 6 animals to n-RFI) were considered for the analysis of the feed efficiency phenotype in the Nelore steers (*cut-off in Table 1). This criteria was based on previous observations indicating that species of ruminal bacteria that exhibit a heritable component show high presence (≥ 50 %) across animals (Sasson et al, 2017).

Table 1. Summary of sequencing data derived from GIT contents of Nelore steers.

	Steers (n)	Good's coverage	After filtering and clean-up		After normalization		After cut-off*	
			Reads	OTUs	Reads	OTUs	Reads	OTUs
Rumen liquid	27	0.990 ± 0.003	35213 ± 11737	1442 ± 166	5009 ± 44	773 ± 76	4091 ± 225	442 ± 35
Rumen solid	27	0.990 ± 0.004	30028 ± 8941	1247 ± 168	5034 ± 75	715 ± 75	4247 ± 256	451 ± 35
Small intestine	27	0.992 ± 0.005	28242 ± 14295	625 ± 121	5076 ± 34	362 ± 110	4958 ± 223	222 ± 36
Cecum	27	0.995 ± 0.002	50138 ± 42872	939 ± 279	5058 ± 35	491 ± 107	4312 ± 420	263 ± 38
Feces	27	0.995 ± 0.003	67401 ± 85626	907 ± 281	5052 ± 26	413 ± 82	4535 ± 270	238 ± 24

Values represent mean and standard deviation. *Reads and OTUs that were detected in at least a half of steers of each GIT portion (at least 13 animals).

Bacterial microbiota changes across the gastrointestinal tract

Alpha diversity was measured using Chao1 richness, Simpson's and Shannon's diversity indexes and results demonstrated that these indices varied across different portions of the GIT. Maximum values of Chao richness and Shannon diversity were observed in the RL and SR communities ($1,296 \pm 207$ SD and 5.21 ± 0.26 , respectively), while minimal values of Chao richness were obtained in the SI and fecal communities (641 ± 189) and of Shannon index in SI community (3.78 ± 0.73). The Simpson diversity index showed the inverse trend, with maximum values observed in SI community (0.071 ± 0.042) and minimal values in RL, SR and cecum communities (0.022 ± 0.01) (Figure 1).

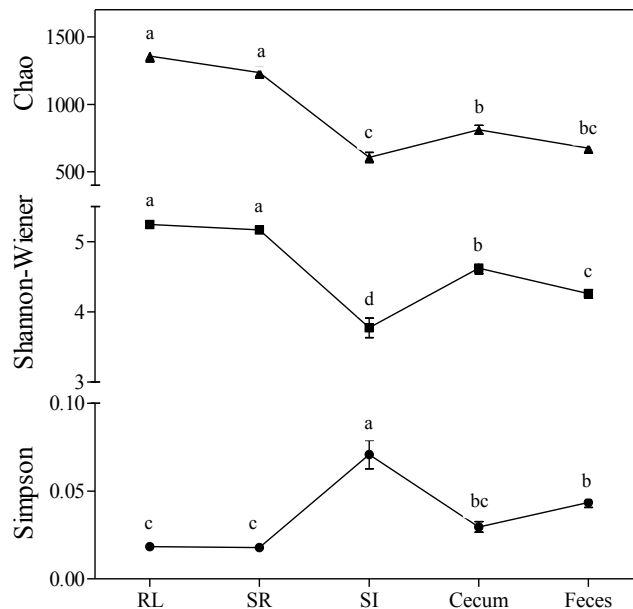


Figure 1. Changes in alpha-diversity of bacterial communities across the GIT of Nelore steers. Triangles represent Chao richness, squares represent Shannon-Wiener diversity and circles represent Simpson diversity index. To each index, means followed by at least one same letter did not differ at 5 % level of significance by the Tukey 's test. RL, rumen liquid; RS, rumen solids and SI, small intestine.

Beta diversity analysis showed that the Bray-Curtis dissimilarities of the bacterial communities differed according to GIT portion (Anosim, $P < 0.001$). Three main clusters were observed comprising the rumen (RL and RS fractions), SI and large intestine (cecum and feces). However, even the overlapping bacterial communities (RL/RS and cecum/feces) were different from each other (Anosim, $P < 0.001$) (Figure 2).

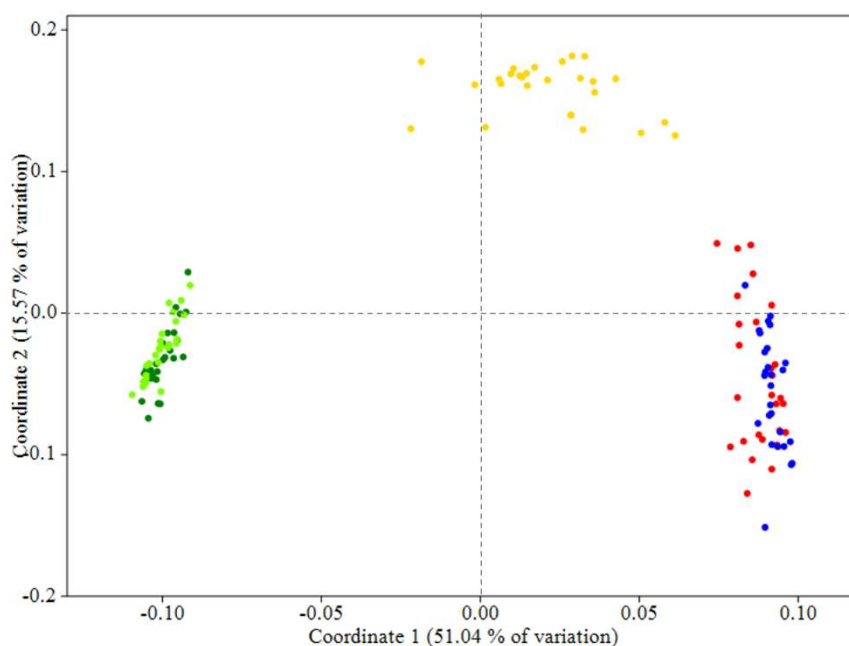


Figure 2. Principal Coordinate Analysis (PCoA) using the Bray-Curtis dissimilarity metric for bacterial communities in the GIT of Nelore steers. Individual points represent GIT sample from different steers and different colors represent distinct GIT portions/fractions. Dark green, light green, yellow, red and blue points represent rumen liquids, rumen solids, small intestine, cecal and fecal samples of the Nelore steers, respectively.

Taxonomic analysis of the GIT bacterial communities revealed 5230 unique OTUs (mean $551 \pm \text{SD } 188$ per sample after normalization) that were assigned to 24 phyla, 50 classes, 84 orders, 163 families and 402 genera. Nearly $1.66 (\pm 1.01 \%)$, $2.48 (\pm 1.26 \%)$, $2.91 (\pm 1.34 \%)$, $5.19 (\pm 1.89 \%)$ and $27.65 (\pm 5.18 \%)$ of the OTUs could not be assigned to any phylum, class, order, family or genus, respectively. Firmicutes represented the predominant phylum in the bacterial community across the GIT of Nelore steers (relative abundance $66.68 \pm \text{SD } 9.15 \%$), followed by Bacteroidetes in RL, RS, cecum and feces samples (relative abundance of $20.50 \pm \text{SD } 1.53 \%$, 20.57 ± 1.59 , 711.34 ± 1.55 and 12.17 ± 1.74 , respectively) and members of the phylum Actinobacteria in SI samples ($13.90 \pm 2.05 \%$) (Figure 3).

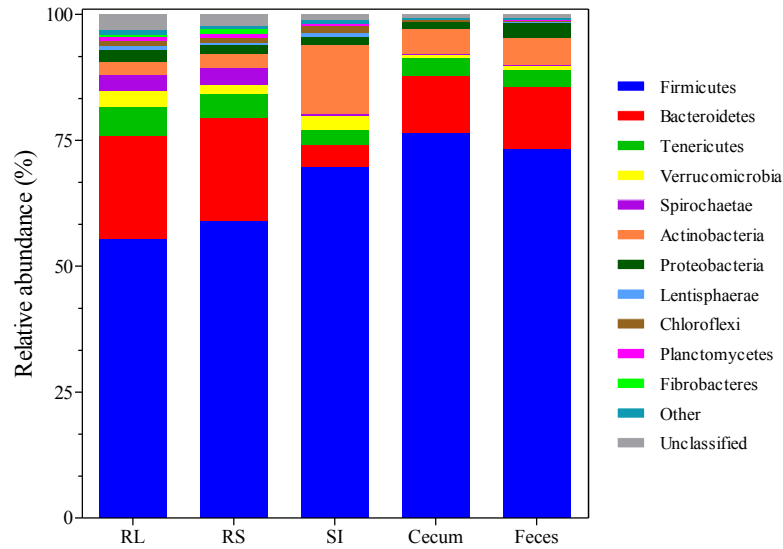


Figure 3. Bacterial composition at phylum-level across the GIT of Nelore steers. Each bar represents the mean bacterial community composition in rumen liquids (RL), rumen solids (RS), small intestine (SI), cecum and fecal samples. Other corresponds to the sum of phyla that showed relative abundance < 0.5 %.

The most highly represented families across the GIT included the Lachnospiraceae (24.61 ± 6.58 %) and Ruminococcaceae (20.87 ± 4.22 %). Prevotellaceae was also predominant in ruminal bacterial communities (10.06 ± 2.25 %), however it was less representative in other portions of the GIT of Nelore steers (3.15 ± 0.76 %) (Figure 4).

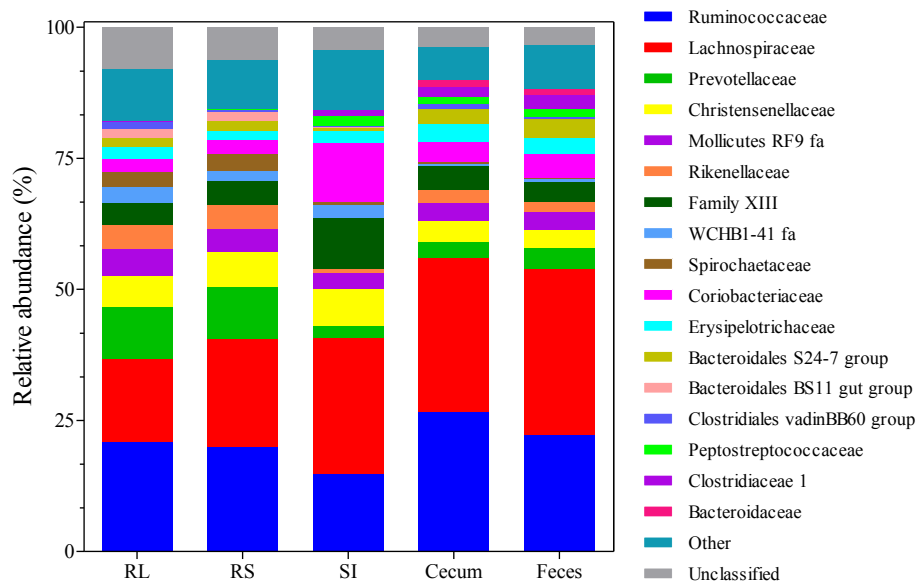


Figure 4. Bacterial composition at family-level across the GIT of Nelore steers. Each bar represents the mean bacterial composition in rumen liquids (RL), rumen solids

(RS), small intestine (SI), cecum and fecal samples. Other corresponds to the sum of phyla that showed relative abundance < 1 %.

To gain a more in-depth understanding of the differences between the microbial communities across the GIT of Nelore steers, we used venn diagram to analyze OTUs from each portion of the GIT that were detected in at least half of the Nelore steers (13 steers) under study. We found 1241 OTUs that were distributed across all samples, 9.91 % which were exclusive to RL, 8.94 % to RS, 10.56 % to SI, 8.3 % to cecum and 4.03 to the feces (Figure 5).

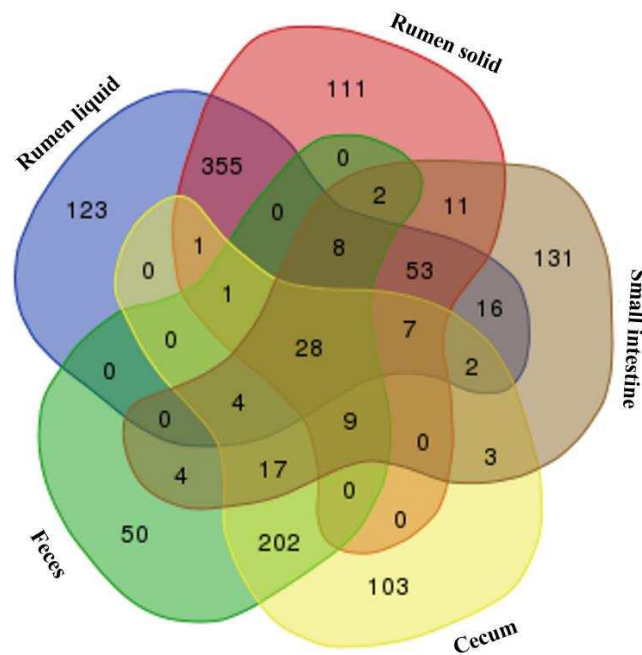


Figure 5. Venn diagram showing the number of bacterial OTUs shared between rumen (liquid and solids), small intestine, cecum and feces communities. Only bacterial OTUs that were identified in at least 50 % of the Nelore steers (13 animals) from each GIT portion are represented.

Taxonomic classification of the 28 shared OTUs distributed between all five portions of the GIT of Nelore steers showed that these sequences belonged to the order Clostridiales (24 OTUs) and Coriobacteriales (3 OTUs). One OTU shared by all GIT portions could not be assigned to any phylum. The highest classifiable level for the shared OTUs, as well the relative abundance across the GIT were represented in a heatmap (Figure 6). These results indicated that the majority of the shared OTUs showed higher abundance in the bacterial community colonizing the SI portion of the GIT and also revealed that the abundance of these shared OTUs was very similar in the cecum and feces of the Nelore steers.

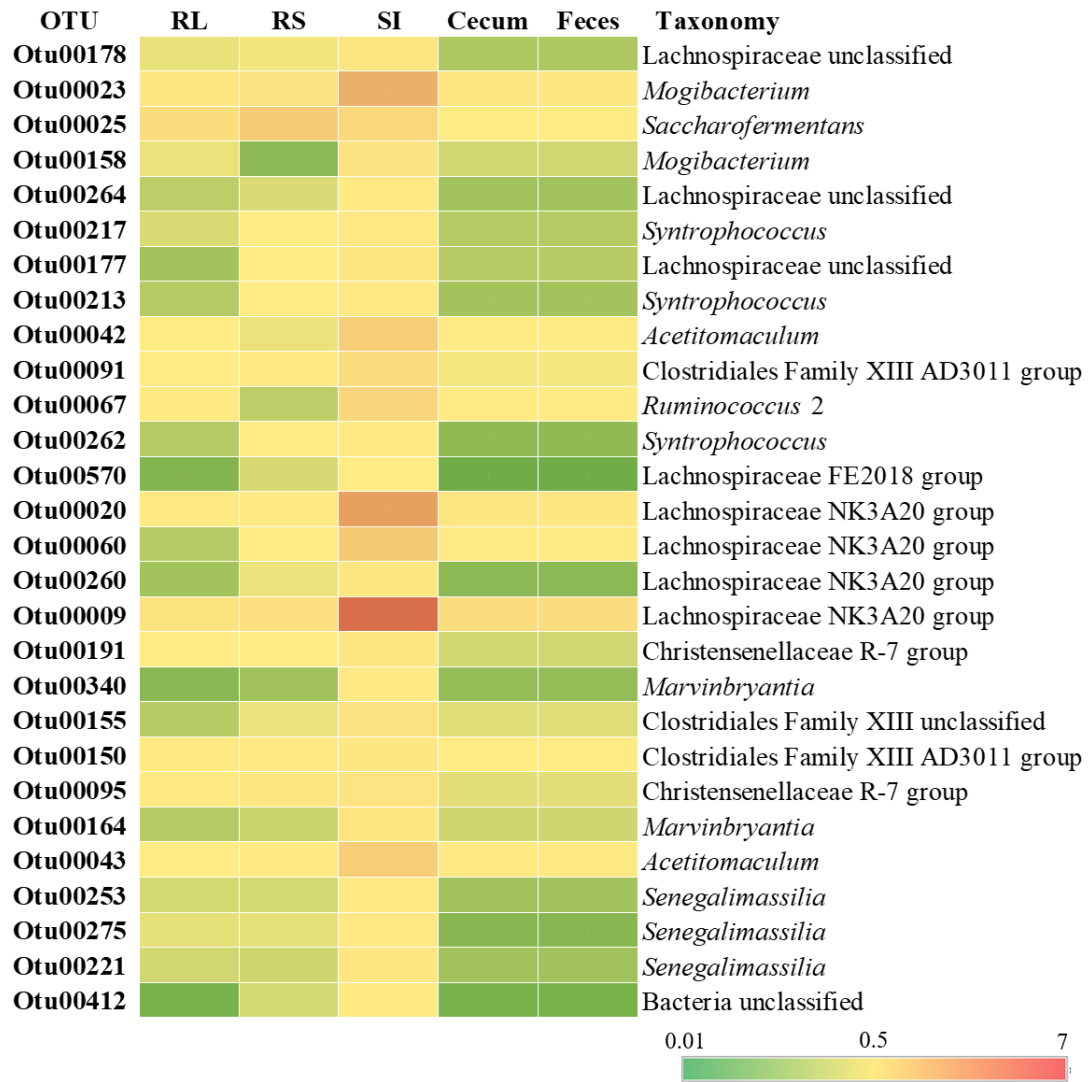


Figure 6. Heatmap representing the relative abundance (%) of OTUs shared between rumen liquid (RL), rumen solids (RS), small intestine (SI), cecum and fecal communities. Taxonomy for each OTU is given at the highest classifiable level.

Analysis of the microbial fermentation profile across the GIT portions evidenced highest concentration (65.13 mmol/l) of total volatile fatty acids (VFAs) in the cecum and feces, while SI showed total VFA concentration almost six times lower than other GIT portions (ANOVA, $P < 0.05$) (Table 3). In the rumen, cecum and feces, the proportions of acetic, propionic and butyric acids were greater than other VFAs. In SI samples succinic acid was present at a high proportion, in addition to acetic and propionic acids (Table 3).

Table 3. Fermentation profiles of Nelore steers GIT portions.

Parameter	Rumen		Small intestine		Cecum		Feces	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acetic acid (%)	69.36a	0.37	55.52b	2.99	65.95a	1.29	69.39a	0.85
Propionic acid (%)	14.51a	0.38	16.71a	2.41	15.65a	0.75	13.17a	0.35
Butyric acid (%)	6.88c	0.19	0.81d	0.29	9.01b	0.56	12.46a	0.62
Isobutyric acid (%)	3.77ab	0.17	3.08b	0.70	5.82a	0.98	2.11b	0.18
Valeric acid (%)	0.97b	0.04	-	-	1.96a	0.16	1.96a	0.16
Isovaleric acid (%)	3.86a	0.19	-	-	1.60b	0.18	0.61c	0.07
Succinic acid (%)	0.65b	0.07	23.88a	2.00	-	-	0.31b	0.11
A/C ratio*	4.87ab	0.14	8.21a	1.80	4.49b	0.24	5.40ab	0.19
Total VFA ** (mmol/l)	51.80b	3.15	11.57c	1.28	66.96a	3.19	63.30a	3.25

The values represent the mean and standard error of mean (SEM). To each parameter, means followed by at least one same letter did not differ at the 5 % level of significance by the Tukey 's test. *A/C = Acetic to propionic acid ratio, ** Total concentration of volatile fatty acids, (-) not detected.

Specific fecal bacterial OTUs are associated with the p-RFI and n-RFI phenotype in Nelore steers

Most studies associating changes in microbial communities with feed efficiency in cattle have focused on the analysis of ruminal samples, in which sample collection depends on invasive methods or the slaughter of the animal. To evaluate if the fecal community could also be used as a proxy to assess differences in feed efficiency in Nelore steers, we conducted OTU analyses to compare the fecal community of efficient (negative Residual Feed Intake, n-RFI) and inefficient (positive-RFI, p-RFI) steers. Our results of alpha diversity analysis of fecal bacterial communities demonstrated that Chao1 richness, Simpson's and Shannon's diversity did not vary significantly in response to RFI group (t-test $P > 0.05$) (Table 4).

Table 4. Alpha-diversity metrics of the bacterial community in fecal samples of Nelore steers according to RFI group.

	p-RFI	n-RFI	P-value
Chao	710 ± 188	632 ± 181	0.213
Shannon	4.31 ± 0.28	4.2 ± 0.36	0.421
Simpson	0.04 ± 0.01	0.04 ± 0.02	0.981

Values represent mean and standard deviation.

However, considering the OTUs that were identified in at least half of steers classified in each feed efficiency group (7 animals to p-RFI and 6 animals to n-RFI), the Venn diagram analysis showed that 99 out of 417 OTUs evaluated, were exclusive of the p-RFI steers (corresponding to 5.16 % of relative abundance), while the n-RFI steers showed 41 exclusive OTUS representing 1.54 % of total relative abundance (Figure 7).

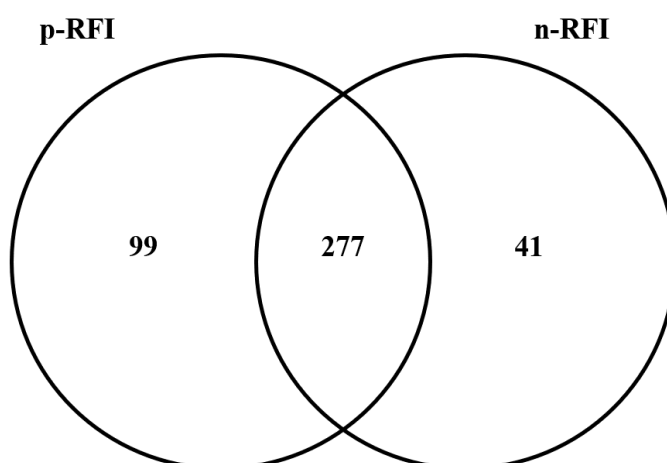


Figure 7. Venn diagram showing the number of bacterial OTUs, shared in the fecal samples of p-RFI and n-RFI Nelore steers. Only bacterial OTUs that were identified in at least 50 % of the steers from each feed efficiency group (at least 7 animals to p-RFI and 6 animals to n-RFI) are represented.

The most abundant exclusives OTUs from p-RFI and n-RFI steers were assigned to different species, with OTU0034 (assigned *Alloprevotella*) being the most abundant in p-RFI steers and OTU00234 (assigned *Turicibacter*) showing greatest abundance in n-RFI steers (Table 5).

Table 5. Most abundant exclusive bacterial OTUs identified in fecal samples of Nelore steers showing low (p-RFI) or high (n-RFI) feed efficiency.

	Taxonomy	Relative abundance (%)	SEM	
p-RFI	Otu00034	<i>Alloprevotella</i>	0.640	0.243
	Otu00053	Ruminococcaceae UCG-005	0.569	0.250
	Otu00068	Bifidobacteriaceae uncultured	0.335	0.291
	Otu00033	Erysipelotrichaceae UCG-003	0.325	0.256
	Otu00209	Bacteroidales unclassified	0.224	0.096
	Otu00476	Clostridium sensu stricto 1	0.120	0.068
	Otu00382	<i>Bacteroides</i>	0.113	0.069
	Otu00129	<i>Blautia</i>	0.097	0.041
	Otu00579	<i>Erysipelatoclostridium</i>	0.089	0.033

	Otu00364	Lachnospiraceae unclassified	0.089	0.065
n-RFI	Otu00234	Turicibacter	0.234	0.110
	Otu00706	Christensenellaceae R-7 group	0.101	0.053
	Otu00341	<i>Butyrivibrio</i> 2	0.087	0.037
	Otu00142	<i>Saccharofermentans</i>	0.079	0.062
	Otu00600	<i>Anaerovibrio</i>	0.073	0.052
	Otu00222	<i>Alloprevotella</i>	0.071	0.021
	Otu00897	<i>Roseburia</i>	0.056	0.027
	Otu01035	<i>Prevotella</i> 1	0.051	0.018
	Otu00790	Erysipelotrichaceae UCG-003	0.046	0.021
	Otu00427	Lachnospiraceae unclassified	0.046	0.021

(*) Taxonomy for each OTU is given at the highest classifiable level. SEM, standard error of mean.

Differences in relative abundance of the OTUs that were shared between steers classified in the p-RFI and n-RFI groups (66.43 % of the total OTUs) were represented on a volcano plot (Figure 8). Out of the 277 shared OTUs, 46.57 % was enriched in n-RFI steers, but only three OTUs were significantly more abundant in these steers (White's non-parametric t-test, $P < 0.05$), whereas 53.43 % of the OTUs were enriched in p-RFI steers, and only seven OTUs were significantly more abundant (White's non-parametric t-test, $P < 0.05$).

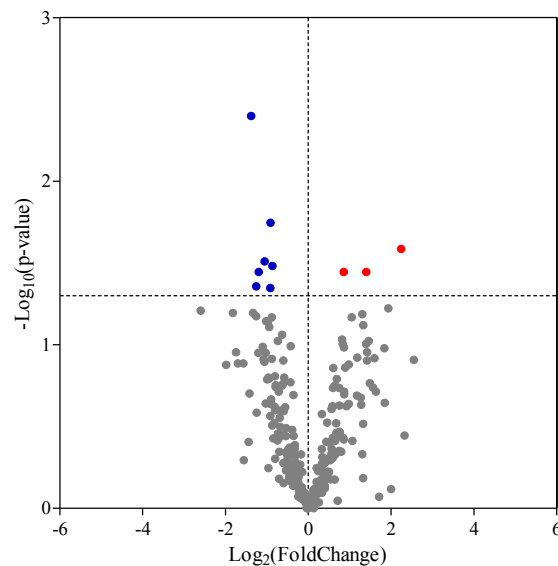


Figure 8. Differences in relative abundance of shared OTUs from fecal community of Nelore steers. Each point represents an OTU and points that showed $\text{Log}_2(\text{FoldChange}) > 0$ were OTUs enriched in the n-RFI steers, while points that showed $\text{Log}_2(\text{FoldChange}) < 0$ were OTUs enriched in the p-RFI steers. Red points are OTUs statistically more abundant in n-RFI steers and blue points represent OTUs that were statistically more abundant in p-RFI steers (White's non-parametric t-test, $P < 0.05$).

Among the OTUs that were statistically more abundant in n-RFI steers, OTU00065 (assigned as unclassified Lachnospiraceae) represented almost 1 % of the relative abundance of OTUs in this group. In the p-RFI steers, the OTU00153 (assigned as *Coprococcus* 3) showed relative abundance that was more than 2.5 times greater in p-RFI than in n-RFI steers (Table 6).

Table 6. Bacterial OTUs that were more abundant (White’s non-parametric t-test, $P < 0.05$) in n-RFI (grey lines) or in p-RFI (white lines) steers.

OTU	p-RFI	SEM	n-RFI	SEM	Taxonomy
Otu00895	0.017	0.006	0.081	0.029	Bacteroidales S24-7 group ge
Otu00065	0.487	0.087	0.880	0.135	Lachnospiraceae unclassified
Otu00883	0.013	0.004	0.035	0.009	Ruminococcaceae unclassified
Otu00153	0.240	0.047	0.092	0.017	<i>Coprococcus</i> 3
Otu00253	0.044	0.007	0.023	0.005	<i>Senegalimassilia</i>
Otu00359	0.113	0.023	0.055	0.005	Clostridium sensu stricto 1
Otu00089	0.220	0.037	0.121	0.019	Christensenellaceae R-7 group
Otu00441	0.030	0.007	0.013	0.004	<i>Atopobium</i>
Otu00703	0.032	0.008	0.013	0.003	<i>Blautia</i>
Otu00545	0.069	0.014	0.036	0.007	Lachnospiraceae NK4A136 group

The values represent relative abundance (%) and the standard error of mean (SEM). (*) Taxonomy for each OTU is given at the highest classifiable level.

Discussion

The gastrointestinal tract (GIT) of animals is a specialized tube that differentiates anatomically in defined and adapted regions (stomach, small intestine and large intestine) and is colonized by microorganisms capable of metabolizing ingested dietary substrates (Turnbaugh and Gordon, 2009). Ruminants have forestomachs (rumen, reticulum, and omasum) that are responsible to digest most of the dietary components in volatile fatty acids (VFAs), whose absorption represents the major source of energy for ruminants maintenance and production (Jami and Mizrahi, 2012; Russell and Hespell, 1981). Because the rumen is the primary site of feed fermentation, most of the studies that evaluate the ruminant microbiome focus on the ruminal ecosystem (Firkins and Yu, 2015; Li et al., 2012; Li and Guan, 2017; Pitta et al., 2010; Shabat et al., 2016; Tajima et al., 2001). However, recent studies have demonstrated relevant associations between the GIT microbiota and ruminant productivity phenotypes (Lindholm-Perry et al., 2016; Myer et al., 2015b, 2015c, 2016).

In the present study, we characterized the bacterial composition of the GIT from 27 Nelore steers, a beef cattle breed that stands out due to its relevance not only to Brazilian economy, but also to the world meat production. We evaluated, for the first time, changes in fecal microbiota of the Nelore steers that could be linked to the feed efficiency phenotype. Our data revealed significant differences in the bacterial community (beta diversity) among the evaluated segments (Figure 2) and supports the idea that passage of digesta through adjacent GIT sections affects the composition of the gut microbial communities (Oliveira et al., 2013). Differences in microbiome composition of distinct GIT components has been reported not only for ruminants (Dias et al., 2017; Perea et al., 2017), but also for other animals, such as chicken (Clavijo and Vives Flórez, 2017) and mice (Montealegre et al., 2016).

The diversity and abundance of gut microorganisms also vary considerably according to host development and anatomical location, mainly because of varying physical-chemical conditions (e.g., pH, redox potential, oxygen availability), the availability of nutrients and sites for adhesion, host secretions (mucins), and exposure to exogenous compounds that cause disturbance in the ecosystem (e.g., antibiotics, dietary changes, pathogens) (Carbonero et al., 2014; Moya and Ferrer, 2016). The differences in composition, richness (Chao index), diversity (Shannon index) and species dominance (Simpson) observed in the passage from the rumen to the small intestine (Figure 1) are related to drastic changes in chemical (acidification and host enzymes secretion) and physical (temperature and pressure) conditions. The secretion of enzymes by the host in the glandular stomach (abomasum) and the small intestine allows the digestion of the microbial mass, which provides protein to the host (Russell and Hespell, 1981). In addition, the time for digestion of feeds in the small intestine is shorter (faster passage rate), which limits the establishment and adaptation of the microbiota to the extreme physical and chemical conditions present in this portion of the GIT (Carbonero et al., 2014). Therefore, the small intestine also presented the lowest total VFA concentration of all GIT segments analyzed in the current study (Table 3). Microbial fermentation seems to be reestablished in the large intestine, whose proportion of acetate, propionate and butyrate was similar to the ruminal ecosystem (Table 3), which agrees with our observation that microbial diversity increases in the distal segments of the ruminant GIT (Figure 1).

Despite the divergences observed among the microbial communities of the GIT segments, the families Lachnospiraceae and Ruminococcaceae were the most abundant across all GIT (Figure 4). The Lachnospiraceae family includes species of bacteria with fibrolytic and proteolytic activity, such as *Lachnospira multiparus* and *Butyrivibrio fibrisolvens*, while members of the Ruminococcaceae family comprise cellulolytic bacteria, such as *Ruminococcus albus* and *Ruminococcus flavefaciens* (Russell and Rychlik, 2001). Considering the functional role of these bacterial groups in the degradation of plant biomass, their relative abundance in the GIT segments represents an increment in the energy obtained by the host during the digestion of structural carbohydrates that escape from ruminal degradation (Myer et al., 2015c; Oliveira et al., 2013). In addition, we observed that 28 out of 1241 OTUs, were common to all segments of the GIT (Figure 5). The sum of relative abundance of these shared OTUs varied from 2.99 % in cecal and fecal communities to 28.26 % in small intestine community (Figure 6). These results show that some bacterial species are able to colonize or survive in all portions of the GIT. All shared OTUs represented sequences from Gram-positive and were found in greater abundances in the small intestine. The digestive conditions and processes that occur in the small intestine could function as a “filter” that limits the growth of ruminal microorganisms throughout the entire ruminant GIT (Moya and Ferrer, 2016; Myer et al., 2016; Oliveira et al., 2013).

Due to the fact that collecting GIT contents depend on invasive techniques or sacrifice of animals, some studies have suggested the use of fecal samples to explore potential associations between the GIT microbiota and host phenotypes and conditions (Nash et al., 2017; Tajima et al., 2012; Tang et al., 2018; Turnbaugh and Gordon, 2009). Thus, the current work is the first to evaluate the relationship between fecal microbiota composition and the feed efficiency of Nelore steers. We observed that differences between the microbiota of negative and positive RFI steers (high and low efficiency steers, respectively) are concentrated in OTUs belonging to the Lachnospiraceae (*Blautia*, *Coprococcus*, *Butyrivibrio* and *Roseburia* genera), Prevotellaceae (*Alloprevotella* and *Prevotella* genera), Coriobacteriaceae (*Atopodium* and *Senegalimassilia* genera) and Ruminococcaceae families (*Saccharofermentans* genus) (Table 3 and 4), which represent functional groups that are typically involved in the degradation of dietary substrates and VFA production in ruminants (Jami and Mizrahi, 2012; Li and Guan, 2017; Russell and Rychlik, 2001). For example, the

Prevotella and *Butyrivibrio* genera are well known for producing hydrolytic enzymes, such as cellulases, xylanases and beta-glucanases that degrade plant structural polysaccharides mainly in the ruminal ecosystem (Cotta and Hespell, 1986; Kamra, 2005). *Butyrivibrio* is related to butyrate production, that represents one of the main sources of energy to enterocytes and exerts effects on GIT epithelial cells, such as stimulation for cell proliferation and differentiation (Guilloteau et al., 2010; Wächtershäuser and Stein, 2000). Butyrate also has an effect on the expression of leptin in bovine adipocytes, affecting feed intake and energy expenditure of the host (Soliman et al., 2007). In addition, ruminal abundance of members of the Lachnospiraceae and Ruminococcaceae families, as well as the *Prevotella* genus have been reported as associated with host feed efficiency (Carberry et al., 2012; McCann et al., 2014; Myer et al., 2015a; Shabat et al., 2016).

Our results expand the knowledge about the composition of the Nelore GIT microbiome and reveals specific taxa potentially associated with feed efficiency. We demonstrated differences between microbial communities colonizing adjacent sections of the GIT and showed that fecal samples harbor considerable diversity of microbial groups that are associated with different fermentation products and feed efficiency phenotypes. This is promising, since collection of fecal samples is non-invasive and a more practical approach to allow comparison of a larger number of animals for traits of interest. Analysis of fecal samples has been applied to population-scale studies of the human microbiome project, making it possible to associate changes in the GIT microbiota with the health status of individuals and several physiological and psychological conditions, including obesity, autism, humor, kidney stone, biogeography and eating habits (Lloyd-Price et al., 2017; Nash et al., 2017; Tang et al., 2018; Turnbaugh and Gordon, 2009)

Thus, our findings suggest that analysis of microbial communities in fecal samples could be useful for monitoring functional groups related to GIT fermentation and feed efficiency in cattle. However, a larger sample size will probably be needed to obtain more accurate estimations of the animal phenotypes and in-depth analysis of the differences in microbial populations. Thus, large-scale studies assessing the fecal microbial community of cattle herds could enable the identification of microbial groups potentially associated with a causal effect on ruminant feed efficiency. Expanding the knowledge about the effects of the microbial community on the well-

being and health of the hosts will be also important for the development of new management, nutrition and manipulation strategies of the GIT microbial community.

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Conclusões e considerações finais

Novilhos Nelore com alta e baixa eficiência alimentar apresentam diferenças específicas na composição da microbiota ruminal e fecal. As diferenças se concentram em estirpes das famílias Lachnospiraceae e Ruminococcaceae, bem como gênero *Prevotella*, os quais são grupos funcionais essenciais para a fermentação dos componentes da dieta e, conseqüente disponibilização de energia para o hospedeiro. Adicionalmente foi observado que o perfil de correlação entre os parâmetros bioquímicos e a microbiota ruminal é diferente para os animais de alta e baixa eficiência alimentar.

Os dados obtidos neste trabalho ainda possibilitaram a expansão dos conhecimentos sobre a microbiota ao longo do trato gastrointestinal (TGI) de novilhos Nelore, evidenciando alterações significativas na diversidade e composição das comunidades do rúmen, intestino delgado e ceco. O rúmen apresentou a maior diversidade bacteriana e o intestino delgado foi o compartimento com menor diversidade, bem como concentração de ácidos orgânicos. Esses resultados evidenciam que a rápida passagem da digesta, além da acidificação e secreção de enzimas pelo hospedeiro no intestino delgado limitam o estabelecimento dos processos fermentativos nesse sítio. Os processos fermentativos e diversidade bacteriana são reestabelecidos nas porções distais do TGI. Apesar das diferenças na composição (beta diversidade), algumas estirpes bacterianas foram observadas em todas porções avaliadas e as famílias Lachnospiraceae e Ruminococcaceae foram as mais abundantes ao longo de todo TGI de novilhos Nelore.

Esses resultados sugerem que bovinos Nelore de alta e baixa eficiência alimentar apresentam diferenças tanto na microbiota ruminal quanto fecal. Sendo assim, por se tratar de uma forma não invasiva e de fácil coleta, a utilização de amostras fecais seria uma alternativa para estudos em larga escala que possibilitariam a ampliação e melhor determinação dos efeitos da comunidade microbiana sobre o bem-estar, saúde e produtividade de ruminantes.