UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

RICHARD ROBERTO LOBO

Inclusão de extrato de erva mate na dieta de cordeiros: efeitos na saúde, desempenho, nutrição, metanogênese e qualidade da carne ovina

Pirassununga
2019

RICHARD ROBERTO LOBO

Inclusão de extrato de erva mate na dieta de cordeiros: efeitos na saúde, desempenho, nutrição, metanogênese e qualidade da carne ovina

Versão corrigida

Dissertação apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do titulo de Mestre em Ciências do programa de Mestrado em Zootecnia.

Área de Concentração: Qualidade e Produtividade animal

Orientador: Prof. Dr. Ives Claudio da Silva Bueno
Co-orientador: Prof. Dr. Rafael Silvio Bonilha Pinheiro

Pirassununga

2019

Ficha catalográfica elaborada pelo
Serviço de Biblioteca e Informação, FZEA/USP,
com os dados fornecidos pelo(a) autor(a)

L799i

Lobo, Richard Roberto
Inclusão de extrato de erva mate na dieta de cordeiros: efeitos na saúde, desempenho, nutrição, metanogênese e qualidade da carne ovina / Richard Roberto Lobo ; orientador Ives Claudio da Silva Bueno ; coorientador Rafael Silvio bonilha Pinheiro. -- Pirassununga, 2019.
121 f.

Dissertação (Mestrado - Programa de Pós-Graduação em Zootecnia) -- Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo.

1. ADITIVOS ALIMENTARES PARA ANIMAL. 2. RUMINANTES. 3. MATE. 4. PRODUÇÃO ANIMAL. I. Bueno, Ives Claudio da Silva, orient. II. Pinheiro, Rafael Silvio bonilha, coorient. III. Título.

Permitida a cópia total ou parcial deste documento, desde que citada a fonte - o autor

UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

CERTIFICADO

Certificamos que a proposta intitulada "INCLUSÃO DE EXTRATO DE ERVA MATE NA DIETA DE TERMINAÇÃO PARA CORDEIRO: EFEITOS NA METANOGÊNESE E NA QUALIDADE DA CAR", protocolada sob o CEUA nº 3497040618, sob a responsabilidade de **Ives Cláudio da Silva Bueno** *e equipe; Richard Roberto Lobo; Yuli Andrea Peña Bermudez* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP (CEUA/FZEA) na reunião de 17/10/2018.

We certify that the proposal "INCLUSION OF YERBA-MATE EXTRACT IN LAMB FINISHING DIET: EFFECTS ON METHANOGENESIS AND MEAT QUALITY", utilizing 36 Ovines (36 males), protocol number CEUA 3497040618, under the responsibility of **Ives Cláudio da Silva Bueno** *and team; Richard Roberto Lobo; Yuli Andrea Peña Bermudez* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 10/17/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 07/2018 a 12/2019 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Ovinos	sexo:	Machos	idade:	2 a 3 meses	N:	36
Linhagem:	Raça de corte			Peso:	20 a 25 kg		

Resumo: No Brasil, a ovinocultura de corte vem crescendo gradativamente ao decorrer dos anos. Essa atividade é extremamente importante, pois os animais conseguem se adaptar muito bem em diferentes condições edafoclimáticas, e nosso país, pelas condições presentes, tem uma enorme chance de chegar entre os principais países produtores de carne ovina. Os ruminantes como um todo são animais que produzem quantidades significativas de gases do efeito estufa através da fermentação ruminal, e que podem provocar mudanças climáticas graves no planeta, portanto existe uma grande preocupação quanto à mitigação desses gases aliada a um incremento na produção. Além de fatores ambientais, o produtor procura alguns sistemas de produção que agreguem valor ao seu produto, e a substituição de aditivos químicos por algo mais natural é extremamente desejado. Quando realizada a inclusão de extrato de erva mate na dieta de bovinos, os animais produzem carne com maior maciez e com uma composição que pode impedir o desenvolvimento de doenças degenerativas, como o câncer colorretal, mas será que essas características também serão observadas em ovinos? Portanto, o presente projeto irá verificar a eficácia do extrato de erva mate quanto a modulação da microbiota ruminal e a sua consequente produção de gases, além de realizar testes de qualidade de carne, perfil de metabólitos e estabilidade oxidativa. Espera-se que a inclusão de extrato de erva-mate module a fermentação ruminal, de forma a modificar a microbiota assim reduzindo a produção de gases, concomitantemente é esperado que o extrato gere efeitos positivos quanto a estabilidade da carne e auxilie na formação de antioxidantes endógenos, que consequentemente irá reduzir os riscos à saúde do consumidor e não cause prejuízos a saúde do animal.

Local do experimento: O experimento in vitro será conduzido no Laboratório de Fermentabilidade Ruminal (LFR) do Departamento de Zootecnia (ZAZ) da Faculdade de Zootecnia e Engenharia de Alimentos (FZEA) da Universidade de São Paulo (USP), para tal, os doadores de conteúdo ruminal para esta fase experimental serão 8 bovinos machos fistulados do ZAZ/FZEA/USP. A segunda etapa será realizada no Galpão de metabolismo de Responsabilidade do Prof. Dr. Marcus Antonio Zanetti, onde serão confinados individualmente 36 ovinos em gaiolas metabólicas de 60 cm de largura por 110 cm de comprimento, o que permitirá que os animais possam desenvolver todos os comportamentos naturais, como alimentar-se, deitar, ruminar, dormir, etc. O abate será realizado no Abatedouro Escola da FZEA e as análises qualitativas de carne, as análises de metabolômica e metagenômica serão desenvolvidas em parcerias com a Faculdade de Engenharia de Ilha Solteira (Unesp), Instituto de Química de São Carlos (USP) e da Embrapa Pecuária Sudeste, respectivamente.

Pirassununga, 31 de outubro de 2018

Profa. Dra. Daniele dos Santos Martins
Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP

Profa. Dra. Cristiane Gonçalves Titto
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP

Folha de avaliação

Autor: LOBO, Richard Roberto

Título: Inclusão de extrato de erva mate na dieta de cordeiros: efeitos na saúde, desempenho, nutrição, metanogênese e qualidade da carne ovina

Dissertação apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do titulo de Mestre em Ciências do programa de Mestrado em Zootecnia.

Área de Concentração: Qualidade e Produtividade animal

Data: ___ / ___ / ___

Banca Examinadora

Prof. Dr. ____

Instituição: ___________________________ Julgamento: ________________

Assinatura: ____

Prof. Dr. ____

Instituição: ___________________________ Julgamento: ________________

Assinatura: ____

Prof. Dr. ____

Instituição: ___________________________ Julgamento: ________________

Assinatura: ____

DEDICATÓRIA

Dedico este trabalho, com o mesmo carinho e respeito, aos meus país, Roberto e Elaine, e aos meus irmãos, Ryan, Yasmim e Ysabelli.

AGRADECIMENTOS

Primeiramente, à Deus, pela sabedoria e força no desenvolvimento deste trabalho.

À Universidade de São Paulo, à Faculdade de Zootecnia e Engenharia de Alimentos e ao Departamento de Zootecnia pela oportunidade de realização do mestrado.

Ao meu orientador, Prof. Dr. Ives Claudio da Silva Bueno, por todo apoio e orientação.

Ao meu co-orientador, Prof. Dr. Rafael Silvio Bonilha Pinheiro, pelo apoio, orientação e amizade ao longo desses 7 anos de convivência.

Aos pesquisadores e colaboradores da Embrapa Pecuária Sudeste, Dr. Alexandre Berndt, Dra. Luciana Correia de Almeida Regitano, Dr. Wadomiro Barioni Junior, Dra. Flavia Aline Bressani Donatoni, e aos pós-graduandos e estagiários do grupo de trabalho do Dr. Alexandre Berndt e do Laboratório de Biotecnologia Animal.

Ao Prof. Dr. Daniel Cardoso, do Instituto de Química de São Carlos, por acreditar no nosso projeto e investir fundos do seu próprio laboratório e tempo para nos auxiliar, assim como agradeço à sua equipe composta pela Dra. Banny Silva Barbosa Correia e Caroline Ceribeli.

Ao Prof. Dr. Adibe Abdalla e Prof. Dr. Helder Louvandini, do Centro de Energia Nuclear na Agricultura da USP, que gentilmente cederam os equipamentos de coleta de metano, dos quais foram usados e geraram importantes resultados para este trabalho.

À Dra. Alexandra Rosa e Dra. Mirele Poletti, pela disciplina que inspirou grande parte das análises realizadas, pelo incentivo e apoio.

Ao Laboratório de Fermentabilidade Ruminal e sua equipe composta pela técnica de Laboratório Priscila Maldonado e seus alunos de pós-graduação e estagiários, que sempre me auxiliaram e tornaram a vida de pós-graduação mais amena e prazerosa.

Ao Prof. Dr. Antonio Faciola e sua equipe, da Universidade da Florida, por me receber, pelo aprendizado e apoio.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pela concessão da bolsa (#2017/25319-0), auxílio financeiro ao projeto (#2018/10308-6) e bolsa de intercambio (#2018/10328-7). Também

agradecemos a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, código de financiamento 001).

Aos trinta e seis animais que fizeram parte neste experimento, que foram sacrificados em prol do desenvolvimento do conhecimento científico.

Aos meus amigos, Rafaela, Gabriela, Flavia, Katieli, Yuli, Danny, Carol, Leticia e Vanderlei Junior, que sempre me apoiam e me incentivam, desde a minha chegada em Pirassununga.

Aos meus amigos da Republica Manguaça, a melhor rep kitnet de Pirassununga.

Aos meus amigos, Camila, Shirlene, Keneth e Marina, que me receberam tão bem durante minha curta estadia em São Carlos.

Ao meu amigo, parceiro, companheiro, e *n* outros títulos, Felipe de Oliveira, por todo o incentivo, por me aturar, e por estar tão presente apesar da distância.

Aos meus pais, Roberto e Elaine, e meus irmãos, Ryan, Yasmim e Ysabelli, pelo amor e apoio incondicional.

Aos meus familiares, por todo incentivo.

Nomes, datas e boas lembranças desta maravilhosa etapa não faltaram. Enfim, agradeço indistintamente a todos que participaram, direta e indiretamente, para o desfecho deste mestrado.

"If you want to go fast, go alone.
If you want to go far, go together."

African Proverb

RESUMO

LOBO, R. R. **Inclusão de extrato de erva mate na dieta de cordeiros: efeitos na saúde, desempenho, nutrição, metanogênese e qualidade da carne ovina.** 2019. 99 f. Dissertação (Mestrado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2019.

O objetivo deste trabalho foi avaliar crescentes níveis de inclusão de extrato de erva-mate como aditivo na nutrição animal e seus efeitos na saúde, performance, nutrição e digestibilidade das dietas, metanogênese *in vivo*, diversidade do microbioma ruminal e qualidade de carne. Para realização deste trabalho, foram utilizados trinta e seis cordeiros machos não castrados e cruzados (Texel x Dorper x Santa Inês) com peso inicial médio de 23,77± 3,70 kg. Os animais foram divididos em nove grupos, os 4 animais de cada grupo tinham pesos similares, e foram sorteados aleatoriamente para um dos 4 tratamentos, que era composto de um controle e outros 3 tratamentos com níveis crescentes de inclusão de extrato de erva-mate (1, 2 e 4%). Os três primeiros dias, todos os animais receberam o tratamento controle, e no terceiro dia foram coletados liquido ruminal via sonda orogástrica e sangue para obtenção de valores iniciais, então começaram a receber o tratamento proposto. Pesagens periódicas foram realizadas para acompanhamento e diariamente era mensurado o consumo de matéria seca, sendo que um resíduo de 10% era desejado para caracterizar uma alimentação *ad libtum*. No 30° dia de confinamento, 24 dos 36 animais foram equipados com sacolas de coletas totais de fezes, que ficaram em adaptação por 3 dias e em coleta por 5 dias para o ensaio de digestibilidade aparente das dietas. No 39° dia os mesmos 24 animais foram equipados com os aparatos para mensuração de metano, utilizando a técnica do gás traçador SF6, foram 3 dias de adaptação aos equipamentos e 5 dias de coletas. Os animais permaneceram em confinamento por 53 dias, ao final deste período, uma última pesagem foi realizada e medidas de biometria corporal foram realizadas, coleta de liquido ruminal e sangue foram realizadas. Após 16h de jejum os animais foram abatido e mensurações de rendimento de carcaça e biometria de carcaça foram realizadas. Uma amostra de músculo *Longissimus thoracis* foi coletada e imediatamente armazenada em nitrogênio líquido após o abate para análise de metabolomica. Uma outra amostra, após 24h de refrigeração foi coletada e armazenada à vácuo para as análises de qualidade de carne. O delineamento experimental foi em blocos casualizados e os resultados foram analisados pelo SAS studio 15.1 pelo procedimento MIXED. Os resultados mostram que níveis moderados (até 2% de inclusão de extrato de erva-mate pode favorecer o sistema imune dos animais e o desempenho geral. Assim como aumentar a ingestão de matéria seca e digestibilidade de alguns nutrientes, como fibra total e FDN. Maior musculatura e menor capa de gordura foi observada nos animais que recebiam o extrato de mate, assim como redução das gorduras sanguíneas. Nenhum efeito sobre a emissão de metano por unidade de produção foi identificada e o extrato não causou mudança na alfa diversidade do microbioma ruminal.

Palavras-chave: Extrato de planta. Aditivo natural. Pequenos ruminantes.

ABSTRACT

LOBO, R. R. **Inclusion of the yerba-mate extract on growing lambs' diet: effects on health, performance, nutrition, methanogenesis, and meat quality.** 2019. 99 f. M.Sc. Dissertation – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2019.

The objective of this study was to evaluate increasing levels of inclusion of yerba mate extract as an additive in animal nutrition and its effects on health, performance, nutrition and digestibility of diets, *in vivo* methanogenesis, ruminal microbiome and meat quality. Thirty six male uncastrated and crossbred lambs (Îlle de France x Dorper x Santa Inês) with initial weight of 23.77 ± 3.70 kg were used for this study. The animals were divided into nine groups, the 4 animals in each group had similar weights, and were randomly assigned into one of the 4 treatments, which consisted of a control and 3 other treatments with increasing levels of yerba mate extract inclusion (1, 2, and 4%). The first three days, all animals received the control treatment, and on the third day ruminal fluid was collected via orogastric tube and blood to obtain baseline values, then began to receive the proposed treatment. Periodic weighting procedures were performed for monitoring, and daily dry matter intake was measured, 10% residue was desired to characterize an ad libtum diet. On the 30th day of period, 24 of the 36 animals were equipped with total fecal collection bags, which were adapted for 3 days and collected for 5 days for the apparent digestibility of diets. On the 39th day, the same 24 animals were equipped with methane measuring devices, using the SF6 tracer gas technique, 3 days of adaptation to the equipment and 5 days of collection. The animals were kept for 53 days, at the end of this period, a last weighing was performed and body biometric measurements were performed, ruminal fluid and blood collection were performed. After 16h of fasting the animals were slaughtered and carcass yield and carcass biometrics measurements were performed. A *Longissimus thoracis* muscle sample was collected and immediately stored in liquid nitrogen after slaughter for metabolomic analysis. Another sample after 24h of refrigeration was collected and stored under vacuum for meat quality analysis. The experimental design was randomized blocks and the results were analyzed by SAS studio 15.1 by the MIXED procedure. Results show that moderate levels (up to 2% inclusion) of yerba mate extract may improve animal immune system and overall performance. As well as increasing dry matter intake and digestibility of some nutrients such as total fiber and NDF. Higher musculature and lower fat cover were observed in animals receiving mate extract, as well as reduction in blood fat. No effect on methane emission per unit of production was identified and the extract did not change the alpha diversity of the rumen microbiome.

Key words: Plant extract. Natural additive. Small ruminant.

CAPÍTULO 1 – Introdução e Revisão bibliográfica 1
INTRODUÇÃO 1
REVISÃO DE BIBLIOGRÁFICA 3
Aditivos na nutrição animal 3
Aditivos 3
Erva-mate 4
Compostos presentes na erva-mate 5
Impacto das plantas não convencionais utilizadas na produção animal 6
Ruminantes 7
Origem 7
Microbiologia do rúmen 9
Nutrição de ruminantes 16
Impacto ambiental da produção animal 19
Gases do efeito estufa 19
Produção entérica de metano 21
Consumo humano de carne 25
Introdução 25
Características qualitativas da carne 26
Problemas relacionados à indústria da carne de ruminantes 29
HIPÓTESE 31
OBJETIVOS 31
REFERÊNCIAS 32
CAPÍTULO 2 – Dietary inclusion of yerba-mate extract in growing lamb nutrition: effects on blood parameters, animal performance, and carcass metrics 44
RESUMO 44
ABSTRACT 45
INTRODUCTION 46
MATERIAL AND METHODS 48
RESULTS 53
DISCUSSIONS 59
CONCLUSIONS 63
REFERENCES 64
CHAPTER 3 – Yerba-mate extract and its effect on digestibility, ruminal fermentation, methane production and microbiome of growing lamb 69
RESUMO 69
ABSTRACT 70
INTRODUCTIO 71
MATERIAL AND METHODS 73

RESULTS **81**
DISCUSSIONS **90**
CONCLUSIONS **94**
REFERENCES **94**
CHAPTER 4 - Effect of levels of dietary inclusion of yerba-mate extract on physicochemical properties of lamb meat ***100***
RESUMO **100**
ABSTRACT **101**
INTRODUCTION **102**
MATERIAL AND METHODS **104**
RESULTS **109**
DISCUSSION **114**
CONCLUSION **118**
REFERENCES **118**

CAPÍTULO 1 – Introdução e Revisão bibliográfica

INTRODUÇÃO

Um dos principais pilares que sustentam a economia brasileira é o agronegócio, que obteve uma média de contribuição anual de 24,62% do PIB total entre os anos de 1996 e 2017 (CEPEA/ESALQ, 2018).

Dentre as atividades do agronegócio, tem-se a ovinocultura com elevada potencialidade no processo de desenvolvimento da pecuária brasileira. Desde a década de 1980, essa criação tem se destacado principalmente nas regiões desfavorecidas do mundo devido a capacidade de adaptação destes animais frente às mais variadas condições edafoclimáticas. Alguns países com baixos índices de renda e carência de alimentos possuem elevado número de cabeças de ovinos (XIMENES; CUNHA, 2012).

Segundo dados do IBGE (2017), o Brasil possui atualmente pouco mais de 18 milhões de cabeças de ovinos, sendo que a região nordeste detém 63,1% desse total, seguida das regiões sul (23,9%), centro-oeste (5,7%), norte (3,7%) e sudeste (3,7%). O estado de São Paulo possui um rebanho de 377 mil ovinos, sendo responsável por 56,1% do total de animais da região sudeste.

Quando levamos em consideração a extensão territorial brasileira, o rebanho ovino não se apresenta em quantidade expressiva quando comparado a outras criações, como, por exemplo, o rebanho bovino, que tem um efetivo de pouco mais de 218 milhões de cabeças e de suínos com quase 40 milhões de cabeças (IBGE, 2017). Nosso país exibe dificuldades para abastecer a demanda interna de carne ovina, sendo necessária a importação deste produto de países como Argentina, Austrália, Uruguai e Nova Zelândia.

Devido às suas condições climáticas favoráveis, às características territoriais e ao potencial de crescimento do rebanho nacional, o Brasil poderá se tornar um dos grandes produtores mundiais de ovinos de corte. A procura por alimentos saudáveis é uma

tendência mundial. Com isso, aumenta-se a perspectiva para avanços ainda maiores na exportação, pelo fato de que a carne ovina brasileira, em sua grande maioria, é produzida à pasto e sem uso de aditivos químicos que poderiam comprometer a saúde humana (MENEZES, 2017).

A partir disso, entender os sistemas de produção, melhorar seus processos e fornecer produtos que garantam sustentabilidade e qualidade é um trabalho que move a comunidade científica e profissional ligada à ovinocultura.

REVISÃO DE BIBLIOGRÁFICA

Aditivos na nutrição animal

Aditivos

Aditivos nutricionais são produtos usados na nutrição animal com o intuito de gerar melhorias no aproveitamento dos alimentos pelos animais ou modular positivamente a qualidade dos produtos de origem animal. Antibiótico é uma classe de aditivos usados na nutrição como uma estratégia para modular a microbiota do trato gastrointestinal de animais de produção, tendo em vista a melhoria da utilização de nutrientes e modulação da composição dos produtos (HASHEMI; DAVOODI, 2011).

É importante realizar a diferenciação entre antibiótico usados como aditivos e antibióticos usados para o tratamento e prevenção de doenças (CASTANON, 2007), visto que os aditivos nutricionais são utilizados em baixas concentrações (em torno de 2,5 a 50 ppm) e se também forem usados para o tratamento de doenças há possibilidade do desenvolvimento de populações bacterianas resistentes, essa preocupação gerou o banimento da utilização de antibióticos em dietas de animais de produção em países da União Europeia e Estados Unidos. No conglomerado europeu, foi publicada a resolução nº 1831/2003 que proíbe, pelo Conselho do Parlamento Europeu a utilização de antibióticos a partir de 2006 (EU, 2003). Já no país norte americano, a aprovação do banimento foi realizada pela organização que regula a Administração de Alimentos e Drogas (U.S.FDA, 2013), no qual dava o prazo de banimento até meados de 2017.

Consequentemente, a aplicação de esforços para substituir esses antibióticos por probióticos, pré-bióticos, simbióticos, enzimas, ácidos orgânicos, minerais orgânicos, oligossacarídeos e outros aditivos, foi enorme nessas últimas décadas (FULTON; NERSESSIAN; REED, 2002). Uma saída que a comunidade científica atualmente utiliza bastante, é o uso de extratos de plantas não convencionais como aditivos naturais.

As fontes de vegetais não convencionais representam todos os alimentos que não são tradicionalmente utilizados na alimentação animal ou referem-se a alimentos destinados ao consumo humano e normalmente não são utilizados em dietas comerciais (DEVENDRA, 1985). A utilização de aditivos naturais na nutrição animal é uma alternativa ao uso de compostos químicos e esta corrente de pensamento vem se tornando uma nova meta na produção animal (MAKKAR; FRANCIS; BECKER, 2007).

Muitas dessas plantas têm níveis importantes de compostos fenólicos, que são fundamentais para o desenvolvimento, reprodução e mecanismos de defesa de qualquer planta. Os fenóis fazem parte de um diverso grupo de fitoquímicos derivados da fenilalanina e tirosina, que incluem fenóis simples, ácidos fenólicos (ácido benzoico, ácido cinâmico e seus derivados), cumarinas, flavonoides, estilbenos, taninos hidrolisáveis e condensados, lignanas, ligninas, etc. (NACZK; SHAHIDI, 2004).

Erva-mate

A erva-mate (*Ilex paraguariensis* St. Hilaire) é uma planta típica de regiões subtropicais da América do Sul (BALZAN et al., 2013), amplamente utilizada em países como Brasil, Paraguai, Uruguai e Argentina (MOSIMANN; WILHELM FILHO; SILVA,, 2006).

Os primeiros a fazerem o uso da erva-mate foram os índios Guaranis, que habitavam a região definida pela bacia dos rios Paraná e Uruguai, na época da chegada dos colonizadores espanhóis, e foi a principal atividade econômica da região depois da metade do século XVI até início do século XVII (BERKAI; BRAGA, 2000).

Atualmente, essa planta é consumida na América Latina normalmente na forma de infusão, com o auxílio de uma “cuia” e um aparato metálico denominado “bomba”, a água (quente ou fria) é colocada diversas vezes. Já na América do Norte, a erva é consumida sob a forma de sachês para infusão ou utilizada na forma de concentrado como

ingrediente na indústria alimentícia (HECK; DE MEJIA, 2007). De acordo com Araújo Filho (2011), são encontrados no mercado diversos produtos à base de erva-mate, como as doses individuais (*tea bags*), o mate concentrado em forma líquida, o mate solúvel, entre outros.

Compostos presentes na erva-mate

A erva-mate possui em sua composição: aminoácidos, minerais (P, Fe e Ca) e vitaminas (C, B1 e B2) (HECK; DE MEJIA, 2007). Vários estudos relatam ou quantificam compostos bioativos presentes na erva-mate, dos quais podemos exemplificar: compostos fenólicos (BASTOS et al., 2007; DE MEJÍA et al., 2010; BERTÉ et al., 2011), alcaloides (CLIFFORD; RAMIREZ-MARTINEZ, 1990; SALDANA et al., 1999) e saponinas (SCHENKEL et al., 1997; GNOATTO; SCHENKEL; BASSANI, 2005; COELHO et al., 2010; GOSMANN et al., 2012).

Diversos estudos vêm sendo realizados com foco nos compostos bioativos da erva-mate. De Souza et al. (2011) identificaram saponinas, xantinas, flavonoides e ácidos fenólicos em folhas de erva-mate, utilizando a técnica de cromatografia líquida de ultra eficiência. A biomassa residual, mais especificamente a casca da árvore, foi estudada como fonte de compostos bioativos por Pagliosa et al. (2010), que observaram que a capacidade antioxidante e os teores de fenóis totais foram superiores aos encontrados em folhas.

Alguns desses compostos têm funções conhecidas e são largamente estudados quando envolve a saúde humana, como: antioxidante, antimicrobiano, antiobesidade, anti-inflamatório, antidiabético e preventivo a doenças cardiovasculares (HECK; DE MEJIA, 2007).

Impacto das plantas não convencionais utilizadas na produção animal

Uma extensiva investigação sobre a utilização dessas plantas e seus efeitos secundários na nutrição animal tem sido realizada (WANAPAT et al., 2012), principalmente com foco no chá verde (*green tea*), por causa de sua composição de antioxidantes e taninos. A utilização da suplementação de chá verde na nutrição animal, melhorou os índices produtivos e sanitários de ovinos (ZHONG et al., 2015) infestados com *Haemonchus contortus* destinados à produção de carne. Um outro exemplo de aplicação de plantas não convencionais que pode ser citado é o pastejo em *Hedysrum coronarium* (comumente conhecida como sula) mostrou um impacto positivo na produtividade em ovinos (BONANNO et al., 2007a) e caprinos leiteiros (BONANNO et al., 2007b).

A inclusão de erva-mate peletizada, na proporção de 500 gramas por dia, para vacas leiteiras que recebiam pastagem e concentrado no cocho, produziu efeitos significativos na produção de gases, pois, os animais que receberam a suplementação da planta produziram menor volume de gases que os animais controle (HARTEMINK et al., 2015).

No trabalho de Po, Xu e Celi (2012), a inclusão erva-mate na proporção de 2,5% do total de ingestão proporcionou efeitos positivos quanto a concentração de gorduras, proteínas e sólidos totais no leite de ovelhas mestiças Dorper no período de periparto, porém também apresentou uma redução do teor de lactose no leite. Este mesmo trabalho mostrou que o peso da prole de ovelhas que receberam a suplementação de erva-mate foi inferior, mas de maneira importante a performance não foi afetada.

Zawadzki et al. (2017) estudaram a inclusão de teores entre 0 a 1,5% de extrato de erva-mate na alimentação dos bovinos de corte e mostraram que a inclusão da planta não afetou o desempenho animal e as características de carcaça, porém a carne

apresentou-se mais macia, tanto na análise de força de cisalhamento quanto na análise sensorial e, portanto, obteve maior aceitabilidade dos consumidores.

Este mesmo trabalho mostrou que a carne apresentou características mais saudáveis pela presença de maiores teores de ácido linoleico conjugado (CLA), creatina e histidina, além de ter aumentado a estabilidade oxidativa, apresentando também antioxidantes endógenos mais altos como carnitina e carnosina, o que promove uma menor tendência à formação de radicais livres no trato gastrointestinal durante a digestão, após o consumo da carne, assim reduzindo o risco de câncer colorretal.

Contudo, outros estudos sobre a erva-mate e sua influência na produção e desempenho animal devem ser realizadas, para complementar os conhecimentos já adquiridos, como sugerido por Po, Xu e Celi (2012).

Ruminantes

Origem

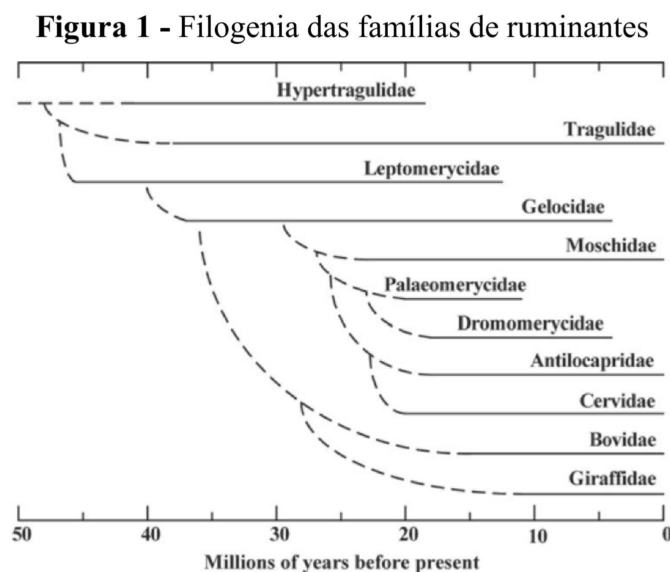
Os ruminantes são animais que apresentam anatomicamente 3 pré-estômagos, sendo estes chamados de rúmen, retículo e omaso. Os dois primeiros desempenham uma importante função na fermentação anaeróbica dos alimentos, pois são câmaras de fermentação que precedem o principal sítio digestivo enzimático, chamado abomaso, que normalmente é conhecido como o estômago verdadeiro, pois é o que mais se aproxima anatômica e fisiologicamente do estômago dos animais monogástricos.

A filogenia desses animais ainda não é bem resolvida, mas um dos possíveis cenários e atualmente mais bem aceito é a teoria apresentada por Gentry (2000), que está apresentada na Figura 1.1. A teoria mostra-nos que provavelmente um animal primitivo foi gerado, sendo ele o *Hypertragulidae* (WEBB; TAYLOR, 1980), por volta de 50 milhões de anos atrás na região sudeste da Ásia (HERNÁNDEZ-FERNÁNDEZ; VRBA, 2005). Pouco depois, os *Tragulidae* e *Leptomerycidae* surgiram, novamente na região

asiática (COLBERT, 1941; MÉTAIS et al., 2001), com uma leve dispersão sobre a América do Norte (WEBB, 1998).

Esses primeiros grupos de ruminantes que apareceram apresentavam tamanho de um coelho (MÉTAIS; VISLOBOKOVA, 2007). Estudos paleontológicos sugerem que os primeiros ruminantes eram muito pequenos, reclusos, onívoros e habitavam florestas (WEBB, 1998). Esse animal ancestral não ruminavam e nem tinha microbiota ruminal funcional para fermentação de fibras até 40 milhões de anos atrás, segundo indicações da morfologia dental (JANIS, 1976) e técnicas moleculares (JERMANN et al., 1995).

Figura 1 - Filogenia das famílias de ruminantes



Fonte: Hackmann e Spain (2010)

O restante das famílias surgiu por volta de 18 a 23 milhões de anos atrás, (JANIS, 1982) na região conhecida como Eurásia (*Antilocapridae*, *Cervidae*, *Moschidae*, *Dromomerycidae*, *Bovidae* e *Palaeomerycidae*) e África (*Giraffidae*) (GENTRY, 2000). Várias dessas famílias (*Antilocapridae*, *Moschidae* e *Dromomerycidae*) se dispersaram pela América do Norte logo depois do seu aparecimento (JANIS; MANNING, 1998; WEBB, 1998).

Por volta de 5 a 11 milhões de anos atrás, as regiões de pastagens se ampliaram (JACOBS et al., 1999) e algumas espécies começaram a incluir maiores quantidades de

gramíneas na dieta, de acordo com o sugerido por estudos de padrões de desgaste dentário e morfologia craniodental (SEMPREBON et al., 2004; SEMPREBON; RIVALS, 2007). Os animais que surgiram nesta época foram então classificados como "*grazers*" (ou comedores de pastagem), segundo a classificação proposta por Hofmann (1989), dentre as espécies deste grupo, encontram-se: bovinos, ovinos domésticos e selvagens, várias espécies de antílopes da Savana Africana e os bisões norte americanos e europeus (HOFMANN, 1989). A partir desse ponto, os ruminantes pertencentes as família *Bovidae* evoluíram para os ruminantes domésticos, assim como são conhecidos na atualidade.

Microbiologia do rúmen

O rúmen é o habitat ideal para o crescimento anaeróbico de microrganismos, as características que qualificam o rúmen para tal título são: ingestão de alimentos supre as demandas de nutrientes, a temperatura é cuidadosamente regulada, água e saliva criam um ambiente úmido e com pH controlado, ácidos graxos de cadeia curta são absorvidos pela parede do rúmen impedindo que esses produtos finais possam se tornar inibidores de crescimento microbiano e o processo de autorregulagem da passagem de conteúdo ruminal permite relativamente rápidas taxas de crescimento microbiano (RUSSELL, 2002, NAGAJARA, 2016).

O ambiente ruminal é anaeróbico e, segundo Nagajara (2016), os gases componentes desse ambiente são dióxido de carbono (CO_2, ~65%) e metano (~35%), e pequenas quantidades de outros gases (H_2, N_2, O_2, etc.). O CO_2 é produzido pela atividade microbiana e pelo processo de neutralização de ácidos pelo bicarbonato proveniente da saliva e sangue. Os traços de O_2 e N_2 são contaminações que adentraram o ambiente junto com o alimento ou água. A ausência de O_2 faz com que o rúmen seja altamente anaeróbico com potencial redox de -350 a 150 mV.

O ecossistema ruminal possui uma diversidade de microrganismos que estão em uma relação simbiótica com o animal hospedeiro (OZUTSUMI et al., 2005). A microbiota é composta por microrganismos pertencentes à três domínios, *Eubacteria* (bactérias), *Archaea* (arqueias, dentre elas as metanogênicas) e Eukarya (protozoários e fungos), com concentrações na ordem de 10^{10}, 10^{6} e 10^{4} células por mL, respectivamente, e as populações bacterianas são as mais vulneráveis às mudanças físico-químicas do rúmen (MCALLISTER et al., 1990).

Bactérias ruminais

O ambiente ruminal possui uma variedade de gêneros bacterianos, no qual constituem a maioria de microrganismos anaeróbicos (PITTA et al., 2010), conforme demonstrado na Tabela 1.1. A competição entre bactérias no rúmen é determinada por vários fatores, podendo ser citados a preferência por determinados substratos, exigência energética para manutenção e resistência a algumas substâncias produzidas no metabolismo que podem ser tóxicas (RUSSEL et al., 1979).

As dietas de ruminantes possuem naturalmente uma base forrageira, o principal componente desse substrato é composto por celulose que não é aproveitado diretamente pelos animais, e alguns microrganismos são adaptados para a utilização de celulose como substrato para seu desenvolvimento e com consequente liberação de produtos do processo de digestão da celulose que podem ser utilizados pelo hospedeiro, e têm uma importante função nutricional ao hospedeiro (RUSSEL et al., 2009).

Para a utilização de carboidratos solúveis, como o amido, outro grupo de microrganismos tem atuação, são as bactérias amilolíticas. Dietas de alto teor de concentrados possuem uma grande quantidade de material altamente fermentável e estão correlacionadas com doenças metabólicas, como acidose (GRESSLEY et al., 2011), depressão da gordura do leite e abcessos de fígado (OWENS et al., 1998). Um dos

principais microrganismos que está relacionado a este problema é o *Streptococci bovis*, quando a dieta muda drasticamente, esses microrganismos mudam seu metabolismo e produzem ácido lático, sendo este um ácido mais forte, o que consequentemente faz com que o pH ruminal caia, assim sendo prejudicial ao ruminante (RUSSEL; HINO, 1985).

Tabela 1.1. Principais classes de bactérias normalmente encontradas no rúmen

Microrganismo	Classificação GRAM	Produtos de fermentação
Bactérias que degradam celulose		
Fibrobacter succinogenes	-	Succinato, Acetato, Formato
Butyrivibrio fibrisolvens	-	Acetato, Formato, Lactato, Butirato, H_2, CO_2
Ruminococci albus	+	Acetato, Formato, H_2, CO_2
Clostridium lochheadii	+	Acetato, Formato, Butirato, H_2, CO_2
Bactérias amiloliticas		
Bacteriodes ruminicola	-	Formato, Acetato e Succinato
Ruminobacter amylophilus	-	Formato, Acetato e Succinato
Selenomonas ruminantium	-	Acetato, Propionato, Lactato
Succinomonas amylolítica	-	Acetato, Propionato, Succinato
Streptococci bovis	+	Lactato
Bactérias lipolíticas		
Anaerovibrio lipolytica	-	Acetato, Propionato, Lactato
Bactérias que degradam acetato		
Selenomonas lactilytica	-	Acetato, Succinato
Megasphaera elsdenii	+	Acetato, Propionato, Butirato, Valerato, H_2, CO_2
Bactérias que degradam pectina		
Lachnospira multiparus	+	Acetato, Formato, Lactato, H_2, CO_2
Bactérias que utilizam ácido lático		
Megasphaera elsdenii	-	Lactato

Fonte: Castillo-González et al. (2014)

Outro grupo bastante importante é das bactérias que degradam lactato, esses microrganismos desempenham um importante papel no rúmen de animais que recebem dieta de alto teor de concentrado, esses microrganismos metabolizam o ácido lático e auxiliam no controle do pH ruminal (MACKIE; HEATH, 1979; COUNOTTE et al., 1981). Esse tipo de bactéria aumenta quando o animal se alimenta de uma dieta com 70% concentrado ou mais (BROWN et al., 2006).

Como já foi mencionado, a dieta natural de ruminantes é constituída majoritariamente por plantas forrageiras, e a pectina é um composto que faz parte da estrutura dessas plantas e constitui 10 a 20% dos carboidratos da forragem utilizada na nutrição de ruminantes. Esse composto é degradado por bactérias e protozoários (DEHORITY, 1969). Bactérias ruminais produzem e liberam enzimas pectinolíticas no ambiente ruminal, e essa enzima é responsável por hidrolisar a pectina em galactourinídeos insaturados (DUSKOVA; MAROUNEK, 2001).

No rúmen, a proteína proveniente da dieta e polissacarídeos estruturais são degradados na proporção de 50 a 70%, e o processo de lise é realizado por enzimas produzidas pelos microrganismos ruminais (COTTA; HESPELL, 1986). Da mesma forma, o lipídio da dieta ingerida é modificado, de ácidos graxos insaturados para ácidos graxos saturados, por dois processos, sendo eles: lipólise e biohidrogenação (NAGAJARA, 2016).

Arqueias metanogênicas

Esses microrganismos são agrupados no filo *Euryarchaeota* e fazem parte do domínio *Archaea* e são divididos em cinco ordens: *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*, *Methanococcales* e *Methanopyrales* (QIAO; TAN; WANG, 2015), conforme demonstrado na Tabela 1.2. As arqueias metanogênicas compõe um grande e diverso grupo de arqueias e sua população varia na ordem de 10^7 a

10^9 células por grama de conteúdo ruminal para ruminantes recebendo dietas de alto teor de concentrado a até 10^9 a 10^{10} células por grama de conteúdo ruminal para animais recebendo uma dieta de volumosos (ATTWOOD et al., 2011).

Algumas espécies de metanogênicas foram isoladas e estudos sobre o substrato utilizado por esses microrganismos foram mais bem descritos. Estudos mostram que esses microrganismos podem possuir mais que uma via de produção de CH_4, como exemplo, podemos citar os trabalhos desenvolvidos por Leahy et al. (2010; 2013), nos quais descrevem que as duas das principais arqueias metanogênicas que habitam o rúmen e abomaso (*M. gottschalkii* e *M. ruminantium*) apresentam duas vias metabólicas completas para a produção de CH_4, sendo uma a partir da redução do H_2 + CO_2 e outra da oxidação do formato, respectivamente, outros exemplos podem ser observados na Tabela 1.2.

Tabela 1.2. Descrição do substrato utilizado, temperatura ótima e habitate dos principais gêneros de arqueias metanogênicas

Ordem	Família	Gênero	Substrato	Temperatura ótima (°C)	Habitat
Methanobacteriales	Methanobacteriaceae	Methanobacterium	H_2, (formato)	37–45	1; 2; 3 e 4
		Methanobrevibacter	H_2, formato	37–40	1; 5; 6 e 7
		Methanosphaera b	H_2 + metanol	37	5
		Methanothermobacter	H_2, (formato)	55–65	1
	Methanothermaceae	Methanothermus	H_2	80–88	8
Methanococcales	Methanococcaceae	Methanococcus	H_2, formato	35–40	9
		Methanothermococcus	H_2, formato	60–65	10
	Methanocaldococcaceae	Methanocaldococcus	H_2	80–85	10
		Methanotorris	H_2	88	10
Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	H_2, formato	40	1; 4 e 13
		Methanoculleus	H_2, formato	20–55	1; 2; 6; 8; 9 e 11
		Methanofollis	H_2, formato	37–40	1
		Methanogenium	H_2, formato	15–57	2; 5; 6 e 9;
		Methanolacinia	H_2	40	9
		Methanoplanus	H_2, formato	32–40	11
	Methanospirillaceae	Methanospirillum	H_2, formato	30–37	1 e 9
	Methanocorpusculaceae	Methanocorpusculum	H_2, formato	30–40	1 e 2
		Methanocalculus b	H_2, formato	30–40	11

continua

continuação

Tabela 1.2. Descrição do substrato utilizado, temperatura ótima e habitate dos principais gêneros de arqueias metanogênicas

Ordem	Família	Gênero	Substrato	Temperatura ótima (°C)	Habitat
Methanosarcinales	Methanosarcinaceae	Methanosarcina	(H_2), Metilamina, Acetato	35–60	1; 2; 4 e 9
		Methanococcoides	Metilamina	23–35	9
		Methanohalobium	Metilamina	40–55	12
		Methanohalophilus	Metilamina	35–40	12
		Methanolobus	Metilamina	37	12
		Methanomethylovorans	Metilamina	20–50	1 e 2
		Methanimicrococcus	H_2 + Metilamina	39	5
		Methanosalsum	Metilamina	35–45	12
	Methanosaetaceae	Methanosaeta	Acetato	35–60	1 e 2
Methanopyrales	Methanopyraceae	Methanopyrus	H_2	98	10

(1) digestão anaeróbica; (2) sedimentos de água doce; (3) solos pantanosos; (4) rúmen; (5) Trato gastrointestinal animal; (6) arrozais; (7) tecidos lenhosos em decomposição; (8) fontes termais; (9) sedimentos marinho; (10) sedimentos geotérmicos marinho; (11) campos de petróleo; (12) sedimentos hipersalinos; (13) águas subterrâneas;
Fonte: Liu e Whitman (2008)

A presença desses microrganismos no rumem é de extrema importância, apesar deles terem pouca representatividade no total de biomassa produzida pelos microrganismos do rumem, as metanogênicas auxiliam para o normal funcionamento e manutenção da fermentação ruminal (JANSSEN; KIRS, 2008). O excesso de H_2 produzido no rúmen é utilizado para reduzir o CO_2 e formar CH_4, este processo recicla o NAD oxidado e permite a continuidade das vias fermentativas (KOZLOSKI, 2002).

Protozoários e fungos ruminais

O rúmen tem uma população diversa de protozoários ciliados, porém sua função na fermentação ruminal não é bem esclarecida, já que alguns experimentos demonstram que esses microrganismos não são fundamentais no rúmen, no entanto, constituem mais da metade da massa microbiana (RUSSELL, 2002), algumas espécies e seus produtos de fermentação estão demonstrados na tabela 1.3.

Esse mesmo autor demonstra que a presença desses eucariotos no rúmen pode ser benéfica e ao mesmo tempo prejudicial ao hospedeiro. Se a dieta é composta de níveis

baixos de nitrogênio, a predação de bactérias e a lise de protozoários auxiliam na reciclagem de proteína microbiana do rúmen, e a contagem de protozoários está diretamente relacionada os teores de amônia ruminal. Se o animal for alimentado com dietas a base de grãos, os protozoários conseguem absorver o amido da dieta, formando assim reservas e regulando o pH, protegendo o animal contra acidose.

Tabela 1.3. Protozoários e fungos ruminais e descrição de suas respectivos produtos de fermentação

	Produtos da fermentação
Protozoário	
Protozoários celulolíticos	
Enoploplastron triloricatum	
Eudiplodinium maggii	
Diploplastron affine	Açúcar reduzido
Epidinium ecaudatum	
Diplodinium monacanthum	
Diplodinium pentacanthum	
Protozoários proteolíticos	
Entodinium caudatum	Amônia, AGCC
Eudiplodinium médium	
Fungos	
Fungos celulolíticos	
Neocallimastix frontalis	Lactato, formato, acetato, succinato, etanol
Piromyces communis	Celobióse, celooligosacarídeo
Orpinomyces joyonii	Glicose

Fonte: Castillo-González et al. (2014)

Alguns trabalhos, demonstram uma importante função dos fungos como degradadores de componentes da parede celular de plantas ingeridas pelos ruminantes, estas estruturas são mais rígidas (lignina, principalmente) e são colonizadas a partir de zoósporos que entram em contato e aderem à este material, desenvolvendo assim um sistema rizomicelial que penetra na parede da planta e libera internamente polissacarídases que degradam o material (AKIN; BORNEMAN, 1990; HO; ADBULLAH, 1999)

Assim como os protozoários os fungos ruminais não são totalmente compreendidos, alguns trabalhos indicam que eles auxiliam na degradação de carboidratos no rúmen já que esses microrganismos são responsáveis por produzir enzimas hidrolíticas no rúmen que hidrolisam a maioria dos componentes da biomassa vegetal ingerida (NAGAJARA, 2016).

Nutrição de ruminantes

Os ruminantes têm a capacidade de maximizar a utilização dos carboidratos celulósicos por causa do seu trato digestivo. Desta maneira, os produtos da fermentação terão maior eficiência de uso (principalmente a energia dos alimentos). Porém, segundo Van Soest (1994), a fermentação pré-gástrica possui as suas desvantagens: apenas 50-70% do nitrogênio microbiano estão disponíveis para o organismo animal, o restante está ligado a estruturas da parede celular e a ácidos nucleicos; a amônia é sempre um subproduto; as proteínas de alta qualidade são quebradas em proteínas de menor qualidade; e a fermentação gera calor e metano.

A avaliação de alimentos e aditivos para o melhor entendimento do mecanismo de digestão é extremamente importante para alcançar o máximo de eficiência e desempenho na produção de ruminantes (JOBIM et al., 2011), já que a alimentação animal é responsável por um dos maiores custos de produção, podendo chegar até quase 65% dos custos totais da produção (GABLER; TOZER; HEINRICHS, 2000).

Para tal, a avaliação de parâmetros de digestão em ruminantes geralmente é realizada em análises *in vivo*, sendo esta técnica bastante acurada (VALENTE; DETMANN; SAMPAIO, 2015). Porém, vários fatores, tais como: bem-estar animal e custos experimentais, vem criando problemas para a experimentação *in vivo*, visando sanar esses problemas, técnicas *in situ* e *in vitro* vêm sendo criadas e aperfeiçoadas, tendo

esta última uma grande representatividade nos resultados publicados nos últimos anos (BRODERICK; COCHRAN, 2000; KRIZSAN et al., 2013; RAMIN et al., 2013).

Avaliação in vitro de alimentos para ruminantes

A técnica de produção de gases (THEODOROU et al., 1994; adaptada por MAURÍCIO et al., 1999 e BUENO et al., 2005) é uma metodologia *in vitro* bastante eficiente para estudar o valor nutritivo de alimentos por possuir alta correlação com a digestibilidade e a degradabilidade dos mesmos (MAURÍCIO et al., 1998; BUENO et al., 1999).

Esta técnica baseia-se em medir a produção de gases liberados pela fermentação de uma amostra incubada em líquido ruminal tamponado. Isso, possibilita simular o ambiente ruminal *in vitro* e medir o desaparecimento do alimento no decorrer do tempo, já que a degradabilidade do alimento está fortemente correlacionada com a produção de gases (BUENO, 2002). Segundo Bueno (2002), a produção de gases *in vitro* está mais relacionada à fermentação que ocorre no rúmen que à digestibilidade que ocorre no trato todo, o que inclui processos de digestão enzimática, absorção e fermentação no ceco.

A produção de gases possui vários benefícios, nos quais podemos citar: ser rápida, manter a uniformidade físico-química do ambiente de fermentação, número de tratamentos pode ser grande, não corre o risco de intoxicação dos animais e assim pode-se fazer o uso de doses acima do limite recomendado, menor custo e a necessidade de poucos animais fistulados (MALAFAIA et al., 1998). Porém, existem algumas desvantagens, dentre as quais podemos citar a precisão da técnica, pequenas variações podem gerar efeitos acumulativos sobre a produção de gases (ALVES, 2010), podendo conduzir a erros de estimativas e o sistema *in vitro* de avaliação de alimentos faz uma simulação da fermentação ruminal, porém o processo de fermentação no rúmem é muito dinâmico e quando é realizada a avaliação *in vitro*, esse dinamismo é reduzido, podendo

o experimento, se não bem planejado, gerar resultados diferentes dos esperados *in vivo* ou *in situ*.

Avaliação in situ de alimentos para ruminantes

O termo *in situ* é uma expressão latina que significa na tradução literal "*no lugar*", no contexto biológico refere-se ao estudo de um determinado fenômeno no exato local onde acontece. No caso da avaliação de alimentos para ruminantes a análise é realizada no próprio rúmem, assim submetendo o alimento estudado às condições reais de digestão e dinamismo ruminal (VANZANT; COCHRAN; TITGEMEYER, 1998).

Entre as técnicas *in vitro* e *in situ*, a segunda é mais recomendada pela comunidade científica (MARINUCCI et al., 1992; VANZANT; COCHRAN; TITGEMEYER, 1998), por causa que o alimento é incubado dentro do rúmem de animais canulados, onde naturalmente todo o processo de fermentação ocorre e presume-se que o processo de análise é mais acurado que o *in vitro* (VAREL; KREIKEMEIER, 1995; ZHOU et al., 2012). Além disso, o processo *in situ* não sofre o acumulo de produtos finais assim como os sistemas *in vitro* estão sujeitos, já que o epitélio ruminal atua na absorção de alguns desses produtos e a liberação de gases pela eructação, produtos esses que quando acumulados em grandes quantidades podem alterar o processo de degradação (CHERNEY et al., 1993).

Avaliação in vivo de alimentos para ruminantes

Essa metodologia baseia-se na avaliação do aproveitamento de um alimento, que é o balanço da matéria perdida na passagem através do trato digestório (VAN SOEST, 1994). Para isso, deve ser considerada a avaliação do consumo e produção de fezes dos animais.

Segundo Safwat et al. (2015), este processo analítico pode ser realizado de duas formas, direto ou indiretamente, sendo a primeira opção realizada pela coleta e

mensuração dos alimentos ingeridos e fezes produzidas pelo animal que foi submetido à dieta teste. A desvantagem do método é o risco de contaminação das amostras de fezes, as mensurações de produção total de fezes nem sempre são acuradas e são bastante trabalhosas. O método indireto baseia-se na utilização de marcadores internos ou externos na dieta e mensuração destes no alimento e fezes, este procedimento é indicado apenas em situações nas quais não é possível a coleta total ou com animais a pasto (SALMAN et al., 2010), este processo pode estimar a produção de fezes e consequentemente proporcionar o cálculo de digestibilidade aparente (NIEVES et al., 2008; BOVERA et al., 2012; 2013). Entretanto, para que se obtenha bons resultados os marcadores internos e externos devem ser facilmente e uniformemente misturáveis na dieta, e devem ser totalmente indigestíveis no trato gastrointestinal e ser totalmente recuperados nas fezes (SALMAN et al., 2010).

As fezes coletadas contêm não apenas o alimento não digerido, mas também produtos metabólicos como bactérias e perdas endógenas do metabolismo animal. Sendo assim, a digestibilidade aparente de um nutriente X é o balanço deste nutriente nos alimentos consumidos menos as suas excreções nas fezes, segundo a equação:

$$Digestibilidade\ aparente\ de\ X = \frac{Consumo\ de\ X - Excreção\ de\ X}{Consumo\ de\ X}$$

Impacto ambiental da produção animal

Gases do efeito estufa

Dentre os diferentes gases de efeito estufa (GEE), a agricultura e pecuária contribuem de forma significativa com a emissão de três deles: gás carbônico (CO_2), metano (CH_4) e óxido nitroso (N_2O), sendo que o primeiro citado é proveniente da queima de combustíveis fósseis e renováveis, então a contribuição deste gás pela agropecuária é

relativamente pequena, mesmo em países onde estes setores são altamente mecanizados, geralmente não excede a 5% do CO_2 (SAUERBECK, 2001).

No Brasil, a liberação de CO_2 pelas queimas de resíduos agrícolas, pastagens, cerrados e florestas associadas ao desmatamento aumentam consideravelmente a liberação desse gás. O fogo libera carbono da biomassa durante a combustão e acentua diretamente a liberação de carbono do solo, do qual a vegetação foi queimada (MONTEIRO, 2009).

O CH_4 é resultante da fermentação anaeróbica da matéria orgânica em ambientes alagados, fermentação entérica, tratamento anaeróbico de resíduos animais e queima de biomassa. Já o N_2O é proveniente da utilização de fertilizantes nitrogenados, da fixação biológica de nitrogênio, da mineralização da matéria orgânica, da adição ou depósito de dejetos animais nos solos, da lixiviação de solos e da queima de resíduos agrícolas. Nos solos, a emissão de N_2O ocorre como consequência do processo microbiológico de nitrificação e desnitrificação (MONTEIRO, 2009).

Segundo um documento emitido pelo Painel Intergovernamental sobre Mudanças Climáticas, as concentrações de dióxido de carbono, metano e óxido nitroso na atmosfera têm aumentado notadamente no mundo como resultado de atividades humanas desde 1750 (IPCC, 2006). Este mesmo documento relatou que os incrementos de CO_2 são devidos primordialmente ao uso de combustíveis fósseis e às mudanças no uso do solo, enquanto os aumentos das concentrações de CH_4 e N_2O são principalmente devido à produção agropecuária.

Adicionalmente, a produção de CH_4 não contribui apenas ao aumento de gases poluentes na atmosfera, mas também gera perdas de 2 a 12% da energia ingerida pelo animal, em particular para vacas leiteiras de alta produção, no qual pelo menos 6% da energia ingerida é perdida (QIAO; TAN; WANG, 2015). Portanto, trabalhar para mitigar

CH_4 não é apenas uma questão ambiental e de cumprimento de normas internacionais descritas no protocolo de Kyoto, mas também melhorar a utilização de energia e consequentemente o desempenho do animal hospedeiro.

Tabela 1.4. Reações envolvidas no metabolismo sintrófico

Reação	**ΔG (kJ/mol)**
Propionato$^-$ + $3H_2O$ → acetato$^-$ + HCO_3^- + H^+ + $3H_2$	+76,1
Butirato$^-$ + $2H_2O$ → 2acetato$^-$ + H^+ + $2H_2$	+48,6
$2H_2$ + HCO_3^- + H^+ → CH4 + $3H_2O$	-130,4
4formato$^-$ + H^+ + H_2O → $3HCO_3^-$ + CH4	-130,4
Acetato$^-$ → HCO_3^- + H^+ + CH4	-36,0

Fonte: Ferry (2011)

Para a pecuária brasileira, o principal gás que devemos dar atenção é o metano, pois no Brasil, no ano de 1994, esta atividade foi responsável pela emissão de 13,17 milhões de toneladas de CH_4, sendo que apenas a pecuária foi responsável por 9,7 milhões de toneladas desse total. Da produção de metano provindo da pecuária, 96% são provenientes dos processos entéricos (incluindo bovinos, bubalinos, ovinos, caprinos, muares, asininos, equinos e suínos) e o restante, dos dejetos (BRASIL, 2006).

Produção entérica de metano

Arqueias metanogênicas produzem metano no rúmen a partir de um reduzido número de substratos, sendo eles: H_2 + CO_2 (mais importante e conhecido processo), formato (HUNGATE et al., 1970; REA et al., 2007), acetato (OPPERMANN et al., 1961) e metanol (NEUMANN et al., 1999). Os três primeiros são provenientes do processo de fermentação de carboidratos e o metanol proveniente da fermentação da pectina. A cultura desses microrganismos em laboratório é difícil, porém é um passo importante para gerar entendimento do metabolismo, fisiologia e via metabólica de produção de CH_4, para tentar sanar este problema da cultura de arqueias metanogênicas, técnicas de análise global de sistemas biológicos vêm sendo aplicadas e geram bons resultados na

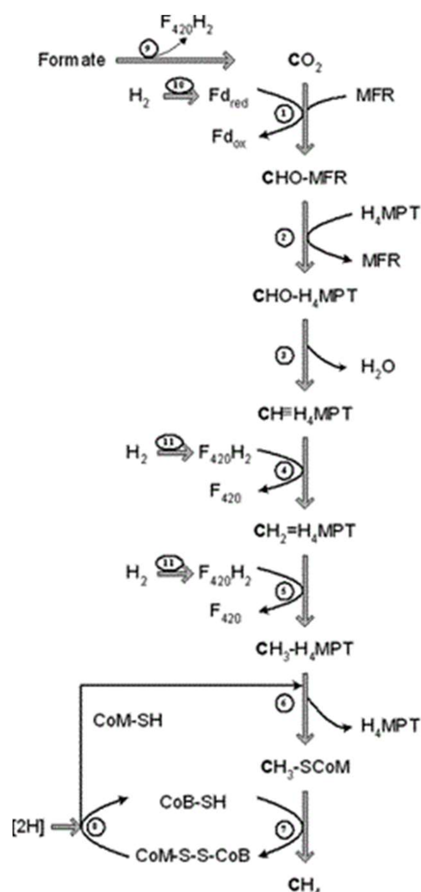
compreensão dos processos metabólicos e fisiológicos presentes no rúmen (QIAO; TAN; WANG, 2015).

Via de utilização de CO_2 e formato como substratos (LIU; WHITMAN, 2008)

A maior parte das arqueias metanogênicas são capazes de reduzir CO_2 para metano, sendo estas chamadas de hidrogenotrópicas, uma parte delas conseguem usar o formato como um doador de elétrons, neste caso, quatro moléculas de formato são oxidadas à CO_2 pela ação da enzima formato desidrogenase ($F_{420}H_2$) (Figura 1.2).

Inicialmente o CO_2 se liga ao metanofuran (MFR) pela ação da formil-metanofuran desidrigenase (enzima 1) e então é reduzido à formil. Nesse primeiro passo, ferrodoxina (Fd) é reduzida pela direta doação de elétron do H_2. E então o formil reage com a Fd reduzida, gerando uma molécula de Fd oxidada e formilmetanofuran (CHO-MFR).

Figura 1.2. Ciclo de produção de CH_4 a partir de CO_2 e formato



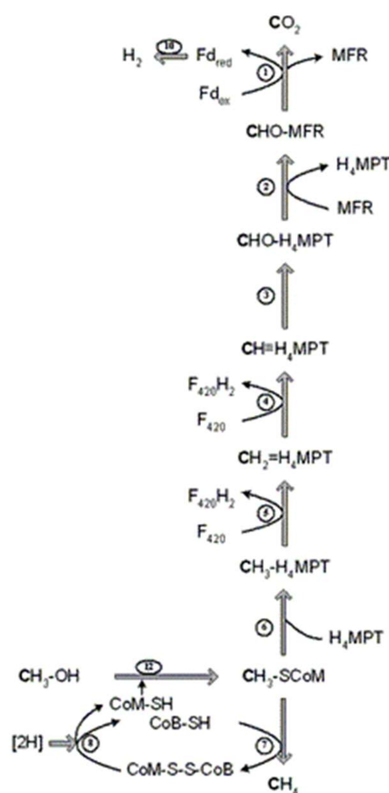
Fonte: Liu e Whitman (2008)

O grupamento formil previamente formado é transferido para uma molécula de tetrahydromethanopterin (H_4MPT) pela ação da enzima formil transferase (enzima 2), formando o formilmetanofuran-tetrahydromethanopterin (CHO-H_4MPT), o grupamento formil da molécula é então desidratada pela cyclohydrolase (enzima 3), formando um grupamento metenil (CH≡H_4MPT).

O grupamento metenil então reage com a forma reduzida da coenzima reduzil hidrogenase ($F_{420}H_2$) pela ação de uma enzima redutase (enzima 4), gerando metilene-tethahydromethanopterin (CH_2=H_4MPT) que então é reduzido novamente pela ação da redutase (enzima 5), formando metil-tethahydromethanopterin (CH_3-H_4MPT).

Desta molécula, o grupamento metil é transferido para a coenzima M (CoM-SH) pela ação da enzima metiltransferase (enzima 6), formando uma molécula de metil-coenzima M (CH_3-SCoM). O último processo de redução é então realizado pela metil coenzima M redutase (enzima 7), assim liberando uma molécula de gás CH_4 e um heterodissulfeto de coenzima M e B (CoM-S-S-CoB). Esse heterodissulfeto é então reduzido pela heterodissulfeto redutaze (enzima 8) gerando CoM-SH e CoB-SH que podem retornar para um novo ciclo.

Figura 1.3. Ciclo de produção de CH_4 a partir de metanol



Fonte: Liu e Whitman (2008)

Durante o esse ciclo de formação de CH4, duas reações são exotérmicas (transferência do grupo metil para a CoM e a redução do heterodissulfeto) e envolvem o processo de conservação de energia.

Via de utilização de metanol como substrato

(LIU; WHITMAN, 2008)

As archeas metanogênicas que conseguem utilizar compostos contendo grupamentos metil, como aminas metiladas (mono, di, tri e tetrametilamina) e sulfetos metilados (metanotiol e dimetilsulfeto), e são conhecidas como metilotroficas (Figura 1.3). O grupo de arqueias dessa classe são restritas aos microrganismos da

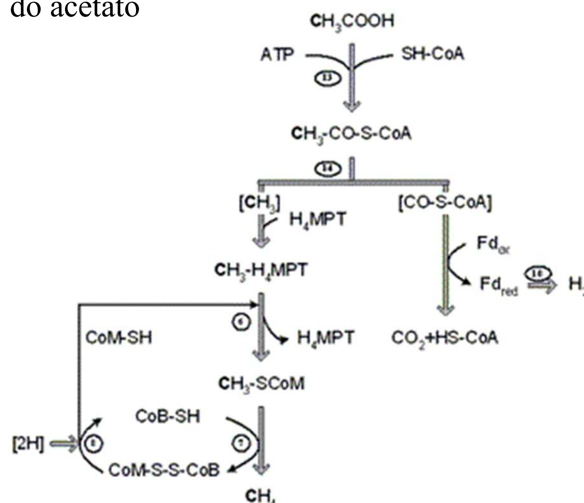
ordem *Methanosarcinales*, com exceção dos organismos do gênero *methanosphaera* que também utilizam o mesmo tipo de substrato.

O grupo metil de uma molécula é transferido para uma proteína corrinoide cognata e então transferida para a CoM-SH. O complexo CH_3-CoM-SH entra no ciclo de produção de CH_4 e é reduzido à CH_4 e heterodissulfeto de coenzima M e B.

Os elétrons requeridos para a redução da CoM-SH à CH_4 são normalmente adquiridos da oxidação de grupos metil adicionais à CO_2. Este processo é o inverso do apresentado em arqueias metanogênicas hidrogenotrópicas, e utiliza três grupos metil para a formação de cada CO_2 e é chamado de processo desproporcional, já que a oxidação de uma parte do substrato é usada para reduzir o restante.

Via de utilização de acetato como substrato (LIU; WHITMAN, 2008)

Figura 1.4. Ciclo de produção de CH_4 a partir do acetato



Fonte: Liu e Whitman (2008)

O acetato é o maior produto intermediário da cadeia alimentar anaeróbica e dois terços do CH_4 biologicamente gerado é derivado desse substrato (Figura 1.4). Porém, apenas dois gêneros são conhecidos por utilizarem esta via metabólica (*Methanosarcina* e *Methanosaeta*).

Essa via metabólica oxida o grupo carboxila do acetato e complexa a esse composto com uma coenzima A (SH-CoA), pela ação da acetato quinase (enzima 13). Esse complexo então sofre a ação da enzima carbonila desidrogenase/acetil-CoA sintetase (enzima 14), gerando CH_2 e um complexo carbonila-CoA (Co-S-CoA), este é então é oxidado, gerando CO2 e HS-CoA, o elétron

gerado é então utilizado para o processo de redução da Fd oxidada, a molecula de Fd reduzida é então oxidada e o processo gera uma molécula de H_2 e Fd oxidada.

O CH_2 gerado pela quebra do primeiro complexo formado é então ligado à H_4MPT, formando CH_2-H_4MPT, que então é transformado em CH_4 pelo mesmo conjunto de reações explicadas anteriormente.

Consumo humano de carne

Introdução

Estudos antropológicos mostram que a evolução do *Homo sapiens* ocorreu com grande participação da carne de caça na dieta, em períodos de tempo entre 3 a 4 milhões de anos, sendo, inclusive fundamental para o grande desenvolvimento do nosso sistema nervoso central (MEDEIROS, 2008).

As exigências nutricionais de proteína para a espécie humana foram estudadas pela FAO e foi demonstrado que os indivíduos adultos do sexo masculino necessitam diariamente de cerca de 55 g de proteína. Já os indivíduos adultos do sexo feminino necessitam em torno de 45 g por dia, porém, estas exigências podem ser alteradas de acordo com o estado físico e de saúde (BERDER, 1992).

A carne tem grande importância nutricional, é uma fonte de proteínas de altíssima qualidade, além de possuir uma composição com aminoácidos essenciais na nutrição humana e de ser uma excelente fonte de ferro, zinco e vitaminas do complexo B (BERDER, 1992).

O consumo elevado de produtos de origem animal (principalmente alimentos processados) está ligado ao aparecimento de algumas doenças, como doenças cardiovasculares, cânceres, alterações no colesterol, entre outras (BERDER, 1992; SINHA et al., 2009), porém, o consumo moderado de proteína animal, sem

processamento, traz excelentes incrementos nutricionais (SINHA et al., 2009), como apresentado no parágrafo anterior.

Características qualitativas da carne

A qualidade da carne é um combinado de características como sabor, suculência, textura, maciez e aparência, associadas à carcaça com pouca gordura, muito músculo e preço acessível (SILVA SOBRINHO, 2001).

Potencial hidrogeniônico (pH)

O pH é um dos fatores cruciais na transformação do músculo em carne, já que está diretamente ligado ao estabelecimento do *rigor mortis*, e tem seus efeitos percebidos tanto na carne fresca como em seus derivados (OSÓRIO; OSÓRIO, 2000). O pH do músculo de ovinos vivos varia entre 7,3 e 7,5 (ZEOLA et al., 2002), após o abate, ocorre o decréscimo do pH podendo chegar até a 5,4 entre duas e oito horas após a sangria. A queda do pH acontece porque, após o abate ainda há continuidade da glicólise aeróbica até que sejam esgotadas as reservas de ATP e de oxigênio. Após o esgotamento, começa a glicólise anaeróbica utilizando glicogênio muscular e tendo como consequência a formação de ácido lático, o que acidifica o meio, conferindo sabor ligeiramente ácido e odor característico. A carne ovina atinge pH final (entre 5,5 e 5,8) de 12 a 24 horas após o abate (SILVA SOBRINHO, 2005).

Cor

A cor é considerada o primeiro fator determinante na hora da compra, mesmo não sendo um fator que influencia a palatabilidade ou o seu valor sensorial; esse fator reflete a quantidade e o estado químico da mioglobina, seu principal pigmento (MAGNO, 2014). Esta característica de qualidade da carne pode ser influenciada, além da exposição ao oxigênio e forma da mioglobina, pela quantidade de mioglobina presente no músculo, que se altera em decorrência da utilização, da localização anatômica e da idade do animal.

Porém, estas não são as únicas fontes de variação da cor da carne, pois a ausência de queda do pH da carne gera carnes DFD (sigla em inglês para *dark, firm and dry*, ou seja, carne escura, firme e seca), desenvolvendo menor retenção de água, consistência mais rígida e coloração escura, em virtude da menor refração de luz e da maior ação enzimática. Já quando a queda do pH é excessiva, a carne libera mais água, fica com consistência flácida e coloração menos intensa (FEIJÓ et al., 1999).

Capacidade de retenção de água

É definida como a capacidade da carne em reter sua umidade ou água durante a aplicação de forças externas, como corte, aquecimento, trituração e prensagem (FERNANDES DE SÁ, 2004). Se a capacidade de retenção de água do tecido muscular for baixa, ocorrerá perda de peso durante o resfriamento e estocagem, o que consequentemente resultará em perdas no valor nutritivo através do exsudado liberado, resultando em uma carne mais seca e menos macia, típicas de carnes DFD (DABÉS, 2001).

Esta característica está relacionado com a velocidade de queda do pH durante a glicólise *post-mortem* e com a formação de ácido lático. A capacidade de retenção de água é menor em pH 5,2-5,3, ou seja, no ponto isoelétrico (pI) da maior parte das proteínas musculares. Se o pH fica acima do pI, desaparecem as cargas positivas ficando um excesso de cargas negativas que determina a repulsão dos filamentos, deixando mais espaço para as moléculas de água escapar (ROÇA, 2009).

Maciez

Este fator é definido como as medidas físicas da resistência da carne cozida à compressão ou cisalhamento (*tenderness*) e resistência à mastigação detectada por provadores (*sensory tenderness*) (DRANSFIELD, 1994). A maciez da carne está diretamente ligada ao conteúdo de colágeno e da idade do animal. Quanto mais velho o

animal, maior o número de ligações cruzadas termoestáveis do colágeno, fazendo com que este tipo de fibra seja menos solúvel na cocção, assim resultando em uma carne mais dura (BRIDI; CONSTATINO, 2010).

Com o avanço da idade, a quantidade de exercício faz com que os músculos apresentem maior quantidade de colágeno. O grau de acabamento das carcaças e o teor de gordura intramuscular também influenciam na maciez da carne ovina. O grau de acabamento de gordura na carcaça reduz o risco de encurtamento dos sarcômeros pelo frio das câmaras de resfriamento, por promover proteção aos músculos fazendo com que a temperatura caia gradativamente (BRIDI; CONSTATINO, 2010).

Perfil lipídico da carne de ruminantes

O perfil lipídico da carne de ruminantes possui uma grande gama de ácidos graxos que não estão presentes nos alimentos desses animais, isso ocorre pela transformação desses compostos no rúmen, através do metabolismo ruminal de lipídios (TORAL et al., 2018). Em condições normais, os ruminantes possuem sua base alimentar constituída por plantas forrageiras e leguminosas, que disponibilizam toda a carga lipídica necessária para o animal, levando em consideração a dieta citada, os ruminantes possuem uma base lipídica alimentar composta em grandes proporções de ácidos graxos insaturados (JENKINS et al., 2008; BUCCIONI et al., 2012).

Ao serem ingeridos, esses lipídios sofrem uma alta metabolização, sendo causada pela ação de lipases produzidas por microrganismos ruminais (JENKINS et al., 2008), seguida pelo processo de biohidrogenação, que consiste na isomerização e saturação de ácidos graxos, sendo este processo realizado por bactérias, com a finalidade de reduzir a toxicidade desses compostos ao crescimento microbiano (JENKINS et al., 2008).

O processo de biohidrogenação pelas bactérias não é perfeito, e comumente processos incompletos são realizados, deixando assim inúmeros metabólitos

intermediários chegarem no duodeno e serem absorvidos e consequentemente incorporados nos tecidos e gordura do leite (SHINGFIELD et al, 2013). Sendo que foi provado por trabalhos recentes, que fatores endógenos de algumas plantas possuem a habilidade de modular este processo de fermentação lipídica (LEE et al., 2009; BUCCIONI et al., 2012).

O processo de modulação do metabolismo de lipídios, como foi dito, é um processo muito relacionado com a população microbiana ruminal, sendo que a alteração desse microbioma irá modular a geração de precursores (ácidos graxos de cadeia curta) que serão transportados para o musculo ou para a glândula mamária e consequentemente mudar a composição de ácidos graxos produzidos pelo processo de síntese *de novo* de ácidos graxos (BERNARD et al., 2008; SHINGFIELD et al, 2013)

Problemas relacionados à indústria da carne de ruminantes

As principais características observadas por consumidores é a aparência, textura e sabor, e a aparência é uma característica determinante na compra ou não do produto (GRAY; GOMAA; BUCKLEY, 1996; FAUSTMAN; PHILLIPS, 2001). Carnes devem ter aspecto fresco, com coloração vermelha brilhante, a perda deste aspecto importantíssimo, origina uma carne com tons amarronzados, é um processo natural (FAUSTMAN; PHILLIPS, 2001; BEKHIT; FAUSTMAN, 2005), porém causa menor saída deste produtos em supermercados e açougues, e consequentemente desvalorização do produto (LI; LIU, 2012).

A cor depende principalmente da concentração e estado redox da mioglobina, hemoglobina e citocromos, já que esses componentes estão diretamente relacionados com a pigmentação da carne (FAUSTMAN; CASSENS, 1990; BEKHIT; FAUSTMAN, 2005). A mioglobina é a proteína solúvel em água e uma das mais abundantes

relacionadas a coloração, na sua composição possui ferro heme e o estado oxidativo deste componente pode alterar a cor da carne (LI; LIU, 2012).

Após o corte da carne, o oxigênio reage com a mioglobina (coloração vermelha arroxeada), formando a oximioglobina (vermelho brilhante), quando o grupamento heme da oximioglobina e mioglobina é oxidado (perdem um elétron) ocorre a mudança de cor, para vermelho amarronzado, pela formação da metamioglobina (GRAY; GOMAA; BUCKLEY, 1996; FAUSTMAN; PHILLIPS, 2001; MANCINI; HUNT, 2005).

Um outro problema bastante recorrente na indústria da carne é a peroxidação lipídica (oxidação de ácidos graxos insaturados em sua forma livre ou em ésteres) na carne, é um indesejável processo na indústria (LI; LIU, 2012). Esse processo ocorre durante todo o período de estocagem e inconveniente proporciona cheiro e gosto de ranço em produtos cárneos (CAMPOS et al., 2006; CASTELLINI; DAL BOSCO; BERNARDINI, 2001; STETZER et al., 2008).

A oxidação lipídica também ocorre *in vivo*, este processo gera produtos que podem interagir com proteínas e gerar danos à saúde do animal (LI; LIU, 2012), e em uma carne com processo muito avançado de oxidação lipídica pode estar relacionado no desenvolvimento de doenças ligadas aos radicais livres nos consumidores (FRANKEL, 1984; HALLIWELL; CHIRICO, 1993).

Como é sabido, o número de insaturações dos ácidos graxos (duplas ligações) está diretamente ligado com a taxa de peroxidação lipídica, sendo que ácidos graxos poli-insaturados (PUFA) são oxidados mais rápidos que ácidos graxos monoinsaturados, isso ocorre pelo fato de que remover o hidrogênio do carbono metileno gasta menos energia do que remover o hidrogênio de carbono metil (RICHARD, 2006).

Altas quantidades de ferro e PUFA são características desejais no ponto de vista da saúde dos consumidores (DIPLOCK et al., 1998), visto que ferro é um nutriente

importantíssimo na nutrição humana e o baixo consumo de PUFA está relacionado com altas taxas de doenças degenerativas. No entanto, existe uma grande preocupação quanto a cor e estabilidade lipídica da carne, visto que o ferro é um precursor do processo de peroxidação lipídica (VENKATARAMAN; SCHAFER; BUETTNER, 2004; SCHAFER; KELLEY; BUETTNER, 2003).

Com este empasse, envolvendo a nutrição humana e indústria da carne, criou-se uma nova área de trabalho para pesquisadores e cientistas na Zootecnia e Engenharia de Alimentos, visando aumentar a quantidade de ácidos graxos benéficos ao consumidor sem ter prejuízos na qualidade e estabilidade dos produtos de origem animal.

HIPÓTESE

A hipótese do presente estudo foi de que a adição de erva-mate na dieta de cordeiros melhoraria a saúde dos animais e consequentemente o desempenho, alteraria a microbiota ruminal e em consequência disso, reduziria emissão de metano, melhoraria a utilização dos nutrientes da dieta e teria efeitos benéficos na qualidade da carne.

OBJETIVOS

Objetivo geral

Avaliar o uso do níveis crescentes de extrato de erva-mate na alimentação de ovinos de corte, como uma estratégia para melhorar a saúde dos animais e seu potencial como modulador da microbiota ruminal, e seus efeitos na metanogênese, digestibilidade da dieta e qualidade da carne.

Objetivos específicos

Capitulo 1

- Avaliar o efeito da inclusão de níveis crescentes de erva-mate nos parâmetros bioquímicos e de contagem de células sanguíneas.
- Avaliar a performance de animais alimentados com níveis crescentes de extrato de erva-mate.

- Avaliar mensurações corpóreas e de carcaça de animais alimentados com níveis crescentes de extrato de erva-mate.

Capitulo 2

- Avaliar o efeito da inclusão de níveis crescentes de extrato de erva-mate na digestibilidade aparente das dietas de cordeiros confinados.
- Avaliar emissão de metano de cordeiros alimentados com níveis crescentes de extrato de erva-mate.
- Avaliar a alfa diversidade do microbioma ruminal e suas alterações ocasionadas pela inclusão de níveis crescentes de extrato de erva-mate na dieta de cordeiros.

Capitulo 3

- Avaliar os efeitos da inclusão de extrato de erva-mate na qualidade de carne físico-quimica da carne e composição centesimal.
- Avaliar o metaboloma do músculo e suas possíveis interações com os tratamentos.

REFERÊNCIAS

ALVES, T. C. **Desenvolvimento ponderal, características da carcaça e eficiência da nutrição energética e proteica no metabolismo ruminal de búfalos e produção de gases in vitro**. 2010. 146 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2010.

ARAUJO FILHO, H. C. Classificação de amostras de erva-mate de acordo com o seu envelhecimento por microextração em fase sólida (MEFS). **Revista de Química Industrial**, Rio de Janeiro, n. 732, p. 19-23, 2011.

ATTWOOD, G. T. et al. Exploring rumen methanogen genomes to identify targets for methane mitigation strategies. **Animal Feed Science and Technology**, Amsterdam, v. 166-167, p. 65-75, 2011.

BALZAN, S. et al. Lipid-lowering effects of standardized extracts of Ilex paraguariensis in high-fat-diet rats. **Fitoterapia**, Amsterdam, v. 86, p. 115-122, 2013.

BASTOS, D. H. M. et al. Yerba mate: pharmacological properties, research and biotechnology. **Medicinal and Aromatic Plant Science Biotechnology**, Middlesex, v. 1, p. 37-46, 2007.

BEKHIT, A. E. D.; FAUSTMAN, C. Metmyoglobin reducing activity. **Meat Science**, Amsterdam, v. 71, p. 407-439, 2005.

BERDER, A. **Meat and met products in human nutrition in developing countries**. Rome: FAO, 1992. (Food and Nutrition Paper 53).

BERKAI, D.; BRAGA, C. A. **500 anos de história da erva-mate**. Porto Alegre: Cone Sul, 2000.

BERNARD, L.; LEROUX, C.; CHILLIARD, Y. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. **Advances in Experimental Medicine and Biology**, New York, v. 606, p. 67-108, 2008.

BERTÉ, K. A. S. et al. Chemical composition and antioxidant activity of yerba-mate (Ilex paraguariensis A. St.-Hil., Aquifoliaceae) extract as obtained by spray drying. **Journal of Agricultural and Food Chemistry**, Washington, v. 59, n. 10, p. 5523-5527, 2011.

BONANNO, A. et al. Grazing sulla and/or ryegrass forage for 8 or 24 hours daily. Effects on ewes feeding behaviour. In: SYMPOSIUM OF THE EUROPEAN GRASSLAND FEDERATION, 14., 2007, Ghent, Belgium, **Proceedings…** Ghent, Belgium, 2007a. p. 208-211.

BONANNO, A. et al. Intake and milk production of goats grazing Sulla forage under different stocking rates. **Italian Journal of Animal Science**, Abingdon, v. 6, supl. 1, p. 605-607, 2007b.

BOVERA, F. et al. Effects of water restriction on growth performance, feed nutrient digestibility, carcass and meat traits of rabbits. **Animal**, Cambridge, v. 7, p. 1600-1606, 2013.

BOVERA, F. et al. Use of dietary mannanoligosaccharides during rabbit fattening period: Effects on growth performance, feed nutrient digestibility, carcass traits, and meat quality. **Journal of Animal Science**, Cary, v. 90, p. 3858-3866, 2012.

BRASIL. Ministério da Ciência e Tecnologia. Relatórios de Referência: emissões de metano pela pecuária. In: ______. **Primeiro inventario brasileiro de emissões antrópicas de gases de efeito estufa**. Brasília: MCT, 2006. p. 1-77.

BRIDI, A. M.; CONSTANTINO, C. Qualidade e avaliação de carcaças e carnes bovinas. In: 31° CONGRESSO PARANAENSE DOS ESTUDANTES DE ZOOTECNIA, 31., 2010, Maringá, PR. **Anais...** Maringá, PR, 2010. Disponível em: < http://www.uel.br/grupo-pesquisa/gpac/pages/arquivos/Qualidade%20e%20Avaliacao%20de%20Carcacas%20e%20Carnes%20Bovinas.pdf >. Acesso em: 25 set. 2017.

BRODERICK, G. A.; COCHRAN, R. C. In vitro and in situ methods for estimating digestibility with reference to protein degradability. In: THEODOROU, M. K.; FRANCE, J. (Ed.). **Feeding systems and feed evaluation models**. Wallingford, UK: CAB International, 2000.

BROWN, M. S.; PONCE, C. H.; PULIKANTI, R. Adaptation of beef cattle to high-concentrate diets: performance and ruminal metabolism. **Journal of Animal Science**, Cary, v. 84, p. 25-33, 2006.

BUCCIONI, A. et al. Lipid metabolism in the rumen: new insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. **Animal Feed Science and Technology**, Amsterdam, v. 174, p. 1-25, 2012.

BUENO, I. C. S. **Cinética e síntese microbiana ruminal em ovinos alimentados com fenos de três qualidades distintas**. 2002. 97 p. Tese (Doutorado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2002.

BUENO, I. C. S. et al. Influence of inoculum source in a gas production method. **Animal Feed Science and Technology**, Amsterdam, v. 123-124, p. 95-105, 2005.

BUENO, I. C. S. et al. Uso de líquido ruminal e fezes de bovinos e ovinos como fonte de inóculo para a técnica in vitro de produção de gás. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 36., 1999. Porto Alegre. **Anais...** Porto Alegre: SBZ, 1999. p. 122.

CAMPOS, M. M. et al. Flavour perception of oxidation in beef. **Meat Science**, Amsterdam, v. 72, p. 303-311, 2006.

CASTANON, J. I. R. History of the use of antibiotic as growth promoters in European poultry feeds. **Poultry Science**, Cary, v. 86, p. 2466-2471, 2007.

CASTELLINI, C.; DAL BOSCO, A.; BERNARDINI, M. Improvement of lipid stability of rabbit meat by vitamin E and C. **Journal of the Science of Food and Agriculture**, Garsington, v. 81, p. 47-53, 2000.

CASTILLO-GONZÁLEZ, A. R. et al. Rumen microorganisms and fermentation. **Archives of Medicine Veterinary**, Curitiba, v. 46, p. 349-361, 2014.

CENTRO DE ESTUDOS AVANÇADOS EM ECONOMIA APLICADA DA ESCOLA SUPERIOR DE AGRICULTURA “LUIZ DE QUEIROZ” - CEPEA/ESALQ. **PIB do agronegócio brasileiro**. 2018. Acesso em: <https://www.cepea.esalq.usp.br/br/pib-do-agronegocio-brasileiro.aspx>. Disponível em: 05 jul. 2018.

CHERNEY, D. J. R.; SICILIANO-JONES, J.; PELL, A. N. Forage in vitro dry matter digestibility as influenced by fiber source in the donor cow diet. **Journal of Animal Sciences**, Cary, v. 71, p. 1335-1338, 1993.

CLIFFORD, M. N.; RAMIREZ-MARTINEZ, J. R. Chlorogenic acids and purine alkaloids contents on maté (Ilex paraguaiensis) leaf and beverage. **Food Chemistry**, Amsterdam, v. 35, p. 13-21, 1990.

COELHO, G. C. et al. Quantification of saponins in extractive solution of mate leaves (Ilex paraguariensis A. St. Hil.). **Journal of Medicinal Food**, New Rochelle, v. 13, n. 2, p. 439-443, 2010.

COLBERT, E. H. The osteology and relationships of Archaeomeryx, an ancestral ruminant. **American Museum Novitates**, New York, n. 1135, p. 1-24, 1941.

COTTA, M. A.; HESPELL, R. B. Proteolytic activity of the ruminal bacterium Butyrivibrio fibrisolvens. **Applied Environmental Microbiology**, Washington, v. 52, p. 51-58, 1986.

COUNOTTE, G.; PRINS, R. Regulation of lactate metabolism in the rumen. **Veterinary Research Communications**, Dordrecht, v. 5, p. 101-115, 1981.

DABÉS, A. C. Propriedades da carne fresca. **Revista Nacional da Carne**, São Paulo, v. 25, p. 32-40, 2001.

DE MEJÍA, E. G. et al. Yerba mate tea (Ilex paraguariensis): Phenolics, antioxidant capacity and in vitro inhibition of colon cancer cell proliferation. **Journal of Functional Foods**, Amsterdam, v. 2, n. 1, p. 23-34, 2010.

DE SOUZA, L. M. et al. Comprehensive analysis of mate (Ilex paraguariensis) compounds: development of chemical strategies for matesaponin analysis by mass spectrometry. **Journal of Chromatography A**, Amsterdam, v. 1218, n. 41, p. 7307-7315, 2011.

DEHORITY, B. A. Pectin-fermenting bacteria isolated from the bovine rumen. **Journal of Bacteriology**, Washington, v. 99, p. 189-196, 1969.

DEVENDRA, C. **Non-conventional feed resources in Asia and the Pacific**. 2nd ed. Bangkok: Food and Agriculture Organization of the United Nations, 1985.

DIPLOCK, A. T. et al. Functional food science and defence against reactive oxidative species. **The British Journal of Nutrition**, Cambridge, v. 80, p. 77-112, 1998.

DRANSFIELD, E. Optimisation of tenderisation, ageing and tenderness. **Meat Science**, Amsterdam, v. 36, p. 105-121, 1994.

DUSKOVA, D.; MAROUNEK, M. Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rumen bacterium Lachnospira multiparus. **Letters in Applied Microbiology**, Chichester, v. 33, p. 159-163, 2001.

EUROPEAN UNION - E.U. Regulations (EC) Nº 1831/2003 of the European Parliament and of the Council: on additives for use in animal nutrition. **Official Journal of the Europe Union**, Brussels, 22 Sept. 2003.

FAUSTMAN, C.; CASSENS, R. G. The biochemical basis for discoloration in fresh meat: a review. **Journal of Muscle Foods**, Chichester, v. 1, n. 3, p. 217-243, 1990.

FAUSTMAN, C.; PHILLIPS, A. Measurement of discolouration in freshmeat. **Current Protocols in Food Analytical Chemistry**, Hoboken, p. F3.3.1-F3.3.13, 2001.

FEIJÒ, G. L. D. **Qualidade da carne bovina**. Campo Grande: Embrapa Gado de Corte, 1999. (Curso conhecendo a carne que você consome, 1).

FERNANDES DE SÁ, E. M. A influência da água nas propriedades da carne. **Revista Nacional da Carne**, São Paulo, n. 325, p. 51-54, 2004.

FERRY, J. G. Fundamentals of methanogenic pathways that are key to the biomethanation of complex biomass. **Current Opinion in Biotechnology**, Kidlington, v. 22, n. 3, p. 351-357, 2011.

FRANKEL, E. N. Lipid oxidation: mechanisms, products and biological significance. **Journal of the American Oil Chemists' Society**, Garsington, v. 61, n. 12, p. 1908-1917, 1984.

FULTON, R. M.; NERSESSIAN, B. N.; REED, W. M. Prevention of Salmonella enteritidis infection in commercial ducklings by oral chicken egg-derived antibody alone or in combination with probiotics. **Poultry Science**, Cary, v. 81, p. 34-40, 2002.

GABLER, M. T.; TOZER, P. R.; HEINRICHS, J. A. Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer. **Journal of Dairy Science**, New York, v. 83, n. 5, p. 1104-1109, 2000.

GENTRY, A.W. The ruminant radiation. In: VBRA, E.; SCHALLER, G. B. (Ed.). **Antelopes, deer, and relatives**: fossil record, behavioral ecology, systematics, and conservation. New Haven, CT: Yale Univ. Press, 2000. p. 11-25.

GNOATTO, S. C. B.; SCHENKEL, E. P.; BASSANI, V. L. HPLC method to assay total saponins in Ilex paraguariensis aqueous extrac. **Journal of the Brazilian Chemical Society**, São Paulo, v. 16, n. 4, p. 723-725, 2005.

GOSMANN, G. et al. Phenolic compounds from Maté (Ilex paraguariensis) inhibit adipogenesis in 3T3-L1 preadipocytes. **Plant Foods for Human Nutrition**, New York, v. 67, n. 2, p. 156-161, 2012.

GRAY, J. I.; GOMAA, E. A.; BUCKLEY, D. J. Oxidative quality and shelf life ofmeats. **Meat Science**, Amsterdam, v. 43, p. 111-123, 1996.

GRESSLEY, T. F.; HALL, M. B.; ARMENTANO, L. E. Ruminant nutrition symposium: Productivity, digestion, and health responses to hindgut acidosis in ruminants. **Journal of Animal Sciences**, Cary, v. 89, p. 1120-1130, 2011.

HACKMANN, T. J.; SPAIN, J. N. Invited review: ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. **Journal of Dairy Science**, New York, v. 93, p. 1320-1334, 2010.

HALLIWELL, B.; CHIRICO, S. Lipid peroxidation: its mechanism, measurement, and significance. **The American Journal of Clinical Nutrition**, Bethesda, v. 57, suppl. 5, p. 715S-724S, 1993.

HARTEMINK, E. et al. The effect of Yerba Mate (Ilex Paraguariensis) suplementation nutriente degradability in dairy caws: Na *In sacco* and *In vitro* study. **Asian-Australasian Journal Animal Science**, Seoul, v. 28, n. 11, p. 1606-1613, 2015.

HASHEMI, S. R.; DAVOODI, H. Herbal plants and their derivatives as growth and health promoters in animal nutrition. **Veterinary Research Communications**, Dordrecht, v. 35, p. 169-180, 2011.

HECK, C. I.; DE MEJIA, E. G. Yerba Mate Tea (Ilex paraguariensis): a comprehensive review on chemistry, health implications, and technological considerations. **Journal of Food Science**, Hoboken, v. 72, p. 138-151, 2007.

HERNÁNDEZ FERNÁNDEZ, M. H.; VRBA, E. S. A complete estimate of the phylogenetic relationships in Ruminantia: A dated species-level supertree of the extant ruminants. **Biological Reviews of the Cambridge Philosophical Society**, Chichester, v. 80, p. 269-302, 2005.

HUNGATE, R. E. et al. Formate as an intermediate in the bovine rumen fermentation. **Journal of Bacteriology**, Washington, v. 102, p. 389-397, 1970.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE. **Produção da pecuária municipal – PPM de 2016**. Rio de Janeiro: IBGE, 2017.

INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE - IPCC. Emissions from livestock and manure management. In: EGGLESTON, H. S. et al. **IPCC Guidelines for national greenhouse gas inventories**. Hayama: IGES, 2006. Chap. 10, p. 747-846.

JACOBS, B. F.; KINGSTON, J. D.; JACOBS, L. J. The origin of grass-dominated ecosystems. **Annals of the Missouri Botanical Garden**, St. Louis, v. 86, p. 590-643, 1999.

JANIS, C. M. Evolution of horns in ungulates: Ecology and paleoecology. **Biological Reviews of the Cambridge Philosophical Society**, 57, pp. 261-318, 1982.

JANIS, C. M. The evolutionary strategy of the Equidae and the origins of rumen and cecal digestion. **Evolution**, Garsington, v. 30, p. 757-774, 1976.

JANIS, C. M.; MANNING, E. Antilocapridae. In JANIS, C. M.; SCOTT, K. M.; JACOBS, L. L. (Ed.). **Evolution of tertiary mammals of North America**. Cambridge, UK: Cambridge Univ. Press, 1998. v. 1. p. 491-507.

JANSSEN, P. H.; KIRS, M. Structure of the archaeal community of the rumen. **Applied Environmental Microbiology**, Washington, v. 74, p. 3619-3625, 2008.

JENKINS, T. C. et al. Board-invited review: recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. **Journal of Animal Science**, Cary, v. 86, p. 397-412, 2008.

JERMANN, T. M. et al. Reconstructing the evolutionary history of the artiodactyl ribonuclease superfamily. **Nature**, London, v. 374, p. 57-59, 1995.

JOBIM, C. C. et al. Cinética de degradação ruminal dos fenos de alfafa e Tifton-85 e da silagem de milho. **Ciências Agrárias**, Londrina, v. 32, n. 2, p. 747-758, 2011.

KOZLOSKI, G. V. **Bioquímica dos ruminantes**. Santa Maria: UFSM, 2002. 140 p.

KRIZSAN, S. J. et al. Comparison of some aspects of the in situ and in vitro methods in evaluation of neutral detergent fiber digestion. **Journal of Animal Science**, Cary, v. 91, p. 838-847, 2013.

LEAHY, S. C. et al. The complete genome sequence of Methanobrevibacter sp. AbM4. **Standards in Genomic Sciences**, London, v. 8, p. 215-227, 2013.

LEAHY, S. C. et al. The genome sequence of the rumen methanogen Methanobrevibacter ruminantium reveals new possibilities for controlling ruminant methane emissions. **PLoS One**, San Francisco, v. 5, n. 1, art. e8926, 2010.

LEE, M. R. F. et al. A comparison between red clover and grass silage feeding on fatty acid composition, meat stability and sensory quality of the M. longissimus muscle of dairy cull cows. **Meat Science**, Amsterdam, v. 81, p. 738-744, 2009.

LI, Y.; LIU, S. Reducing lipid peroxidation for improving colour stability of beef and lamb: on farm considerations. **Journal of the Science Food and Agriculture**, Garsington, v. 92, p. 719-726, 2012.

LIU, Y.; WHITMAN, W. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. **Annals of the New York Academy of Science**, New York, v. 1125, p. 171-189, 2008.

MACKIE, R. I.; HEATH, S. Enumeration and isolation of lactate-utilizing bacteria from the rumen of sheep. **Applied Environmental Microbiology**, Washington, v. 38, p. 416-421, 1979.

MAGNO, L. L. **Fatores de influência na qualidade de carne ovina**. 2014. Trabalho de Conclusão de Curso (Graduação) - Universidade Federal de Goiás, Goiânia, 2014. Disponível em: <https://evz.ufg.br/up/66/o/9_FATORES_DE_INFLU%C3%8ANCIA_NA_QUALIDADE_DE_CARNE_OVINA.pdf>. Acesso em: 25 set. 2017.

MAKKAR, H. P. S.; FRANCIS, G.; BECKER, K. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential application in livestock and aquaculture production systems. **Animal**, Cambridge, v. 1, n. 9, p. 1371-1391, 2007.

MALAFAIA, P. A. M. et al. Cinética ruminal de alguns alimentos investigada por técnicas gravimétricas e metabólicas. **Revista Brasileira de Zootecnia**, Viçosa, v. 27, n. 2, p. 370-380, 1998.

MANCINI, R. A.; HUNT, M. C. Current research in meat color. **Meat Science**, Amsterdam, v. 71, p. 100-121, 2005.

MARINUCCI, M. T.; DEHORITY, B. A.; LOERCH, S. C. In vitro and in vivo studies of factors affecting digestion of feeds in synthetic fiber bags. **Journal of Animal Science**, Cary, v. 70, p. 296-307, 1992.

MAURICIO, R. M. et al. Semi automated of in vitro gas production technique using a pressure transducer. In: ANNUAL MEETING BRITISH SOCIETY OF ANIMAL SCIENCE, 1998, Scarboroug. **Proceedings…** Scarborough: BSAS, 1998. p.70.

MAURICIO, R. M. et al. Semi-automated in vitro gas production technique for ruminant feedstuff evaluation. **Animal Feed Science Technology**, Amsterdam, v. 79, p. 321-330, 1999.

MCALLISTER, T. A. et al. Effect of ruminal microbial colonization on cereal grain digestion. **Canadian Journal of Animal Science**, Ottawa, v. 70, p. 571-579, 1990.

MEDEIROS, S. R. **Valor nutricional da carne bovina e suas implicações para a saúde humana**. Campo Grande, MS: Embrapa Gado de Corte, 2008.

MENEZES, T. J. **Levantamento de dados sobre as práticas de melhoramento genético animal aplicadas na ovinocultura de corte**. 2017. Dissertação (Mestrado) - Faculdade de Ciências Agrárias e Tecnológicas, Universidade Estadual Paulista "Júlio de Mesquita Filho", Dracena, 2017.

MÉTAIS, G. et al. New remains of primitive ruminants from Thailand: evidence of the early evolution of the Ruminantia in Asia. **Zoologica Scripta**, Chichester, v. 30, p. 231-248, 2001.

MÉTAIS, G.; VISLOBOKOVA, I. Basal ruminants. In: PROTHERO, D. R.; FOSS, S. E. (Ed.). **The Evolution of the artiodactyls**. Baltimore, MD: The John Hopkins Univ. Press, 2007. p. 189-212, 2007.

MONTEIRO, R. B. N. C. **Desenvolvimento de um modelo para estimativas da produção de gases de efeito estufa em diferentes sistemas de produção de bovinos de corte**. 2009. 75 f. Dissertação (Mestrado) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2009.

MOSIMANN, A. L. P.; WILHELM FILHO, D.; SILVA, E. L. Aqueous extract of Ilex paraguariensis (mate) attenuates the progression of atherosclerosis in cholesterol-fed rabbits. **BioFactors**, Hoboken, v. 26, p. 59-70, 2006.

NACZK, M.; SHANIDI, F. Extraction and analysis of phenolics in food. **Journal of Chromatography A,** Amsterdam, v. 1054, n. 1-2, p. 95-111, 2009.

NAGAJARA, T. G. Microbiology of the rumen. In: MILLEN, D.; ARRIGONI, M. B.; PACHECO, R. D. L. **Rumenology**. Switzerland: Springer International Publishing, p. 63-101, 2016.

NEUMANN, L.; WEIGAND, E.; MOST, E. Effect of methanol on methanogenesis and fermentation in the rumen simulation technique (RUSITEC). **Journal of Animal Physiology and Animal Nutrition,** Berlin, v. 82, p. 142-149, 1999.

NIEVES, D. et al. Faecal digestibility of diets with tropical forages in rabbits. Comparison between direct and indirect methods. **Bioagro,** Cabudare, v. 20, p. 67-72, 2008.

OPPERMANN, R.; NELSON, W. O.; BROWN, R. E. In vivo studies of methanogenesis in bovine rumen: dissimilation of acetate. **Journal of General Microbiology,** London, v. 25, p. 103-111, 1961.

OSÓRIO, M. T. M.; OSÓRIO, J. C. S. Condições de abate e qualidade de carne. In: MONTEIRO, E. M. (Coord.). **Qualidade de carne e dos produtos cárneos**: curso. Bagé: Embrapa Pecuária Sul, 2000. v. 4, cap. 7, p. 77-128.

OWENS, F.N. et al. Acidosis in cattle: a review. **Journal of Animal Sciences**, v. 76, p. 275-286, 1998.

OZUTSUMI, Y. et al. The effect of protozoa on the composition of rumen bacteria in cattle using 16S rRNA gene clone libraries. **Bioscience, Biotechnology, and Biochemistry**, Abingdon, v. 69, p. 499-506, 2005.

PAGLIOSA, C. M. et al. Methylxanthines, phenolic composition, and antioxidant activity of bark from residues from mate tree harvesting (Ilex paraguariensis A. St. Hil.). **Food Chemistry**, Amsterdam, v. 122, p. 173-178, 2010.

PITTA, D. W. et al. Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. **Microbial Ecology,** New York, v. 59, p. 511-522, 2010.

PO, E.; XU, Z.; CELI, P. The effect of Yerba Mate (Ilex paraguarensis) supplementation on the productive performance of Dorper Ewes and their progeny. **Asian-Australasian Journal Animal Science**, Seoul, v. 25, n. 7, p. 945-949, 2012.

QIAO, J.; TAN, Z.; WANG, M. Potencial and existing mechanisms of enteric methane production in ruminants. **Scientia Agricola**, Piracicaba, v. 71, n. 5, p. 430-440, 2014.

RAMIN, M. et al. Short communication: measurements of methane emissions from feed samples in filter bags or dispersed in the medium in an in vitro gas production system. **Journal of Dairy Science,** New York, v. 96, p. 4643-4646, 2013.

REA, S. et al. Methanobrevibacter millerae sp nov and Methanobrevibacter olleyae sp nov., methanogens from the ovine and bovine rumen that can utilize formate for growth. **International Journal of Systematic and Evolutionary Microbiology,** London, v. 57, p. 450-456, 2007.

RICHARD, M. P. Lipid chemistry and biochemistry. In: HUI, Y. H. (Ed.). **Handbook of food science, technology, and engineering**. Boca raton: Taylor & Francis Group, 2006. v. 1. p. 21.

ROÇA, R. O. **Modificações Post-mortem**. 2009. Disponível em: <http://www.fca.unesp.br/Home/Instituicao/Departamentos/Gestaoetecnologia/Teses/Roca105>. Acesso em: 25 set. 2017.

RUSSEL, J. B. **Rumen microbiology and its role in ruminant nutrition.** Ithaca, NY: Cornell University, 2002.

RUSSELL, J. B.; SHARP, W. M.; BALDWIN, R. L. The effect of pH on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. **Journal of Animal Science**, Cary, v. 48, p. 251-255, 1979.

RUSSELL, J. B.; MUCK, R. E.; WEIMER, P. J. Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. **FEMS Microbiology Ecology,** Oxford, v. 67, p. 183-197, 2009.

RUSSELL, J. B.; HINO, T. Regulation of lactate production in Streptococcus bovis: A spiraling effect that contributes to rumen acidosis. **Journal of Dairy Sciences**, v. 68, p. 1712-1721, 1985.

SAFWAT, A. M. et al. Estimating apparent nutriente digestibility of diets containing Leucaena leucocephala or Moringa oleífera leaf meals for growing rabbits by two methods. **Asian-Australian Journal of Animal Science**, Seoul, v. 28, n. 8, p. 1155-1162, 2015.

SALDANA, M. D. A. et al. Extraction of purine alkaloids from maté (Ilex paraguariensis) using supercritical CO2. **Journal of Agricultural and Food Chemistry**, Washington, v. 47, p. 3804-3808, 1999.

SALMAN, A. K. D. et al. **Metodologias para avaliação de alimentos para ruminantes domésticos**. Porto Velho, RO: Embrapa Rondônia, 2010.

SAUERBECK, D. R. CO2 emissions and C sequestration by agriculture – perspective and limitations. **Nutrient Cycling in Agroecosystems**, Dordrecht, v. 60, p. 253-266, 2001.

SCHAFER, F. Q.; KELLEY, E. E.; BUETTNER, G. Oxidative stress and antioxidante intervention. In: RICHARD, G.; RODRIGUEZ, H. (Ed.). **Critical reviews of oxidative stress and aging**: advances in basic science, diagnostics and intervention. New Jersey: World Scientific, 2003. v. 2, p. 849-869.

SEMPREBON, G.; JANIS, C. M.; SOLOUNIAS, N. The diets of the Dromomerycidae (Mammalia: Artiodactyla) and their response to Miocene vegetational change. **Journal of Vertebrate Paleontology**, New York, v. 24, p. 427-444, 2004.

SEMPREBON, G. M.; RIVALS, F. Was grass more prevalent in the pronghorn past? An assessment of the dietary adaptations of Miocene to recent Antilocapridae (Mammalia: Artiodactyla). **Palaeogeography, Palaeoclimatology, Palaeoecology - Journals**, Amsterdam, v. 253, p. 332-347, 2007.

SHINGFIELD, K. J.; BONNET, M.; SCOLLAN, N. D. Recent developments in altering the fatty acid composition of ruminant-derived foods. **Animal,** Cambridge, v. 7, suppl. 1, p. 132-162, 2013.

SILVA SOBRINHO, A. G. Aspectos quantitativos e qualitativos da produção de carne ovina. In: A PRODUÇÃO ANIMAL NA VISÃO DOS BRASILEIROS, 2001, Piracicaba. **Anais...** Piracicaba: FEALQ, 2001. p. 425-446.

SILVA SOBRINHO, A. G. et al. Características de qualidade da carne de ovinos de diferentes genótipos e idades ao abate. **Revista Brasileira de Zootecnia**, Viçosa, v. 34, n. 3, p. 1070-1078, 2005.

SINHA, R. et al. Meat intake and mortality: a rospective study of over half a million people. **Archives of Internal Medicine**, Chicago, v. 169, p. 562-71, 2009.

STETZER, A. J. et al. Effect of enhancement of ageing on flavor and volatile compounds in various beef muscles. **Meat Science**, Amsterdam, v. 79, p. 13-19, 2008.

THEODOROU, M. K. et al. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. **Animal Feed Science and Technology**, Amsterdam, v. 48, p. 185-197, 1994.

TORAL, P. G. et al. Review: modulating ruminal lipid metabolism to improve the fat acid composition of meat and milk. Challenges and opportunities. **Animal**, Cambridge, v. 12, p. 272-281, 2018.

U.S.FDA. **New animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food producing animals:** recommendations for drug sponsors for voluntarily aligning product use conditions with GFI #209. 2013.

VALENTE, T. N. P.; DETMANN, E.; SAMPAIO, C. B. Review: recent advances in evaluation of bags made from different textiles used in situ ruminal degradation. **Canadian Journal of Animal Science**, Ottawa, v. 95, p. 493-498, 2015.

VAN SOEST, P. J. **Nutritional ecology of the ruminant**. 2nd ed. Ithaca: Cornell University Press, 1994. 476 p.

VANZANT, E. S.; COCHRAN, R. C.; TITGEMEYER, E. C. Standardization of in situ techniques for ruminant feedstuff evaluation. **Journal of Animal Science**, Cary, v. 76, p. 2717-2729, 1998.

VAREL, V. H.; KREIKEMEIER, K. K. Comparison of in vitro and in situ digestibility methods. **Journal of Animal Science**, Cary, v. 73 p. 578-582, 1995.

VENKATARAMAN, S.; SCHAFER, R. Q.; BUETTNER, G. R. Detection of lipidradicals using EPR. **Antioxid & Redox Signaling,** New Rochelle, v. 6, p. 631-638, 2004.

WANAPAT, M. et al. Effects of plants containing secondary compounds and plant oils on rumen fermentation and ecology. **Tropical Animal Health Production,** Dordrecht, p. 399-405, 2012.

WEBB, S. D. et al. **Evolution of tertiary mammals of North America:** terrestrial carnivores, ungulates, and ungulate-like mammals. Cambridge, UK: Cambridge Univ. Press, 1998. v. 1, p. 463-476.

WEBB, S. D.; TAYLOR, B. E. The phylogeny of hornless ruminants and a description of the cranium of Archaeomeryx. **Bulletin of the American Museum of Natural History**, New York, v. 167, p. 120-157, 1980.

XIMENES, L. J. F.; CUNHA, A. M. da. Setor de Peles e de couros de caprinos e de ovinos no nordeste. **Banco do Nordeste**, ano 6, n. 1, p. 22, mar. 2012. Disponível em: <https://www3.eco.unicamp.br/neit/images/stories/arquivos/artigos/informe-rural_pelos-e-couros_ano6_n1.pdf>. Acesso em: 25 out. 2017.

ZAWADZKI, A. et al. Mate extract as feed additive for improvement of beef quality. **Food Research International**, Oxford, v. 99, p. 336-347, 2017.

ZEOLA, N. M. B. L. et al. Influência de diferentes níveis de concentrado sobre a qualidade da carne de cordeiros Morada Nova. **Revista Portuguesa de Ciências Veterinárias**, Lisboa, v. 97, n. 544, p. 175-180, 2002.

ZHONG, R. Z. et al. Effects of dietary supplementation with green tea polyphenols on digestion and meat quality in lambs infected with *Haemonchus contortus*. **Meat Science**, Amsterdam, v. 105, p. 1-7, 2015.

ZHOU, B. et al. Evaluation of chemical composition, in situ degradability and in vitro gas production of ensiled and sun-dried mulberry pomace. **Journal of Animal and Feed Sciences,** Jablonna, v. 21, p. 188-197, 2012.

CAPÍTULO 2 – Dietary inclusion of yerba-mate extract in growing lamb nutrition: effects on blood parameters, animal performance, and carcass metrics[1]

RESUMO

Atualmente, pesquisas para avaliar os efeitos de aditivos naturais na nutrição animal é uma área bastante promissora. Neste contexto, o presente estudo foi desenvolvido com o intuito de avaliar uma planta nativa sul-americana e amplamente utilizada na nutrição humana, erva-mate (*Ilex paraguariensis* St. Hilaire), como aditivo alimentar como melhorador de desempenho. Trinta e seis cordeiros machos não castrados foram alimentados com as dietas experimentais que eram compostas de uma dieta controle e outras três dietas contendo níveis crescentes de extrato de erva-mate (1, 2 e 4% de inclusão) durante um período experimental de 53 dias. No início do período, todos os animais foram vermifugados e pesados, o procedimento de pesagem foi realizado regularmente a cada 14 dias. Coleta de sangue foi realizada no início e final do período experimental, e analises de contagem celular e bioquímico foram realizadas. Ingestão de matéria seca foi mensurado diariamente, e ao final do período experimental, os parâmetros de performance puderam ser calculados. Ao final dos 53 dias, os animais foram abatidos e mensurações de carcaça foram tomadas. O experimento foi desenvolvido seguindo as premissas do delineamento em blocos casualizados. Resultados mostram que o extrato de erva-mate não alterou de forma geral o status de saúde dos animais, no entanto, inclusões de até 2% de extrato podem estimular o sistema imune dos animais, pelo incremento de células brancas do sangue e globulinas. Também, inclusões de até 2% de extrato aumentaram levemente a ingestão de matéria seca e os parâmetros de produtividade animal, no entanto, altas doses de inclusão apresentam severas reduções

---

[1] Chapter submitted to Animal Feed Science and Technology.

nos parâmetros de produção animal. Espessura de gordura subcutânea teve uma redução à medida que as doses de extrato foram aumentadas, e a literatura mostra que a erva-mate possui atividade que modula a diferenciação de preadipocitos e redução do acumulo de lipídios nas células. Concluímos então, que moderadas doses de extrato de erva-mate são benéficos à saúde e produtividade de cordeiros em engorda.

Palavras-chave: aditivo natural, extrato de planta, medida corporal, ruminantes, sistema imune.

ABSTRACT

Use of natural feed additives is currently one of the most important research area on ruminant nutrition. In this context, this study aimed to evaluate a plant widely used on human nutrition on South American countries, yerba-mate (*Ilex paraguariensis* St. Hilaire), as a feed additive to improve animal production. Thirty-six male uncastrated growing lambs were used fed experimental diets were composed by a control and 3 other treatments with increased levels of yerba-mate extract (1, 2, and 4% of inclusion) during an experimental period of 53 days. At the beginning, all animals were dewormed and weighted, the weighting procedure were repeated every 14 days. A blood sampling was carried out at the beginning and at the end of the experimental period, cell count and biochemistry analysis were carried out. Daily dry matter intake was measured and, at the end, animal performance parameters could be calculated. After 53 days, all animals were slaughtered and carcass yield and metrics were measured. The experiment was carried out according to the randomized block design statement. Results show that yerba-mate extract does not change general health status of the animals, however, inclusions up to 2% of the extract could stimulate immune system of the animals, by increasing some white blood cells and globulins. Also, inclusions up to 2% of yerba-mate extract could slightly increase feed intake and animal production parameters, however, higher levels of

inclusions showed a severe reduction on animal productive parameters. Fat thickness have a linear decrease by the treatment, for yerba-mate have some biological particles that modulate the differentiation of preadipocytes and reduce accumulation of lipids into those cells. In summary, moderates levels of yerba-mate extract is beneficial to the health and productive parameters of the animals.

Key-words: body metrics; immune system; natural additive; plant extract; ruminants.

INTRODUCTION

The nutrition feedstuff additives are products used in animal nutrition to generate better performance of the animals, modulate microbiome or meat and milk composition on ruminant nutrition. The most used products on ruminant nutrition are additives with some antimicrobial activity (HASHEMI; DAVOODI, 2011). However, Europe Union (EU, 2003) and United States (U.S.FDA, 2013) prohibited some chemicals used for this purpose, and several researchers have been spend a lot of effort to find other components with similar activity, like probiotics, prebiotics, enzymes, organic acids, organic minerals, plant extracts, essential oils, and other products (FULTON; NERSESSIAN; REED, 2002).

A common plant used in human nutrition in South-American countries is the yerba-mate (Ilex paraguariensis St. Hilaire). Its bioactive compounds is composed by phenolic compounds (BERTÉ et al., 2011; DE MEJÍA et al., 2010; BASTOS et al., 2007), alkaloids (CLIFFORD; RAMIREZ-MARTINEZ, 1990; SALDANA et al., 1999) and saponin (GOSMANN et al., 2012; SCHENKEL et al., 1997; COELHO et al., 2010; GNOATTO; SCHENKEL; BASSANI, 2005). Some knowledge on the function of those chemicals have been well described in human nutrition, for instance as antioxidant, antimicrobial, anti-obesity, anti-inflammatory, anti-diabetic and used as well like a cardiovascular disease preventive (HECK; DE MEJIA, 2007). In the other hand, a very

small knowledge concerning the use of the yerba-mate in animal nutrition is available and its effects on animal performance and physiological health.

Usually, some body biometrics of the animals have a high correlationship with productivity (CAM; OLFAZ; SOYDAN, 2010), in this regard, measure animal performance by weighting, taking measurements and measure dry matter intake, will help farmers to evaluate the alimentary efficiency and conversion (MACHILA et al., 2008), and maybe decide using or not some type of additive. Several studies have showed important results involving these kind of metrics as the evaluation of animal productivity (SILVA et al., 2019; LIMA et al., 2018; BOYD et al., 2015; CAM; OLFAZ; SOYDAN, 2010).

In additional, health of the animal is one of the most important feature that influences productivity parameters. A very high correlation between health status and nutritional balance was well described in Goff (2006), an important number of studies trying to figure out nutritional strategies to prevent certain disorders. On this context, when a new substance or product is tested, it is one of the main objective of the researchers understand how this product will affect the animal production and consequently health status.

The hypothesis of the project were that increased levels of yerba-mate extract could increase performance and change positively the health status of lambs. The objective were evaluate the performance parameters, body and carcass yield, and check the health status by blood count and biochemistry analysis of the lambs fed with increased levels of yerba-mate extract.

MATERIAL AND METHODS

All procedures using animal were approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Food Engineering – University of São Paulo (Protocol number CEUA 3497040618).

Location, animals and experimental design

The animals were born and raised until weaning in a commercial farm at Uberaba, Minas Gerais, Brazil, they had access to pasture and feed supplementation on a creep-feeding system. After weaning, they were transported to the College of Animal Science and Food Engineering (FZEA), University of São Paulo (USP), Pirassununga, São Paulo, Brazil, where the experiment were carried out.

Thirty six male lambs, uncastrated (crossbreed Texel x Dorper x Santa Inês) weighting 23.77±3.70 kg were used on this trial. At the first day on the experimental building, all animals were deworming, using a commercial drug with ivermectin as active principle, following the dose recommendations from the package insert. The animals were weighted and assigned into nine groups, each group had four animals with similar weight and those animals were randomly assigned to one of experimental treatments, according to a randomized block design statement. Each animal was placed into an individual tie stall with individual water and feeder access.

Diet and experimental treatment

The animals were fed twice a day at 8h00 and 16h00, diets were calculated following the nutritional requirements for finishing lambs, according to NRC (2007), table 2.1. shows the ingredient and composition of the diet. Before morning feed, the feeder was cleaned up and refusals from the day before were weighted to adjust the diet at least 10% of daily residue. Around 10% of refusals were sampled and stored in an

identified plastic bag on a -20°C freezer, one composed refusal sample was generated by week and dry matter analysis was carried out in order to calculate the daily dry matter intake (DMI).

Table 2.1. Ingredients and composition of the diets, based on percentage of dry matter (% DM)

	Diet1	Diet2	Diet3	Diet4
	---------------- %DM ----------------			
Ingredients				
Corn silage	40.00	40.00	40.00	40.00
Corn meal	33.00	33.00	33.00	33.00
Soybean meal	20.50	20.50	20.50	20.50
Salt	0.15	0.15	0.15	0.15
Bicalcic fosfate	0.15	0.15	0.15	0.15
Mineral*	2.20	2.20	2.20	2.20
Yerba mate extract	0.00	1.00	2.00	4.00
Kaolin	4.00	3.00	2.00	0.00
Diets composition				
Dry matter (%)	68.89	68.83	68.77	68.64
Organic matter (%)	89.56	90.43	91.30	93.04
Crude protein (%)	21.46	21.56	21.66	21.87
Ether extract (%)	2.09	2.09	2.09	2.09
Non-fiber carbohydrate (%)	51.90	52.69	53.49	55.07
Crude fiber (%)	13.87	13.88	13.88	13.88
ADF (%)	18.19	18.20	18.20	18.22
NDF (%)	34.53	34.54	34.54	34.56
Lignin (%)	4.48	4.48	4.48	4.48
Total digestible nutrient (%)	67.35	68.11	68.87	70.39
Gross energy (kcal/kg)	4,188	4,229	4,271	4,354

*Mineral composition per kilogram: calcium (maximum) – 218g; calcium (minimum) – 190g; cobalt (min) – 148mg; copper (min) – 2,664mg; sulfur (min) – 64g; fluoride (max) – 1,600mg; phosphorus (min) – 160g; iodine (min) – 141mg; manganese (min) – 2,220mg; selenium (min) – 37mg; zinc (min) – 7,992mg.

Source: own authorship

Diet ingredients were analysed by AOAC (1990) methods, dry matter (DM, method 934.01), ash (ASH, method 923.03), ether extract (EE, method 920.85), and crude fiber (CF, method 978.10) were measured, crude protein (CP, method 920.87) were analysed by Kjeldahl method. Neutral detergent fiber (NDF) were analysed according to Mertens (2002) using the amylase enzyme method, acid detergent fiber (ADF) and lignin

(LN) were measured according to method 973.18), were measured by Van Soest, Robertson, and Lewis (1991) method. Gross energy (GE) was measured by the calorimetric pump (C200 System, IKA, Staufen, Germany). Organic matter (OM) was calculated by subtracting the ASH from 100, and non-fiber carbohydrates (NFC) was calculated subtracting EE, CP, ASH and CF from 100. Total digestible nutrient (TDN) were calculated by the equation described by Kearl (1982).

Experimental treatments were one control diet (0% of yerba-mate extract) and three diets with increasing inclusion levels of yerba-mate extract (1, 2 and 4%). The extract was produced by Centro Flora, Botucatu, São Paulo, Brazil, from fresh leaves of Ilex paraguariensis by water:ethanol 75:25 v/v extraction at 90°C; the total phenolic concentration was 21.7 eq-g gallic acid/100 g extract and 6% of caffeine and characterization of this same extract was well described on Zawadzki et al. (2017). During the three first days on the experiment, all thirty-six animals were fed the control diet. After this period, the animals started receive their respective experimental diets.

Blood collection and analysis

Sample collections were carried out on the 3° and 49° day of the period, the first sampling was collected when all animals were fed control diet to represent the initial measurements and the second collection was done at the end of the period, to measure the changes on the blood count and biochemistry parameters by the dietary additive. Blood was collected by the jugular vein 2h after the morning feed, using a Vacuntainer® collection tube. In total, three tubes were collected from each animal, first tube with sodium fluoride for the counting cells analysis, second one with EDTA for the blood glucose analysis and the last one without anticoagulant for the biochemistry analysis.

The counting analysis was carried out immediately after the collection by the automatized counter BC-2800 Vet Mindray®. Obtaining the hematocrit (HT, %) and the

red blood cell distribution width (RDW, %), as well counting the amount of hemoglobin (HB, g/dl) and the number of blood platelets (PT, 103/µl), red blood cells (RBC, 106/µl), total and different types of leukocytes (103/µl), as neutrophils, lymphocytes, monocytes, eosinophils and basophils. After that, the mean corpuscular volume (MCV) were calculated as (HT/RBC)*10; mean corpuscular hemoglobin (MCH) were calculated as (HB/RBC)*10; mean corpuscular hemoglobin concentration (MCHC) were calculated as (HB/HT)*10.

Biochemistry analysis tubes were processed immediately after the collection of the blood, by centrifugation with 2,000 g force for 15 min, and the plasma was stored in an identified 2 ml tube in a freezer (-20°C). Commercial kits manufactured by RX Daytona – Randox®, were used: glucose (GLU) (code: GL 3815), triglycerides (TG) (code: TR 3823), total cholesterol (CHOL) (code: CH 3810), high density lipoprotein (HDL-C) (code: CH 3811), blood urea nitrogen (BUN) (code: UR 3825), creatinine (CRE) (code: CR 3814), albumin (ALB) (code: AB 3800), total protein (TP) (code: TP 4001), creatinine kinase (CK) (code: CK 3812), gamma-glutamyl transferase (GGT) (code: GT 3817), and aspartate aminotransferase (AST) (code: AS 3804). Low-density lipoprotein (LDL-C) were calculated as (CHOL)-(HDL-C)-(TG/5); very-low-density lipoprotein (VLDL-C) were calculated as (TG/5), globulin (GLB) were calculated as (TP-ALB); ALB:GLB ratio were calculated as (ALB/GLB); BUN:CRE ratio were calculated as (BUN/CRE); and atherogenic index (AI) were calculated as [(CHOL-(HDL-C))/(HDL-C)].

Performance control and in vivo biometrics

On the day after the arrival of the animals on the experimental building, all thirty six lambs were weighted and the weighting procedure was repeated every 14 days, using

an electronic scale. After control dry matter intake (DMI) and weight gain (WG), food conversion (FC) and efficiency (FE) could be calculated.

On the last weighting procedure of the period, called final weight (FW), at the day before slaughter, body dimension of the animals were taken. Body condition scored (BCS) was measured according to Russel (1984) by palpation of the posterior dorsum of the animal and visual assessment by assigning scores ranging from 1 (vary thin) to 5 (very fat). Length (LH), height of withers (HW), height of rump (HR), and girth (GH), were measured according to Pinheiro and Jorge (2010). The body compactness index (BCI) was calculated as FW/LH.

Slaughter and carcass biometrics

The animals were raised per 53 days until obtain live weight close to 40 kg. Before slaughter, the animals were submitted to 16h of solids fasting and at the end of the time, they were weighted again, this measurement was called body weight at slaughter (BWS). The slaughter was carried out at Experimental Slaughterhouse at FZEA/USP, for stunning was used a pneumatic pistol, the animals were hang up and bled by the jugular vein. The skin, head and paws were removed from the carcasses and evisceration was made, after those steps, measurement of the hot carcasses weight (HCW) was made.

After the HCW procedure, the carcass dimensions were measured before taken them to the cold storage. The measurements were carried out according to Sañudo and Sierra (1986) and Carrasco et al. (2009), outer carcass length (OCL), inner carcass length (ICL), chest girth (CG), chest depth (CD), hind limb width (HLW), hind limb girth (HG), leg width (LW), and pelvic limb length (LL) were measured. The calculations of some index were made: carcass compactness index (CCI) where calculated as CCW/ICL and leg compactness index (LCI) where calculated as LW/LL.

Then, the carcasses were placed into a cold storage (2ºC) for around 24h. On the day after, carcasses were split down into two sides and the half carcasses were divided into forequarter and hindquarter. The carcass were cut between the 12th and 13th rib and fat thickness (FT) were measured using a pachymeter, ribeye area (RA) were measured from the same carcass piece by measuring the area by image analysis on the software ImageJ 1.8.0.

Carcass yield were calculated from the weight measurements of the animal body and carcass: hot carcass yield (HCY) were calculated as (HCW/BWS)*100; cold carcass yield (CCY) were calculated as (CCW/BWS)*100; carcass yield (CY) were calculated as (CCW/FW)*100; and chilling carcass weight loss (CCWL) were calculated as 100-((CCW/HCW)*100).

Statistic analysis

The experiment was carried out following the random block design statement. All procedures were analysed by MIXED procedure of the SAS 15.1 software. First, study of the normality of the residues and influencer points were carried out and then the statistics were performed. Model for animal performance variables were composed by fixed effects (treatment and block) and error. For blood variables, the model were composed by fixed effects (treatment and block), initial values observed for each parameter were tested as covariable (if $P>0.05$ the covariable were removed from the model), and error. Orthogonal contrast (linear, quadratic and cubic) were performed and considered significant if $P \leq 0.05$ and tendency if $0.05<P \leq 0.10$.

RESULTS

Health parameters

The results from blood count cells analysis is reported in Table 2 and all evaluated parameters are within the range of normal values from the literature (BYERS; KRAMER,

2010). Most of the parameters do not had treatment effect, except for some parameters from leukocytes cells, the absolute counting of WBC had increased from 7,00 x 103/µl cells in the control to an average of 7.97 103/µl (data not showed) cells in the treated animals, and the contrast analysis indicated a cubic effect (P=0.052). Its increase is explained by the increasing on lymphocytes (P=0.043) with a cubic effect and segmented neutrophils (P=0.023) with quadratic effect.

Table 2.2. Parameters of blood counting analysis from growing lambs fed increased levels of yerba-mate extract

Parameter[1]	Level of inclusion (%)				MSE[2]	Normal BC values[3]	P value[4]		
	0	1	2	4			Lin	Quad	Cub
Erythrogram									
RBC	14.44	14.41	13.99	14.32	0.28	9-15	0.668	0.387	0.434
HB	14.09	13.92	13.44	13.76	0.30	9-15	0.410	0.279	0.491
HT	41.78	40.77	39.74	40.57	0.75	27-45	0.286	0.139	0.706
MCV	28.80	27.99	28.52	28.62	0.31	28-40	0.890	0.237	0.147
MCH	9.63	9.61	9.57	9.53	0.14	8-12	0.572	0.967	0.937
MCHC	33.63	34.13	33.61	33.87	0.38	31-34	0.881	0.889	0.287
RDW	18.17	18.21	17.97	18.18	0.24	16-20	0.924	0.615	0.554
Leukogram									
WBC	7.00	8.25	7.99	8.41	0.25	4-8	0.003	0.070	0.052
Absolute count (10^3/µl)									
Band neutrophils	0.028	0.018	0.00	0.032	0.014	Rare	0,793	0,110	0,537
Segmented neutrophils	2.02	3.39	3.40	3.18	0.36	0.7-6	0,081	0,023	0,323
Lymphocytes	4.04	5.15	4.44	4.48	0.31	2-9	0.798	0.177	0.043
Monocytes	0.024	0.108	0.076	0.076	0.042	0-0,75	0.599	0.350	0.355
Eosinophils	0.075	0.068	0.091	0.084	0.026	0-1	0.713	0.867	0.599
Basophils	0.025	0.019	0.024	0.020	0.012	0-0.3	0.813	0.975	0.726
Percentage (%)									
Band neutrophils	35.00	37.89	42.78	38.00	3.60	10-50	0,546	0,200	0,591
Segmented neutrophils	0.33	0.22	0.00	0.33	0.16	Rare	1,000	0,124	0,507
Lymphocytes	62.78	55.57	54.78	55.78	3.18	40-55	0.196	0.152	0.631

To be continued

Continuation

Table 2.2. Parameters of blood counting analysis from growing lambs fed increased levels of yerba-mate extract

	Levels of inclusion (%)					Normal	P value[4]		
Parameter[1]	**0**	**1**	**2**	**4**	**MSE[2]**	**BC values[3]**	**Lin**	**Quad**	**Cub**
Monocytes	0.38	1.11	0.89	0.89	0.47	0-6	0.607	0.452	0.500
Eosinophils	1.22	0.78	1.22	1.16	0.35	0-10	0.863	0.742	0.331
Basophils	0.33	0.22	0.33	0.22	0.15	0-3	0.711	0.953	0.532
Thrombogram									
PT	397.44	533.67	549.13	540.67	46.35	100-800	0.068	0.087	0.516

[1]RBC - red blood cells, 10^6/µl; HB – hemoglobin, g/dl; HT – hematocrit, %; MCV - mean corpuscular volume, fL; MCH - mean corpuscular hemoglobin, pg; MCHC - mean corpuscular hemoglobin concentration, %; RDW - red blood cells distribution width, %; WBC - white blood cells, 10^3/µl; PT – platelets, 10^3/µl.
[2]MSE – mean square error.
[3]Reference values for a normal animal from Byers and Kramer (2010).
[4]Values from orthogonal contrast (linear, quadratic, and cubic) are significantly different if P<0.05 and tendency if 0.05<P<0.10.

Source: own authorship

The effect of increasing levels of yerba-mate extract on blood biochemistry was presented in Table 3. Nutritional status of the animal demonstrates a linear increase on total protein of the blood serum (P=0.038) from 5.76 g/dL in the control to 5.99 g/dL on the treatment with the highest level of inclusion of yerba-mate extract. This effect on total protein is caused by the globulin increasing (P=0.047), and it also affected the ratio albumin:globulin (quadratic effect, P=0.044). On fat composition of the blood, triglycerides (P=0.033) and VLDL (P=0.033) had a quadratic effect by the treatment, showing a reduction on triglycerides and VLDL on the blood of animals fed up to 2% of yerba-mate extract, however, no difference (P>0.05) on the others constituents of the blood fat parameters (cholesterol, HDL, LDL, and AI) was observed.

Marker to investigate the integrity of the skeletal and cardiac muscle, creatinine kinase had a tendency to cubic effect (P=0.068), nonetheless result does not make sense, the behaviour of the curve has no biological sense. Liver function marker GGT, shows a

linear increasing (P<0.001) of 23.84% on the quantity of the enzyme between the control and the highest level of extract inclusion. Kidney function markers also shows a slight linear increase on creatinine (P=0.029) and a linear reduction on the urea:creatinine ratio (P=0.063).

Table 2.3. Parameters of blood biochemical analysis from growing lambs fed increased levels of Yerba-mate extract

	Level of inclusion (%)					***P value*[4]**		
Parameter[1]	**0**	**1**	**2**	**4**	**MSE[2]**	**Lin**	**Quad**	**Cub**
Nutritional status								
TP	5.76	5.93	6.05	5.99	0.07	0.027	0.038	0.852
ALB	3.55	3.53	3.56	3.52	0.06	0.760	0.763	0.703
GLB	2.25	2.31	2.44	2.42	0.03	0.001	0.047	0.209
A:G	1.60	1.56	1.47	1.49	0.02	0.004	0.044	0.168
GLU	83.72	81.97	85.05	83.01	1.58	0.993	0.720	0.194
Fat composition								
TG	35.11	32.89	30.98	39.13	2.15	0.139	0.033	0.627
CHOL	44.86	47.48	46.23	46.51	1.91	0.738	0.587	0.449
HDL-C	18.01	19.79	20.01	19.97	1.15	0.392	0.332	0.712
LDL-C	18.16	23.12	20.32	18.37	1.89	0.643	0.139	0.184
VLDL-C	7.02	6.58	6.20	7.83	0.43	0.139	0.033	0.627
AI	1.45	1.41	1.34	1.43	0.14	0,971	0,594	0,839
Muscle integrity								
CK	90.24	110.33	86.93	125.57	10.13	0.045	0.328	0.068
Liver function								
AST	81.97	73.47	75.81	71.18	4.27	0.140	0.582	0.371
GGT	58.21	60.76	64.10	72.09	2.02	<0.001	0.631	0.959
Renal function								
BUN	43.70	45.77	47.28	44.62	2.31	0.842	0.267	0.866
CRE	0.84	0.80	0.99	0.99	0.06	0.029	0.707	0.084
BUN:C	53.10	61.20	47.64	43.23	5.09	0.063	0.555	0.110

[1]TP - total protein, g/dL; ALB - albumin, g/dL; GLB - globulin - g/dL; A:G - ratio albumin:globulin; GLC - glucose, mg/dL; TG - triglicerides, mg/dL; CHOL - cholesterol, mg/dL; HDL-C - high density lipoprotein, mg/dL; LDL-C - low density lipoprotein, mg/dL; VLDL - very low density lipoprotein, mg/dL; CK - creatinin kinase, U/dL; AST - aspartate aminotrasferase, U/dL; GGT - gamma-glutamyl transpeptidase, U/dL; BUN – blood urea nitrogen, mg/dL; CRE - creatinin, mg/dL; BUN:C - ratio blood urea nitrogen:creatinin.
[2]MSE – mean square error.
[3]Values from orthogonal contrast (linear, quadratic, and cubic) are significantly different if P<0.05 and tendency if 0.05<P<0.10.

Source: own authorship

Animal performance

Table 2.4. Performance parameters and body morphometry from growing lamb fed increased levels of yerba-mate extract

Parameter[1]	Unit	Level of inclusion (%)					*P value* of the Contrast		
		0	1	2	4	EPM	Lin	Quad	Cub
Performance									
ILW	kg	24.19	24.02	24.17	23.62	0.22	0.086	0.467	0.4227
FLW	kg	39.94	39.10	40.78	36.41	0.93	0.018	0.087	0.1176
TWG	kg	15.76	15.08	16.61	12.80	0.89	0.037	0.106	0.1499
DWG	g	267	256	282	217	15.2	0.037	0.106	0.1499
DMI	g	1153	1152	1171	1002	49.3	0.032	0.1575	0.6067
DMI LW	%	3.61	3.67	3.60	3.28	0.11	0.020	0.1869	0.8387
FC		3.79	4.05	3.76	4.00	0.14	0.525	0.8741	0.1095
EF		0.265	0.249	0.270	0.257	0.010	0.855	0.9035	0.1040
In vivo measurements									
LWLF	%	4.76	5.22	4.35	3.93	0.29	0.013	0.454	0.094
BCE	cm	3.67	3.83	4.00	3.89	0.17	0.363	0.295	0.802
LH	cm	65.0	64.7	64.4	65.6	1.0	0.651	0.499	0.909
GH	cm	78.6	79.1	78.1	76.9	1.2	0.235	0.695	0.686
HW	cm	56.7	57.7	57.2	56.8	0.7	0.825	0.396	0.511
HR	cm	59.4	61.1	59.7	59.6	0.9	0.741	0.482	0.244
BCI	kg cm^{-1}	0.599	0.601	0.618	0.559	0.013	0.039	0.040	0.324

[1]IW – initial live weight; FW – final live weight; TWG – total weight gain; DWG – daily weight gain; DMI – dry matter intake; DMIW – percentage of dry matter intake related to the average of weight; FC – food conversion; EC – efficiency conversion; LWLF - live weight loss at 16h fasting; BCE - body condition score; LH - length; GH - girth; HW - height of withers; HR - height of rump; BCI - body compactness index;

Source: own authorship

Animal performance results are presented on Table 4. Initial live weight of the animal were 23.88 kg, and the initial live weight from each treatment show no different. Final live weight had a tendency to have a quadratic response (P=0.087) by the treatment, animals fed up to 2% of yerba-mate extract had an increasing on final weight on about 2%. In the other hand, animas fed the highest level had a strong decrease on final weight (around 8.87% comparing to the control and 10.64% comparing to animals fed 2% of extract). Body compactness index had a quadratic effect (P=0.040), corroborating with the previous results of final weight. Linear negative effect was observed for total weight

gain (P=0.037), daily weight gain (P=0.037), dry matter intake (P=0.032), dry matter intake per live weight (P=0.020), and live weight loss after 16h of fasting (P=0.039). However, the highest level of inclusion of extract had the lowest values for those parameters, that result pull down the curve and explain it shape.

Carcass metrics and yield

Table 2.5. Carcass yield and measurements from growing lambs fed increased levels of yerba-mate extract

Parameter[1]	Unit	Level of inclusion (%)					P value[3]		
		0	1	2	4	EPM[2]	Lin	Quad	Cub
Carcass yield									
HCW	kg	17.75	17.33	18.96	16.98	0.48	0.447	0.062	0.034
HCY	%	47.34	47.05	49.67	48.15	0.57	0.133	0.070	0.165
CCW	kg	17.33	16.93	18.57	16.59	0.48	0.460	0.056	0.031
CCY	%	46.22	45.97	48.64	47.02	0.55	0.122	0.046	0.012
CY	%	44.03	43.57	46.52	45.18	0.55	0.039	0.084	0.006
CCWL	%	2.36	2.30	2.06	2.33	0.10	0.771	0.059	0.256
Carcass measurements									
OCL	cm	59.45	59.56	59.00	57.56	0.54	0.011	0.361	0.771
ICL	cm	53.12	53.44	52.00	51.78	0.70	0.103	0.917	0.280
CG	cm	66.56	65.56	67.22	66.89	1.10	0.613	0.981	0.322
CD	cm	25.06	25.44	25.44	25.83	0.38	0.175	0.869	0.697
HLW	cm	19.56	19.94	20.61	20.06	0.32	0.257	0.071	0.425
HG	cm	57.22	58.61	60.78	57.78	0.59	0.537	<0.001	0.132
LW	cm	15.83	15.89	16.33	15.28	0.21	0.078	0.015	0.170
LL	cm	30.00	30.11	29.11	30.00	0.42	0.810	0.218	0.175
CCI	kg cm^{-1}	0.318	0.316	0.356	0.308	0.008	0.614	0.001	0.004
LCI	kg cm^{-1}	0.65	0.66	0.71	0.67	0.01	0.261	0.015	0.111
FT	mm	2.75	2.55	2.21	1.82	0.40	0.099	0.915	0.865
RA	mm^2	1330	1301	1519	1215	79	0.411	0.058	0.088

HCW - hot carcass weight; HCY - hot carcass yeild; CCW - cold carcass weight; CCY - cold carcass yield; CY - carcass yield; CCWL - chilling carcass weight loss; OCL - outer carcass length; ICL - inner carcass length; CG - chest girth; CD - chest depth; HLW - hind limb width; HG - hind limb girth; LW - leg width; LL - pelvic limb length; CCI - carcass compactness index; LCI - leg compactness index; FT - fat thickness; RA - ribeye area.

[2]MSE – mean square error.

[3]Values from orthogonal contrast (linear, quadratic, and cubic) are significantly different if P<0.05 and tendency if 0.05<P<0.10.

Source: own authorship

Carcass yield and metrics were placed in Table 5; parameters of hot carcass weight (P=0.034), cold carcass weight (P=0.031), cold carcass yield (P=0.012), and carcass yield (P=0.006) shows a cubic effect. Quadratic tendency caused by the treatment were observed on the hot carcass yield (P=0.070). On the carcass metrics, the results show linear treatment effect on the outer carcass length (P=0.011) and quadratic effect differences on hind limb width (P=0.071), hind limb girth (P<0.001), and leg width (P=0.015). Index parameters of the carcass had a cubic effect for carcass compactness index (P=0.004) and a quadratic effect for leg compactness index (P=0.015). Fat trickness (P=0.099) and ribeye area (P=0.058) had a linear and quadratic effect, respectively.

DISCUSSIONS

Health parameters

This study tested increasing levels of inclusion of yerba-mate extract into the diet of growing lambs, and its effects on blood profile and animal performance. The blood count and biochemistry parameters will help us to understand the overall health status of the animals. For blood count analysis, a reference range was used as base to result interpretation, because count cells parameters have more consistent data. On the other hand, no reference interval parameter of blood biochemistry was used, for according to Braun, Trumel, and Bézille (2010), a high variation is observed for this values, and most part of the published textbooks, papers or specific articles that present this information are poorly characterize its base animals, analytical methods, and statistical models.

To start a global analysis of it and to evaluate a new feed additive it is important pay attention first to health status of the animals. In general, all blood count parameters are in the normal values comparing to the literature (BYERS; KRAMER, 2010), it suggest a general health status of the animals remained normal although the dietary treatment.

Most part of the blood count parameters had no effect by the treatment, except some WBC parameters. Same response were observed by Beigh et al. (2018), in their study with lambs fed a cocktail of exogenous fibrolytic enzymes, and with Morsy et al. (2016), using red propolis extract to feed Santa Ines ewes, their results show that extract had increase also the number of leukocyte count. In the other hand, no effect of Salix babylonica extrac, exogenous enzymes, and their combination on hematological parameters of lambs was observed on the study of Rivero et al. (2012).

Increasing on WBC occurred because of the increased number of segmented neutrophils and lymphocytes. The WBC system is one of the most important components of the defense system, neutrophils are cells considerate the first barrier against health problems with microorganisms infection, as well with tissue trauma and inflammatory signal (APPELBERG, 2006). Usually those cells are recruited from blood to the site where an infection or even a risk of infection is detected (WEISS; RAMAIAH; WALCHECK, 2010).

Lymphocytes are the main WBC component (in quantity) in the blood. Their three types of cells (T, B, and natural killer cells) are crucial to the immune system, its function is recognize as antigens, producer of antibodies, and killer cells that could damage the organism (WEISS; WARDROP, 2010). These components of the WBC have a major role in the body surfaces protection, especially on the enteric system, mainly protecting mucosa against proliferation of virus infection (TED VALLI; JACOBS, 2010). Related to WBC defense system the body count also with others protection systems, that will be discussed soon.

According to Eckersall (2008), total protein is divided into two subclasses, first one is albumin, this class of serum protein is the main protein in the serum composition, and it is an important regulator of the colloid osmotic pressure and the blood volume.

Albumin is also an important marker for nutritional protein status, because is largely used on the protein muscle turnover. Second, globulin is a class of protein that includes some different types of proteins, as proteins produced by the immune system and liver.

The results of this study show an increase on levels of total blood protein and also an increase on globulins on animals fed up to 2% of yerba-mate extract. Morsy et al. (2016) presented similar results using red propolis extract to feed Santa Ines ewes, their results show that extract had increase globulin concentration, which indicates that isoflavonoids found in the red propolis extract could activate the immune system of the ewes. It must support that hypothesis that bioactive compounds presents on the yerba-mate extract could also have the same effect on the immune systems of growing lambs.

On the lipid composition of the blood, total cholesterol, HDL, and LDL keep at the same range although treatments. In the other hand, triglycerides and VLDL were affected, having both reduction on animals feeding up to 2% of inclusion of yerba-mate extract. This reduction could be explained by inhibition of the synthesis of cholesterol in the liver by flavonoids and phenolic acids (YEH et al., 2009; MURASE et al., 2002; GEBHARDT, 1998), however, in this study inhibition occurred only on VLDL concentration and do not change LDL parameter.

One very sensitive blood marker for muscle damage were used, according to Houpert et al. (1995), the creatinine kinase have a high concentration on the muscle (about 4,000-5,000 U/g) compared to the plasma (50-100 U/g), and a minimal damage could rapidly increase the concentration in the blood. Our results show no biological sense, cause two peaks of blood CK where showed in the animals fed 1 and 4% of yerba-mate extract. Those peaks are out of the normal range, however, no conclusions about it could be taken.

Gamma-glutamyltransferase (GGT) is one marker for kidney damage, enzymes in the plasma range from 20-52 U/dL (Cesar et al., 2007). Increasing on plasma were reported in different papers and associated with hepatobiliary disorders of sheep, as fasciolosis (MATANOVIC et al., 2007; SALEH, 2008), facial eczema (FLAOYEN; SMITH, 1992), and even liver fibrosis due to long-term ingestion of Tephrosia cinerea (CESAR et al., 2007). However, no diagnoses of kidney sickness could be inferred, because it is important use more than only one parameter to identify one sickness.

Animal performance and carcass metrics

Animal performance presented in this study shows great results in general, and corroborate with the results shows in the current literature using Dorper breed and its cross-breeds (MA et al., 2019; NASCIMENTO et al., 2018; WANG et al., 2016). The effect on high levels (4%) of yerba-mate extract results in a reduction on dry matter intake and consequently a reduction in the general performance of the lambs, in the other hand, inclusions up to 2% have a little increment on dry matter intake, total weight gain, and daily weight gain. Corroborating with the study of the Zawadziki et al. (2017), showing that small quantities (up to 1.5% of the dry matter intake) of the yerba-mate extract in the diet does not change the general performance of the steers.

The results agree with the study published by Po, Xu, and Celi (2012), where they fed dorper ewes 2.5% of yerba-mate extract, and its results show a reduction on the feed intake of the diets on few weeks post-partum. The yerba-mate extract used is rich in hydrolysable tannins (ZAWADZIKI et al., 2017), and those compounds have benefits and adverse effects on ruminant nutrition, according to Makkar, Francis, and Becker (2007), inclusion of tannins in a moderate concentration in ruminant nutrition could increase dry matter intake and ruminal undegraded protein. The mechanism of reduce

ruminal degradability of the protein by the tannins is well described in the literature (MAKKAR; FRANCIS; BECKER, 2007).

Although a reduction on the production parameters were observed, the index of food and efficiency conversion does not change, it shows that feed intake had a similar rate of reduction as weight gain. It indicate that the same alimentary cost could be observed, however, on animals fed a right level of yerba-mate extract a long time to reach the slaughter weight must be observed, and consequently a bigger cost could be the result.

Fat thickness had a reduction by the increment of yerba-mate extract in the diet, this result could be explained by the reduction of the dry matter intake, however on a recent study, using mice as human models, shows that yerba-mate could decrease the differentiation of preadipocytes and reduce accumulation of lipids into those cells (Kang et al., 2012). It occurs due to the adipogenesis modulatory characteristic by the yerba-mate, causing regulation of the gene expression levels of pro-adipogenic transcription factors (ARCARI et al., 2013; ARCARI et al., 2009; PANG; CHOI; PARK, 2008).

In the carcass yield parameters, we can observe a increasing on the productive parameters (up to 2%), however, a strong decrease when used high levels (4%) of yerba-mate extract. Carcass metrics show a reduction on the carcass and leg compactness index also on the highest level treatment, it is an indicative of that high levels strongly affect the productive parameters of lambs fed yerba-mate extract.

CONCLUSIONS

In general, the extract do not change the nutritional status of the animals, however, inclusion of yerba-mate extract up to 2% of the dry matter intake, could increase protection by the immune system, due to increased levels of leukocytes cells and globulins in the serum, and reduce VLDL and triglycerides. Also, productive parameters

had a slight increase on animals fed up to 2% of yerba-mate extract, however, high levels of inclusion demonstrate severe losses on animal performance.

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis**. 14th ed. 4 rev. Arlington, VA: The Association, 1990. 2 v.

APPELBERG, R. Neutrophils and intracellular pathogens: beyond phagocytosis and killing. **Trends in Microbiology**, Kidlington, v. 15, p. 87-92, 2006.

ARCARI, D. P. et al. Antiobesity effects of yerba mate extract (Ilex paraguariensis) in high-fat diet-induced obese mice. **Obesity**, Hoboken, v. 17, p. 2127-2133, 2009.

ARCARI, D. P. et al. The in vitro and in vivo effects of yerba mate (Ilex paraguariensis) extract on adipogenesis. **Food Chemistry**, Amsterdam, v. 141, p. 809-815, 2013.

BASTOS, D. H. M. et al. Yerba mate: pharmacological properties, research and biotechnology. **Medicinal and Aromatic Plant Science Biotechnology**, Middlesex, v. 1, p. 37-46, 2007.

BEIGH, Y. A. et al. Blood metabolic profile of lamb fed complete diet supplemented with exogenous fibrolitic enzymes cocktail. **Journal of Animal Health and Production,** Lahore, v. 6, p. 96-102, 2018.

BERTÉ, K. A. S. et al. Chemical composition and antioxidant activity of yerba-mate (Ilex paraguariensis A. St.-Hil., Aquifoliaceae) extract as obtained by spray drying. **Journal of Agricultural and Food Chemistry**, Washington, v. 59, p. 5523-5527, 2011.

BOYD, B. M. et al. Effects of shade and feeding zilaterol hydrochloride to finishing steers on performance, carcass quality, heat stress, mobility, and body temperature. **Journal of Animal Science**, Cary, v. 93, p. 5801-5811, 2015.

BRAUN, J. P.; TRUMEL, C.; BÉZILLE, P. Clinical biochemistry in sheep: A selected review. **Small Ruminant Research**, Amsterdam, v. 92, p. 10-18, 2010.

BYERS, S. R.; KRAMER, J. W. Normal hematology of sheep and goats. In: WEISS, D. J.; WARDROP, K. J. (Ed.). **Schalm's veterinary hematology**. 6th ed. Ames, Iowa: Willey-Blackwell, 2010. p. 836-842.

CAM, M. A.; OLFAZ, M.; SOYDAN, E. Body measurements reflect body weights and carcass yields in karayaka sheep. **Asian Journal of Animal and Veterinary Advances**, Faisalabad, v. 5, p. 120-127, 2010.

CARRASCO, S. et al. Effect of feeding system on growth and carcass characteristics of Churra Tensina light lambs. **Livestock Science**, Amsterdam, v. 121, p. 56-63, 2009.

CESAR, A. S. J. et al. Toxic hepatopath in sheep associated with the ingestion of the legume Tephrosia cinerea. **Journal of Veterinary Diagnostic Investigation**, Thousand Oaks, v. 19, p. 690-694, 2007.

CLIFFORD, M. N.; RAMIREZ-MARTINEZ, J. R. Chlorogenic acids and purine alkaloids contents on maté (Ilex paraguaiensis) leaf and beverage. **Food Chemistry**, Amsterdam, v. 35, p. 13-21, 1990.

COELHO G. C. et al. Quantification of saponins in extractive solution of mate leaves (Ilex paraguariensis A. St. Hil.). **Journal of Medicinal Food**, New Rochelle, v. 13, p. 439- 443, 2010.

DE MEJÍA, E. G. et al. Yerba mate tea (Ilex paraguariensis): Phenolics, antioxidant capacity and in vitro inhibition of colon cancer cell proliferation. **Journal of Functional Foods**, Amsterdam, v. 2, p. 23-34, 2010.

ECKERSALL, P. D. Proteins, proteomics and the dysproteinemias. In: KANEKO, J. J.; HARVEY, J. W.; BRUSS M. L. (Ed.). **Clinical biochemistry of domestic animals**. 6th ed. Burlington: Academic Press, 2008. p. 117-155.

E.U. **Regulations (EC) Nº 1831/2003 of the European Parliament and of the Council:** on additives for use in animal nutrition. Brussels: Official Journal of the Europe Union, 2003.

FLAOYEN, A.; SMITH, B. L. Parenchymal injury and biliary obstruction in relation to photosensitization in sporidesmin-intoxicated lambs. **Veterinary Research Communications**, Dordrecht, v. 16, p. 337-344, 1992.

FULTON, R. M.; NERSESSIAN, B. N.; REED, W. M. Prevention of Salmonella enteritidis infection in commercial ducklings by oral chicken egg-derived antibody alone or in combination with probiotics. **Poultry Science**, Cary, v. 81, p. 34-40, 2002.

GEBHARDT, R. Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (Cynara scolymus L.) extracts. **The Journal of Pharmacology and Experimental Therapeutics**, Baltimore, v. 286, p. 1122-1128, 1998.

GNOATTO, S. C. B.; SCHENKEL, E. P.; BASSANI, V. L. HPLC method to assay total saponins in Ilex paraguariensis aqueous extrac. **Journal of the Brazilian Chemical Society**, São Paulo, v. 16, p. 723-725, 2005.

GOFF, J. P. Major advances in our understanding of nutritional influences on bovine health. **Journal of Dairy Science**, New York, v. 89, p. 1292-1301, 2006.

GOSMANN, G. et al. Phenolic compounds from Maté (Ilex paraguariensis) inhibit adipogenesis in 3T3-L1 preadipocytes. **Plant Foods for Human Nutrition**, New York, v. 67, p. 156-161, 2012.

HASHEMI, S. R.; DVOODI, H. Herbal plants and their derivatives as growth and health promoters in animal nutrition. **Veterinary Research Communications**, Dordrecht, v. 35, p. 169-180, 2011.

HECK, C. I.; DE MEJIA, E. G. Yerba Mate Tea (Ilex paraguariensis): A Comprehensive Review on Chemistry, Health Implications, and Technological Considerations. **Journal of Food Science**, Hoboken, v. 72, p. 138-151, 2007.

HOUPERT, P. et al. In vivo non-invasive quantification of muscle damage following a single intramuscular injection of phenylbutazone in sheep. **Veterinary and Human Toxicology**, Manhattan, v. 37, p. 105-110, 1995.

KANG, Y. R. et al. Anti-obesity and anti-diabetic effect of yerba mate (Ilex paraguariensis) in C57BL/6J mice fed a high-fat diet. **Laboratory Animal Research**, Seoul, v. 28, p. 23-29, 2012.

KEARL, L. C. **Nutrient requirements of ruminants in developing countries**. Logan: International Feedstuffs Institute, 1982. 381 p.

LIMA, A. G. V. O. et al. Intake, digestibility, nitrogen balance, performance and carcass traits of Santa Ines lamb fed with sunflower cake from biodiesel production. **Small Ruminant Research**, Amsterdam, v. 168, p. 19-24, 2018.

MA, T. et al. Growth performance, nutrient digestibility, and slaughter traits of male fattening lambs under different feeding standards. **Animal Nutrition**, Beijing, v. 5, p. 74-79, 2019.

MACHILA, N. et al. Farmer estimation of live body weight of cattle: implications for veterinary drug dosing in East Africa. **Preventive Veterinary Medicine**, Amsterdam, v. 87, p. 394-403, 2008.

MAKKAR, H. P. S.; FRANCIS, G.; BECKER, K. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. **Animal**, Cambridge, v. 1, p. 1371-1391, 2007.

MATANOVIC, K. et al. Hematological and biochemical changes in organically farmed sheep naturally infected with Faciola hepatica. **Parasitology Research**, v. 101, p. 1657-1661, 2007.

MERTENS, D. R. Gravimetric determination of amylase treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. **Journal of AOAC International**, Cary, v. 85, p. 1217-1240, 2002.

MORSY, A. S. et al. Impact of Brazilian red propolis extract on blood metabolites, milk production, and lamb performance of Santa Inês ewes. **Tropical Animal Health and Production**, Dordrecht, v. 48, p. 1043-1050, 2016.

MURASE, T. et al. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipids catabolism in the liver. **International Journal of Obesity and Related Metabolic Disorders**, Hampshire, v. 26, p. 1459-1464, 2002.

NASCIMENTO, U. F. S. et al. Performance and carcass characteristics of lambs ½ Dorper + ½ Santa Inês, slaughtered with different thicknesses of subcutaneous fat. **Revista Brasileira de Saúde e Produção Animal**, Ondina, v. 19, p. 125-135, 2018.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of small ruminants:** sheep, goats, cervids, and new world camelids. Washington, DC: The National Academies Press, 2007. p. 384.

PANG, J.; CHOI, Y.; PARK, T. Ilex paraguariensis extract ameliorates obesity induced by high-fat diet: Potential role of ampk in the visceral adipose tissue. **Archives of Biochemistry and Biophysics**, Maryland Heights, v. 476, p. 178-185, 2008.

PINHEIRO, R. S. B.; JORGE, A. M. Medidas biométricas obtidas in vivo e na carcaça de ovelhas de descarte em diferentes estágios fisiológicos. **Revista Brasileira de Zootecnia**, Viçosa, v. 39, p. 440-445, 2010.

PO, E.; XU, Z.; CELI, P. The effect of yerba mate (Ilex paraguarensis) supplementation on the productive performance of Dorper Ewes and their progeny. **Asian-Australian Journal of Animal Sciences**, Cary, v. 25, p. 945-949, 2012.

RIVERO, N. et al. Effect of exogenous enzymes and Salix babylonica extract or their combination on haematological parameters in growing lambs. **Journal of Animal and Feed Sciences**, Jablonna, v. 21, p. 577-586, 2012.

SALDANA, M. D. A. et al. Extraction of purine alkaloids from maté (Ilex paraguariensis) using supercritical CO2. **Journal of Agricultural and Food Chemistry**, Washington, v. 47, p. 3804-3808, 1999.

SALEH, M. A. Circulating oxidative stress status in desert sheep naturaly infected with Faciola hepatics. **Veterinary Parasitology**, Amsterdam, v. 154, p. 262-269, 2008.

SAÑUDO, C.; SIERRA, I. Calidad de la canal em la espécie ovina. **Ovino**, v. 1, p. 127-153, 1986.

SCHENKEL, E. P. et al. Saponins from maté (Ilex paraguariensis) and other South American Ilex species: ten years research on Ilex saponins. **Ciência e Cultura**, São Paulo, v. 49, p. 359-363, 1997.

SILVA, J. A. et al. Dietery addition of crude form or ethanol extract of brown propolis as nutritional additive on behavior, productive performance and carcass traits of lamb in feedlot. **Journal of Animal and Feed Sciences**, Jablonna, v. 28, p. 31-40, 2019.

TED VALLI, V. E.; JACOBS, R. M. Structure, function, and disorers of lymphoid tissue. In: WEISS, D. J.; WARDROP, K. J. (Ed.). **Schalm's veterinary hematology**. 6th ed. Ames, Iowa: Willey-Blackwell, 2010. p. 367-375, 2010.

U.S.FDA. **New Animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food producing animals**: recommendations for drug sponsors for voluntarily aligning product use conditions with GFI #209. 2013.

VAN SOEST, P. J.; ROBERTSON, J. B.; LEWIS, B. A. Methods for dietary fiber, neutral detergente fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal Dairy Science**, New York, v. 74, p. 3583-3597, 1991.

WANG, B. et al. Effect of urea supplementation on performance and safety in diets of dorper crossbred sheep. **Journal of Animal Physiology and Animal Nutrition**, Berlin, v. 100, p. 902-910, 2016.

WEISS, D. J.; RAMAIAH, S. K.; WALCHECK, B. Neutrophil distribution and function. In: WEISS, D. J.; WARDROP, K. J. (Ed.). **Schalm's veterinary hematology**. 6th ed. New York: Willey-Blackwell, 2010. p. 268-274.

WEISS, D. J.; WARDROP, K. J. **Schalm's veterinary hematology**, 6th ed. New York: Willey-Blackwell, 2010. p. 1232.

YEH, Y. H. et al. Dietary caffeic acid, ferulic acid and coumaric acid supplements on cholesterol metabolism and antioxidant activity in rats. **Journal of Food and Drug Analysis**, Philadelphia, v. 17, p. 123-132, 2009.

ZAWADZKI, A. et al. Mate extract as feed additive for improvement of beef quality. **Food Research International**, Oxford, v. 99, p. 336-347, 2017.

CHAPTER 3 – Yerba-mate extract and its effect on digestibility, ruminal fermentation, methane production and microbiome of growing lamb

RESUMO

O uso de aditivos nutricionais com o intuito de melhorar a utilização de nutrientes e reduzir impactos ambientais é uma pratica bastante utilizada. Neste trabalho, objetivou-se estudar os efeitos de níveis de extrato de erva mate na dieta de cordeiros em confinamento, e seus efeitos na digestibilidade da dieta, metanogênese, diversidade do microbioma e metabolismo ruminal. O experimento foi realizado com trinta e seis cordeiros machos e não castrados, os animais foram divididos em 9 blocos e dentro de cada bloco um animais foi sorteado aleatoriamente para um dos 4 tratamentos (0, 1, 2 e 4% de inclusão de extrato de erva mate). No inicio (até o 3º dia de confinamento) todos os animais receberam a dieta controle (0%) para que pudesse ser feito uma coleta de conteúdo ruminal inicial. No terceiro dia, uma coleta de liquido ruminal foi realizada e o material foi armazenado em freezer -80°C. No 30º dia de confinamento, os 6 grupos com pesos centrais foram submetidos ao ensaio de digestibilidade aparente, no qual foi feita coleta total de fezes para mensuração da digestibilidade total das dietas. Logo em seguida, no 39º dia, os mesmo animais foram submetidos à coleta de metano, pela técnica do SF6. Ao final do confinamento, uma nova amostragem de liquido ruminal foi realizada, e uma parte do material foi armazenada para analise de microbioma, já outra parte armazenada para analise de parâmetros de fermentação ruminal. Após abate, uma outra amostra de liquido ruminal filtrado foi coletada diretamente do rúmen, esse material também foi analisado quanto os parâmetros de fermentabilidade ruminal. A estatística foi realizada no software SAS studio, e foi utilizado o procedimento MIXED. A alfa diversidade foi calculada pelo indice FAITH PD e comparado pelo teste de Krusckal-Wallis. Os animais alimentados com até 2% de extrato de erva mate apresentaram maior ingestão de

nutrientes, e melhoria da digestibilidade de alguns nutrientes como matéria seca, fibra total e fibra em detergente neutro. Os animais alimentados com até 2% de extrato apresentaram acréscimo na ingestão de matéria seca e digestibilidade de alguns nutrientes, e consequentemente a produção de metano foi mais, no entanto nenhuma diferença entre a emissão de metano por unidade de ganho de peso ou de ingestão de matéria seca foi identificado. Ocorreu uma perda na alfa diversidade bacteriana comparando o início com o final do período experimental, no entanto nenhuma diferença entre tratamentos foi identificada, mostrando que mais analises de bioinformática são necessárias para entender a ação do extrato dobre a microbiota. Podemos concluir que o extrato de erva mate (até 2% da dieta) pode ser benéfico aos animais, melhorando ingestão e digestibilidade de nutrientes, no entanto, nenhum efeito na metanogênese e alfa diversidade foi atribuído à aplicação do tratamento.

Palavras-chave: aditivos naturais, extrato de planta, nutrição de ruminantes.

ABSTRACT

The use of nutritional additives in order to improve nutrient utilization and reduce environmental impacts is a widely used practice. The objective of this work was to study the effects of yerba-mate extract levels on the diet of growing lambs, and their effects on diet digestibility, methanogenesis, microbiome diversity and ruminal metabolism. The experiment was carried out with thirty-six male and non-castrated lambs, the animals were divided into 9 blocks and within each block one animal was randomly selected for one of the 4 treatments (0, 1, 2 and 4% inclusion of yerba-mae extract). At the beginning (until the 3rd day of the experiment) all animals received the control diet (0%), that an initial ruminal content sampling could be made. On the third day, a ruminal fluid collection was performed and the material was stored in a -80°C freezer. On the 30th day of experiment, 6 groups with central weights were submitted to apparent digestibility test,

in which total feces collection was performed to measure the total digestibility of the diets. On the 39th day, the same animals were submitted to methane collection by the SF6 technique. At the end of the experiment, a new ruminal fluid sampling was performed, and part of the material was stored for microbiome analysis, while another part was stored for analysis of ruminal fermentation parameters. After slaughter, another sample of filtered ruminal fluid was collected directly from the rumen, this material was also analyzed for ruminal fermentability parameters. The statistics were performed using SAS studio software, and the MIXED procedure was used. Alpha diversity was calculated by the FAITH PD index and compared by the Krusckal-Wallis test. Animals fed up to 2% yerba-mate extract had higher nutrient intake and improved digestibility of some nutrients such as dry matter, total fiber and neutral detergent fiber. The animals fed up to 2% of extract showed increase in dry matter intake and digestibility of some nutrients, consequently methane production was higher, however no difference between methane emission per unit of weight gain or intake of dry matter was identified. There was a loss in bacterial alpha diversity comparing the beginning and the end of the experimental period, however no differences between treatments were identified, showing that more bioinformatics analyzes are needed to understand the action of the extract on the microbiota. It can be concluded, that yerba-mate extract (up to 2% of the diet) may be beneficial to animals by improving nutrient intake and digestibility, however, no effect on methanogenesis and alpha diversity was attributed to the treatment application.

Key word: natural additives, plant extract, ruminant nutrition.

INTRODUCTIO

Rumen is a perfect environment for several communities of microorganisms, at least the ruminal habitat has contains around of 50 genera of bacteria (10^{10}-10^{11} cells/ml), 25 genera of protozoa (10^{4}-10^{6} cells/ml), a considerable amount of archaea (10^{9} cells/ml),

and other microorganisms as fungi (PATRA, 2012; GRUNINGER et al, 2014). That community of organisms is allow to produce a wide array of enzymes, that work in essential roles into ruminant nutrition, for example breaking down lignocellulosic and non-structural carbohydrate (starch, sugar) from feed, nitrogenous compounds (plant protein, amino acids, urea), and lipids (RUSSEL; RYCHLIK, 2001).

The fermentation of the feed by microorganisms of the rumen is an essential process for both, microorganisms and host, because it provides energy (ATP) for the growth of microorganisms and also provide important molecules that are used by the host to produce glucose (gluconeogenesis), lipid (acetate, butyrate), and energy-releasing fuels (acetate, butyrate) (MCCANN; ELOLIMY; LOOR, 2017). Nitrogen metabolism from the microbial community is also an important process, because microorganisms can use non-protein nitrogen to produce microbial protein, and it will be used as one of the greatest source of amino acids for protein synthesis (muscle and milk) in the animal body (BACH; CALSAMIGIA; STERN, 2005).

On this regard, increase the efficacy of the rumen microbiota is a goal for a vast number of groups of researchers, enterprises, and producers, and use feed additives that increase productivity, reduce environmental pollution and increase quality of the animal products is one of the ways to archive those goals. A large number of studies using different plant components to modulate ruminal fermentation and animal health is well established in the literature (BONANNO et al., 2007a; BONANNO et al., 2007b; ZHONG et al, 2015).

One South-American plant commonly used and with desirable characteristics as a natural additive for ruminant nutrition is the yerba-mate. Its bioactive compounds is composed by phenolic compounds (BASTOS et al., 2007; DE MEJÍA et al., 2010; BERTÉ et al., 2011), alkaloids (CLIFFORD; RAMIREZ-MARTINEZ, 1990;

SALDANA et al., 1999), and saponins (SCHENKEL et al., 1997; GNOATTO; SCHENKEL; BASSANI, 2005; COELHO et al., 2010; GOSMANN et al., 2012). Some researchers were studied yerba-mate as a feed additive in animal nutrition (HARTEMINL et al., 2015; PO; XU; CELI, 2012, ZAWADZKI et al., 2017), however more studies are necessary to clarify the pathway of action of the yerba-mate compounds on ruminal fermentation.

The hypothesis of the project were that Yerba-mate (Ilex paraguariensis St. Hilaire) extract can modify ruminal microbiota and consequently increase efficiency of nutrient utilization and reduce losses by methane emission on finishing lamb nutrition. The objective of the work were evaluate ruminal methane emission, apparent digestibility of the nutrients, ruminal fermentation and microbiome from finishing lamb's rumen.

MATERIAL AND METHODS

All procedures using animal were approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Food Engineering – University of São Paulo (Protocol number CEUA 3497040618).

Location, animals and experimental design

The animals were born and raised until weaning in a commercial farm at Uberaba, Minas Gerais, Brazil, they had access to pasture and feed supplementation on a creep-feeding system. After weaning they were transported to the College of Animal Science and Food Engineering (FZEA), University of São Paulo (USP), Pirassununga, São Paulo, Brazil, where the experiment were carried out.

Thirty-six male lamb uncastrated (crossbreed Texel x Dorper x Santa Inês) weighting 23.77±3.70 kg were used on this trial. At the first day on the experimental building, all animals were deworming, using a commercial drug with Ivermectin as active principle, following the doses recommendations from the package insert.

The animals were weighted and assigned into nine groups, each group had four animals with similar weight and those animals were randomly assigned to one of experimental treatments, according to a randomized block design statement. Each animal was placed into an individual tie stall with individual water and feeder access.

Diet and experimental treatment

The animals were fed twice a day at 8 am and 4 pm, diets were calculated following the nutritional needs for finishing lamb, according to NRC (2007), the ingredients and composition are show in the table 2.1. in the previous chapter. Before morning feed the feeder was cleaned up and the residues of feed from the day before was weighted to adjust the diet at least 10% of daily residue. Around 10% of the residue was collected and stored in a identified plastic bag on a -20 °C freezer, one composed residue sample was generated by week and dry matter analyse was carried out, in order to calculate the daily dry matter intake (DMI).

Experimental treatments were one control diet and three diets with increased levels of inclusion of yerba-mate extract (1, 2 and 4%). The extract was produced by Centro Flora, Botucatu, São Paulo, Brazil, from fresh leaves of Ilex paraguariensis A. St-Hil. plant by water:ethanol 75:25 v/v extraction at 90 °C, total phenolic concentration was 21.7 g GAE/100g extract and caffeine concentration 6% (w/w), composition and characterization of this extract was well described on Zawadzki et al. (2017). During the three firsts days on the experiment, all thirty-six animals receipted the control diet. After this period, the animals started eat the experimental diet.

Ruminal fermentation and microbiome sample collection

On the 3rd day of the experiment, all animals were receiving control diet, an initial collection of ruminal fluid were carried out 2 h after the morning feed using an orogastric

tube and a surgical pump. The first 30 ml of the liquid were discarded and the next 45 mL were stored into a 50 mL tube into liquid nitrogen and then stored in a -80°C freezer.

Three days before the end of the experimental period, a second ruminal fluid collection were carried out. Same methodological collection from initial sampling were used, the material were collected and pH were measured (HANNA Instruments HI8424), part were stored on liquid nitrogen and then in a -80 freezer to microbiome analysis. Another part, composed by 2 mL of ruminal fluid were sampled and acidified with 1 mL of sulphuric acid (1N), and stored at -20°C freezer for measurement of ammonia nitrogen by colorimetric analysis (FOLDAGER, 1977). Another 2 mL of ruminal fluid were collected and stored at -20°C freezer for short-chain fat acids (SCFA) analysis. SCFA analysis were carried out by gas chromatography (GC-2014, Shimadzu, Japão), and using capillary column (Stabilwax®, Restek, EUA), a split/splitless injector and dual FID detector at 250°C, following Getachew et al. (2002).

A third sampling collection were carried out after slaughter process, sampling were carried out after 16 h of solid fasting, and a representative sample were collected from different parts of the rumen. Sample collected directly from the rumen, were filtered by two layers of cheesecloths, and then pH were measured, using the same equipment cited above. Two different samples were collected for ammonia nitrogem analysis and SCFA analysis, following the procedures cited above.

Apparent digestibility

Were selected from all groups of animals six groups to the apparent digestibility essay, groups with extreme initial weight were not used on this essay. Animals were equipped with total feces collector bag on the 30th day of the experiment, they were adapted for the equipment for 3 days and the sample collection took place for five consecutive days. Feces were weighted and recorded twice a day just before feeding, feces

were mixed and 10% were collected into an identified plastic bag and stored in a -20°C freezer.

Ten percent of the daily residues and around 150g of the diets were stored also in a -20°C freezer. At the end of the fifth days, one composed sample of residue and feces by animal were obtained. The diets, residues, and feces composition were analysed by AOAC (1990) methods, dry matter (DM, method 934.01), ash (ASH, method 923.03), ether extract (EE, method 920.85), and crude fiber (CF, method 978.10) were measured, crude protein (CP, method 920.87) were analysed by Kjeldahl method. Neutral detergent fiber (NDF) were analysed according to Mertens (2002) using the amylase enzyme method, acid detergent fiber (ADF) and lignin (LN) (method 973.18), according to Van Soest, Robertson, and Lewis (1991) method. Gross energy (GE) was measured by the calorimetric pump (C200 System, IKA, Staufen, Germany). Organic matter (OM) was calculated by subtracting the ASH from 100, and non-fiber carbohydrates (NFC) was calculated subtracting EE, CP, ASH and CF from 100. Total digestible nutrient (TDN) were calculated by the equation described by Kearl (1982).

Methane emission essay

Same 24 animals from the apparent digestibility essay were used in the methane production essay, using the method described in Berndt et al. (2014). At the beginning of the experimental period, all 24 animals receipt by oral via one identified sulphur hexafluoride (SF_6) capsule, that have already a known rate of emission. On the 39th day of the experiment, the animals were equipped with the equipment of CH_4 collection, composed by halter, a support and a cylinder. First 3 days were used as an adaptation period and then 5 days of sample collection were carried out, with 2 more extra days if necessary repeat a measurement.

Collection apparatus were composed first by the support, placed on the dorsum of the animal, and then the cylinder were attached to the support, and a capillary tube from the cylinder to the halter conduct the gas to the storage container. To prepare a cylinder a vacuum pump were used to clean up three times and at the 4th time the vacuum were made. Once placed on the support and valve opened the halter end of the capillary tube placed close to the mouth and nose of the animals start collect the gas from eructation. Collection had a duration of 24 h, and at the end of the period a new cylinder were replaced.

The initial and final cylinder vacuum were measured. SF_6 and CH_4 mensuration were carried out in a chromatography machine (GC-2014, Shimadzo Corporation, Japan), according Johnson et al. (1994). Standard curve were established by standard gas from White Martins Corporation, following the methodology from Westberg et al. (1998). CH4 emission were calculated by:

$$RCH4 = RSF6\ x\ \frac{(CH4)M - (CH4)BG}{(SF6)M - (SF6)BG}\ x\ \frac{MWCH4}{MWSF6}\ x\ 1000$$

where: RCH4 is the rate of ruminal CH_4 emission, g/d; RSF6 is the rate of SF_6 emission by the capsule, mg/d; MWCH4 is the molecular mass of the CH_4, 16; MWSF6 is the molecular mass of the SF_6, 146. Dry matter intake, initial weight, final weight, and total weight gain were recorder for the week that methane assay were carrying out from the 24 animals, and the methane yield by unit of production parameters were calculated.

Microbiome analysis

At the end, 72 samples were collected, half from the beginning and half from the end of the experimental period. Those samples were stored in a -80°C freezer until start ruminal fluid processing. Ruminal fluid frizzed were hand grounded using liquid nitrogen, porcelain crucible, and other tools to break the material, all apparatus used in this procedure were burned at 350°C to sterilise. Powdered sample were mixed and

around 130 mg of the sample were submitted to DNA extraction by the Fecal and Soil Microbiome DNA miniprep kit (D6010, Zymo Research Corporation®). Extra sample were restored into -80°C freezer.

DNA concentration were measured by nanodrop spectrophotometer (ND-1000, Thermo Fisher Scientific), and gel electrophoresis of 1.5% agarose were carried out to measure the DNA quality. PCR amplification of the DNA fragments were carried out using three different forward and reverse primers:

16S rRNA broad spectrum bacterial primer (KLINDWORTH et al., 2013)

forward primer

5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′

reverse primer

5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′

16S rRNA archaea primer (KITTELMANN et al., 2013)

forward primer

5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGAATTGGCGGGGGAGCAC-3′

reverse primer

5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCGGTGTGTGCAAGGAGC-3’

18S rRNA protozoa primer (KITTELMANN et al., 2013)

forward primer

5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGACTAGGGATTGGARTGG-3’

reverse primer

5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATTGCAAAGATCTATCCC-3’

PCR were performed using Kapa HiFi HotStart 2x ReadMix DNA polymerase (Kapa Biosystems LTD., London, UK). Cycle conditions for Bacterial and Protozoa primers were 95°C (3 min), then 25 cycles of 95°C (30 s), 55°C (30 s), 72°C (30 s), then a final extension of 72°C (5 min). For Archaea primers the cycle conditions were 95°C

(3 min), then 25 cycles of 95°C (30 s), 65°C (30 s), 72°C (30 s), then a final extension of 72°C (5 min).

Libraries were purified using AGENTCOURT AMPure XP beads (A63881, Beckman Coulter, Inc., Indianapolis, United States), following to the Illumina 16S metagenomic sequencing library protocol. After obtaining purified libraries, a new 1.5 agarose gel electrophoresis were carried out to measure quality of the amplicons. Dual indices and Illumina sequencing adapters from Illumina Nextera XT index kit v2 (Illumina, San Diego, USA) were added to the target amplicon by new PCR process using the same DNA polymerase from the previous PCR. Cycles condition for all three primers were 95°C (3 min), then 12 cycles of 95°C (30 s), 55°C (30 s), 72°C (30 s), then a final extension of 72°C (5 min). Libraries were purified again with the same protocol cited above.

Mensuration of the libraries carried out for quantity and purity on a nanodrop spectrophotometer (ND-1000, Thermo Fisher Scientific). All sample were diluted to the same concentration as a lowest sample concentration on each primer amplification PCR according to quantification from nanodrop spectrophotometer. All samples from each primer amplification PCR were pooled into two pool of 36 randomized sample using 3 µL from each original sample, generating a total of 6 pooled samples.

Library quantification were carried out by rtPCR, using KAPA Library Quantification Kit and protocol (KK4824, KAPA Biosystems, Wilmington, MA, United States). The dilution and denaturation of the library were made according Illumina MiSeq library preparation guide. Diluted PhiX Illumina control library version 3 were made. Sequencing run was conducted on the Illumina MiSeqTM using 600 cycle MiSeq reagent kit (version 3) with paired 300 bp reads.

Data pre-processing and analysis

Sequences generated by previous methodological procedures were analysed and processed by QIIME 2 (version 2018.8) (Bolyen et al., 2018). Raw reads (6,012,728 paired end reads for bacteria, 5,897,604 for archaea, and 5,394,412 for protozoa) were quality checked, filtered for quality (≥Q25), and trimmed at the position 280 (forward) and 260 (reverse) for bacteria and archaea, and 290 (forward) and 280 (reverse) for protozoa, based on aggregated quality plots generated by QIIME 2. Remaining sequences were used to build high-quality bins by DADA2, who merges and denoises raw sequence reads into Amplicons sequences variants (ASVs) (CALLAHAN et al., 2016). Additionally, chimeric sequences were excluded using the DADA2 algorithm.

Sequences were classified using SILVA database version 132 (SEEDORF et al., 2014) for bacteria, the Rumen and Intestinal Methanogen database (RIM-DB) (FRIEDMAN and ALM, 2012) for archaea, and a curated database (KITTELMANN et al., 2015) for protozoa. Rarefaction curves generated for each dataset were used to standardize the data, using Shannon-Wiener (diversity) index. ASV results tables were used to determine alpha diversity using Faith's Phylogenetic Diversity index (FAITH, 1992).

Statistic analysis

The experiment were carried out following the random block designer statement. All procedures were analysed by MIXED procedure of the SAS Studio. First, study of normality of residues and influencer points were carried out and then the statistics were performed. Model for ingestion of nutrients, apparent digestibility and methane emissions parameters were composed by fixed effects (treatments and block) and error. For fermentation parameters, two different samples collections were carried out, first via orogastric tube and the second directly into rumen after slaughter, as described above, for

this reason a different model were performed, using fixed effects (treatments, block, sample type, and interaction treatment and sample type) and error. Orthogonal contrast (linear, quadratic and cubic) were performed and considered significant if $P \leq 0.05$ and tendency if $0.05 < P \leq 0.10$.

In order to identify differences in the microbial community structure, alpha diversity were contrasted using nonparametric statistical methods Krusckal-Wallis, and considered significant if $P<0.05$.

RESULTS

Apparent digestibility coefficient

Apparent digestibility results are presented in table 3.1., the results from nutrient intake shows a linear reduction on intake of dry matter (P=0.005), organic matter (P=0.018), ether extract (P=0.004), non-fiber carbohydrate (P=0.046), energy (P=0.018), and total nutrient digestible (P=0.025). Cubic effect were observed on intake of total fiber (P=0.045) and acid detergent fiber (P=0.005), and a tendency to a cubic effect on intake of mineral matter (P=0.093), crude protein (P=0.084), neutral detergent fiber (P=0.061), and lignin (P=0.055). In general, inclusions of 1% of yerba-mate extract could increase intake of the nutrients around 8.53%, in the other hand, inclusions of 2% could have a slight decrease on nutrient intake (around 3.77%) and excessive inclusions (4%) of the extract could have a severe negative effect on the nutrient intake, reducing around 11.72%.

Nutrient digestibility coefficient do not have a significant effect (P>0.05) by the treatment on organic matter (77.83%), crude protein (82.07%), acid detergent fiber (44.12%), lignin (47.65%), energy (76.56%), and total nutrient digestible (68.19%). However, linear positive effect were observed on dry matter apparent digestibility (P=0.018), and quadratic effect on total fiber (P=0.008), ether extract (P=0.019), and non-

nitrogen extract (P=0.050) apparent digestibility, in additional, a tendency to a quadratic and cubic effect were observed on neutral detergent fiber (P=0.066) and mineral matter (P=0.051) apparent digestibility, respectively.

Table 3.1. Daily ingestion of nutrient and its apparent digestibility coefficient from growing lambs fed with increased levels of Yerba-mate extract.

		Level of inclusion (%)					P value[3]		
Parameter[1]	**Unit**	**0**	**1**	**2**	**4**	**EPM[2]**	**Lin**	**Quad**	**Cub**
Daily ingestion based on DM									
DM	g	1,316	1,420	1,268	1,134	52,2	0.005	0.177	0.114
MM	g	135.7	135.4	110.4	78.2	4.8	<0.001	0.190	0.093
OM	g	1,181	1,284	1,158	1,056	47.6	0.018	0.177	0.118
CP	g	291.8	317.2	284.4	255.5	10.7	0.005	0.121	0.084
TF	g	185.0	196.6	172.7	165.0	6.2	0.007	0.552	0.045
EE	g	27.8	29.8	26.6	23.7	1.1	0.004	0.193	0.129
NNE	g	672.6	737.6	671.7	610.1	30.1	0.046	0.161	0.169
ADF	g	235.7	268.3	222.2	207.7	8.9	0.003	0.173	0.005
NDF	g	456.3	490.9	434.2	409.2	16.8	0.013	0.371	0.061
Lignin	g	61.3	64.8	56.0	53.0	2.3	0.004	0.653	0.055
Energy	kcal/kg	55.4	60.2	54.3	49.5	2.2	0.018	0.178	0.116
TND	g	852.9	934.3	843.3	762.4	37.1	0.025	0.158	0.138
Apparent digestibility coefficient									
DM	%	75.19	74.63	77.01	77.63	0.95	0.038	0.993	0.214
MM	%	56.27	53.40	59.03	67.62	1.27	<0.001	0.010	0.051
OM	%	77.36	76.86	78.73	78.38	0.97	0.313	0.753	0.289
CP	%	82.21	81.42	81.86	83.16	0.72	0.254	0.248	0.664
TF	%	63.77	65.46	68.39	64.23	1.13	0.785	0.009	0.284
EE	%	73.97	68.75	65.32	70.73	2.28	0.415	0.019	0.786
NNE	%	79.01	78.01	81.95	80.89	1.05	0.079	0.458	0.050
ADF	%	41.10	42.50	45.36	45.77	2.58	0.180	0.613	0.736
NDF	%	61.44	63.91	67.82	67.74	1.20	0.002	0.066	0.334
Lignin	%	49.17	44.72	49.96	50.88	7.71	0.764	0.837	0.625
Energy	%	76.41	75.53	77.36	76.95	1.02	0.495	0.912	0.273
TND	%	67.60	66.75	69.52	68.90	1.33	0.320	0.744	0.242

[1]DM – dry matter; MM – mineral matter; OM – organic matter; CP – crude protein; TF – total fiber; EE – ether extract; NNE – non-nitrogen extract; ADF – acid detergent fiber; NDF – neutral detergent fiber; TND – total nutrient digestible.
[2]MSE – mean square error.
[3]Values from orthogonal contrast (linear, quadratic, and cubic) are significantly different if P≤0.05 and tendency if 0.05<P≤0.10.

Source: own authorship

Ruminal metabolism

Results of ruminal metabolism analysis are presented on table 3.2., no treatment effect were observed (average of the parameter is described into parenthesis) on ruminal

pH (6.82), ruminal ammonia concentrations (27.59 mg dL-1), and the absolute concentration on short-chain fat acids (63.49 mMol), and its concentration of acetate (38.85 mMol), propionate (13.93 mMol), isobutirate (1.25 mMol), and valerate (0.83 mMol). A tendency on quadratic and a cubic effect were observed on absolute concentration of isovalerate (P=0.097) and butyrate (P<0.001). Similar results were observed on relative concentration of the short-chain fat acids, where no effect were observed on acetate (61.35%), propionate (21.32%), isobutirate (2.14%), isovalerate (3.06%), and valerate (1.35%), and cubic effect (P=0.004) were observed on butyric relative concentration (11.03; 9.83; 11.64, and 10.49% to the treatment control, 1, 2, and 4% of yerba-mate extract inclusion, respectively).

Table 3.2. Ruminal fermentation parameters sampled by orogastric tube and after slaughter from growing lambs fed with increased levels of Yerba-mate extract.

	Level of inclusion (T, %)					Sample type (S)			*P value*[3]			
Parameter[1]	0	1	2	4	EPM[2]	SL	OT	EPM[2]	T	S	T*S	Contrast
pH	6.76	6.83	6.83	6.85	0.05	6.95	6.69	0.03	0.486	<0.001	0.598	NS
NH3	27.50	26.46	28.86	29.11	1.72	35.63	20.33	1.22	0.672	<0.001	0.001	NS
Acet	40.33	38.08	38.22	39.32	2.05	29.62	48.36	1.45	0.849	<0.001	0.319	NS
Prop	14.65	13.74	14.13	13.53	0.74	9.92	18.10	0.52	0.728	<0.001	0.348	NS
IsoBut	1.26	1.21	1.23	1.27	0.06	1.33	1.17	0.04	0.908	0.014	0.581	NS
But	7.57	6.27	8.39	6.69	0.43	4.40	10.06	0.30	0.005	<0.001	0.002	Cub (P<0.001)
IsoVal	1.79	1.57	1.61	1.72	0.10	2.11	1.24	0.07	0.334	<0.001	0.032	Quad (P=0.097)
Val	0.80	0.77	0.90	0.84	0.05	0.76	0.90	0.04	0.339	0.008	0.180	NS
CCFAt	68.05	61.59	63.33	63.38	2.98	48.13	80.04	2.11	0.470	<0.001	0.098	NS
AcetProp	2.92	2.83	2.88	3.01	0.10	3.03	2.78	0.07	0.583	0.012	0.506	NS

[1]NH3 - amoniacal nitrogen, mg dL^{-1}; Acet – acetate, mMol; Prop - propionate, mMol; IsoBut - iso butirate, mMol; But - butirate, mMol; IsoVal - iso valerate, mMol; Val - valerate, mMol; SCFA – short-chain fatty acids, mMol; AcetProp - relationship between acetate and propionate.
[2]MSE – mean square error.
[3]P values from the model (T = treatment; S = sample type; T*S = interaction between treatment and sample type) and orthogonal contrast (linear, quadratic, and cubic). Significantly different if P≤0.05 and tendency if 0.05<P≤0.10.

Source: own authorship

Strong effect of sampling type were observed to all parameters (P≤0.012), and an interaction between sampling type and treatment were observed on the concentration of

NH3 (P=0.001), butyrate (P=0.002), and isovalerate (P=0.032). The decomposition of the interaction for the first parameter (NH3), shows an inverse shape of the curve on orogastric sample and slaughter sample, the results for increased levels of yerba-mate inclusion treatment is 21.72ab, 23.88a, 19.29ab, 16.42b mMol and 33.27ab, 29.03a, 38.44bc, 41.79c mMol, respectively. A similar aspect of the shape were observed for butyrate, 10.84a, 9.05b, 12.20a, 8.17b mMol and 4.31ab, 3.49a, 4.59ab, 5.21b mMol, respectively. Also for isovalerate paramenter, where we found 1.39, 1.36, 1.07, 1.13 mMol and 2.20a, 1.78b, 2.14ab, 2.32a mMol, for all four treatments sampled by orogastric system and at the slaughter. Lower-case superscript letter immediately after the number represent the mean comparison analysis.

Productive and methane parameters

Table 3.3. Productive parameters and methane emission results from 24 growing lambs fed with increased levels of Yerba-mate extract.

		Level of inclusion (%)					*P value*[3]		
Parameter[1]	Unit	0	1	2	4	EPM[2]	Lin	Quad	Cub
DMI	g	1192	1257	1200	890	69.0	0.003	0.049	0.822
ILW	kg	35.09	35.19	36.03	31.51	0.73	0.002	0.012	0.289
FLW	kg	36.67	36.77	37.75	32.65	0.81	0.002	0.011	0.271
DWG	g	226	225	245	164	12.5	0.003	0.010	0.172
CH4GD	g day^{-1}	27.30	31.65	30.86	23.34	1.81	0.057	0.012	0.617
CH_4DMI	g kg^{-1}	25.48	25.47	25.77	26.74	1.84	0.603	0.863	0.978
CH_4DWG	g kg^{-1}	133.39	140.89	126.81	132.24	8.99	0.727	0.880	0.301
CH_4LW	g kg^{-1}	0.83	0.88	0.83	0.73	0.06	0.134	0.353	0.629
EBCH_4	MJ kg^{-1}	1.52	1.76	1.72	1.30	0.10	0.057	0.012	0.617
YM	%	7.97	7.95	8.05	8.32	0.58	0.638	0.879	0.969

[1]DMI - dry matter intake; ILW - initial live weight; FLW - final live eight; DWG - daily weight gain; CH4GD - daily emission of methane; CH4DMI - emission of methane by kg of DMI; CH4DWG - emission of methane by kg of weight gain; CH4LW - emission of methane by live weight; EBCH4 - energy loss by methane emission; YM - yield methane.
[2]MSE – mean square error.
[3]Values from orthogonal contrast (linear, quadratic, and cubic) are significantly different if P≤0.05 and tendency if 0.05<P≤0.10.

Source: own authorship

Results of methane emission and performance are presented in table 3.3., at the week of methane collection, feed intake (P=0.049) of the 24 animals had a quadratic effect

by the treatments. A similar result were observed at the initial weight (P=0.012), final weight (P=0.011), and daily weight gain (P=0.010). Daily methane production (P=0.012) and energy loss by methane emission (P=0.012) had also a quadratic effect by the treatment, however, the treatment does not affect methane by productivity unit and yield methane (P>0.05).

Microbiome analysis

Quality control, denoising, and chimera exclusion retained 10,665,497 cleaned fragments (3,308,372 paired end reads for bacteria, 3,969,501 for archaea, and 3,387,624 for protozoa). After exclusion of singletons, 6,652 ASVs (5,706 ASVs for bacteria, 276 ASVs for archaea, and 670 ASVs for protozoa) were retained.

Rarefaction curves (figure 3.1.) using alpha diversity metrics Shannon-Wiener reached a plateau which indicates that the sampling depth was adequate, and additional sequences would not likely result in additional features. In order to compare microbiome diversity, the data was rarefied to 28,000 reads for bacteria, 25,000 reads for archaea, and 19,000 reads for protozoa, based on the rarefaction curves.

Comparisons between microbiomes (figure 3.1, 3.2, and 3.3) using alpha-diversity metrics (Faith's Phylogenetic Diversity index) shows no differences between groups of treatments (control, 1, 2, and 4%) on initial and final sampling (P>0.05). The same response were obtained from contrast by group between initial and final sampling for protozoa and archaea (P>0.05), however, significant differences on alpha-diversity metrics were obtained for bacterial community on the treatment control, 1 and 2% of inclusion of yerba-mate extract (P<0.012).

Figure 3.1. Rarefaction plot of (A) bacteria, (B) archaea, and (C) protozoa, red line is the sampling death.

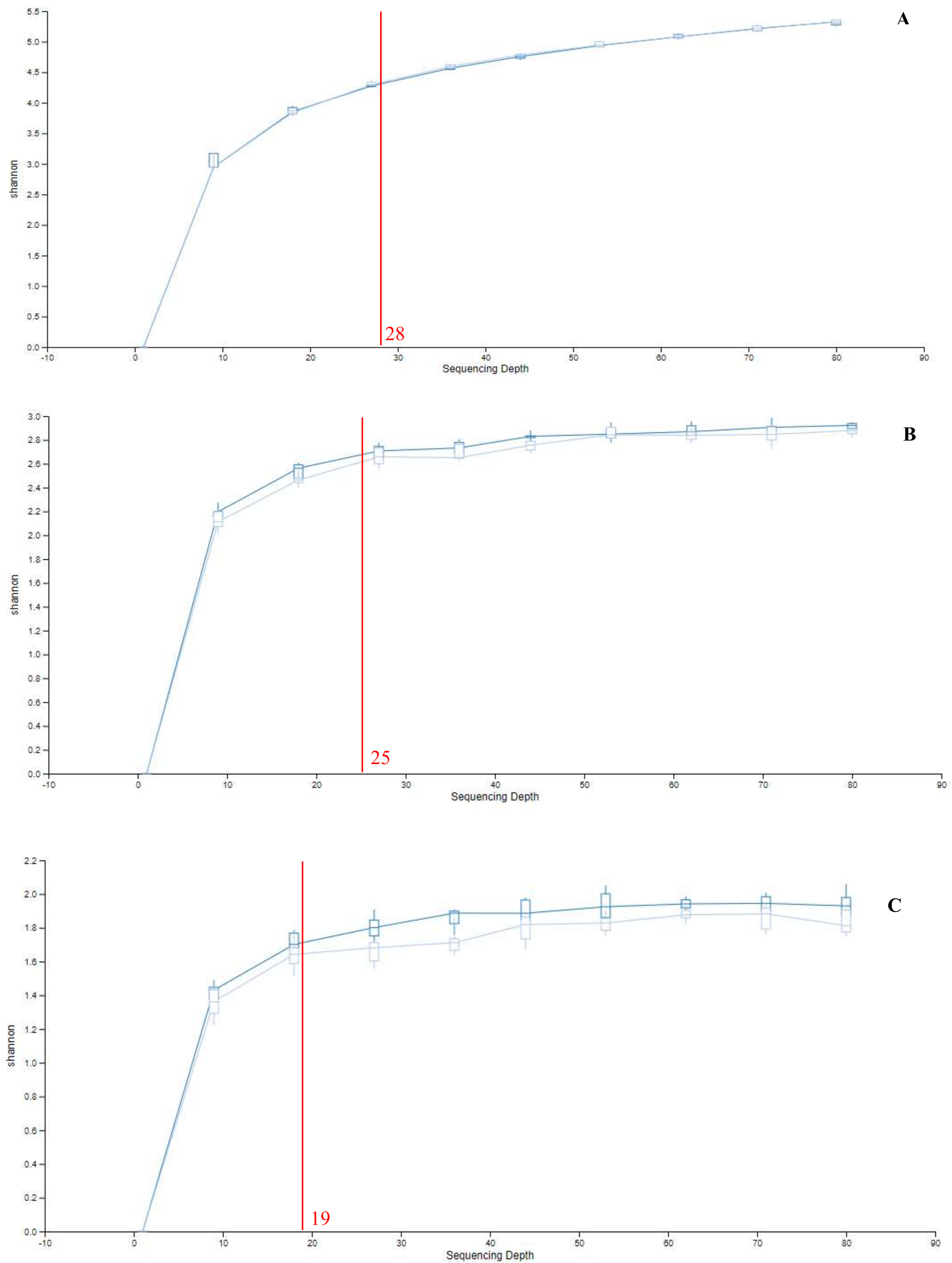
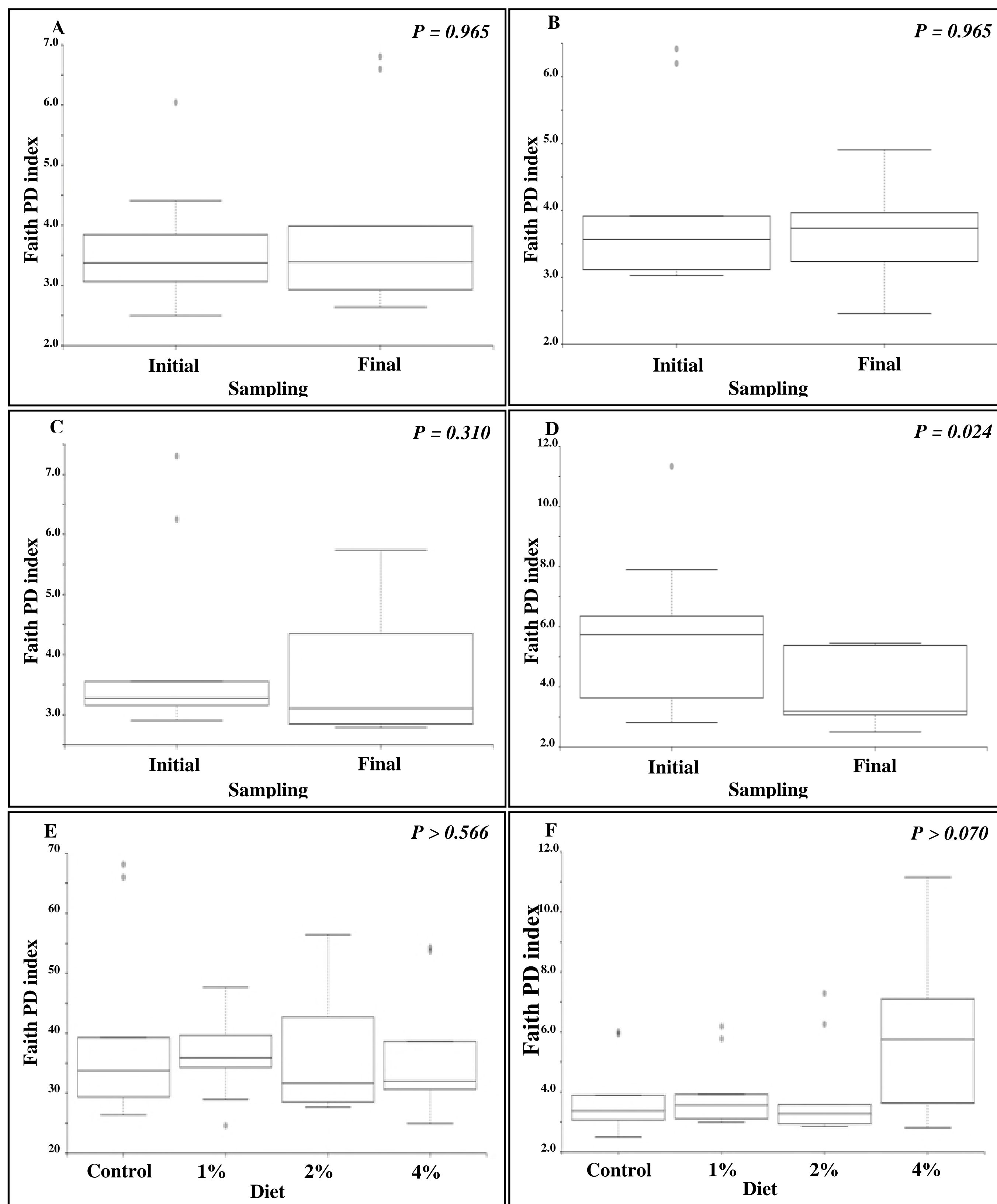
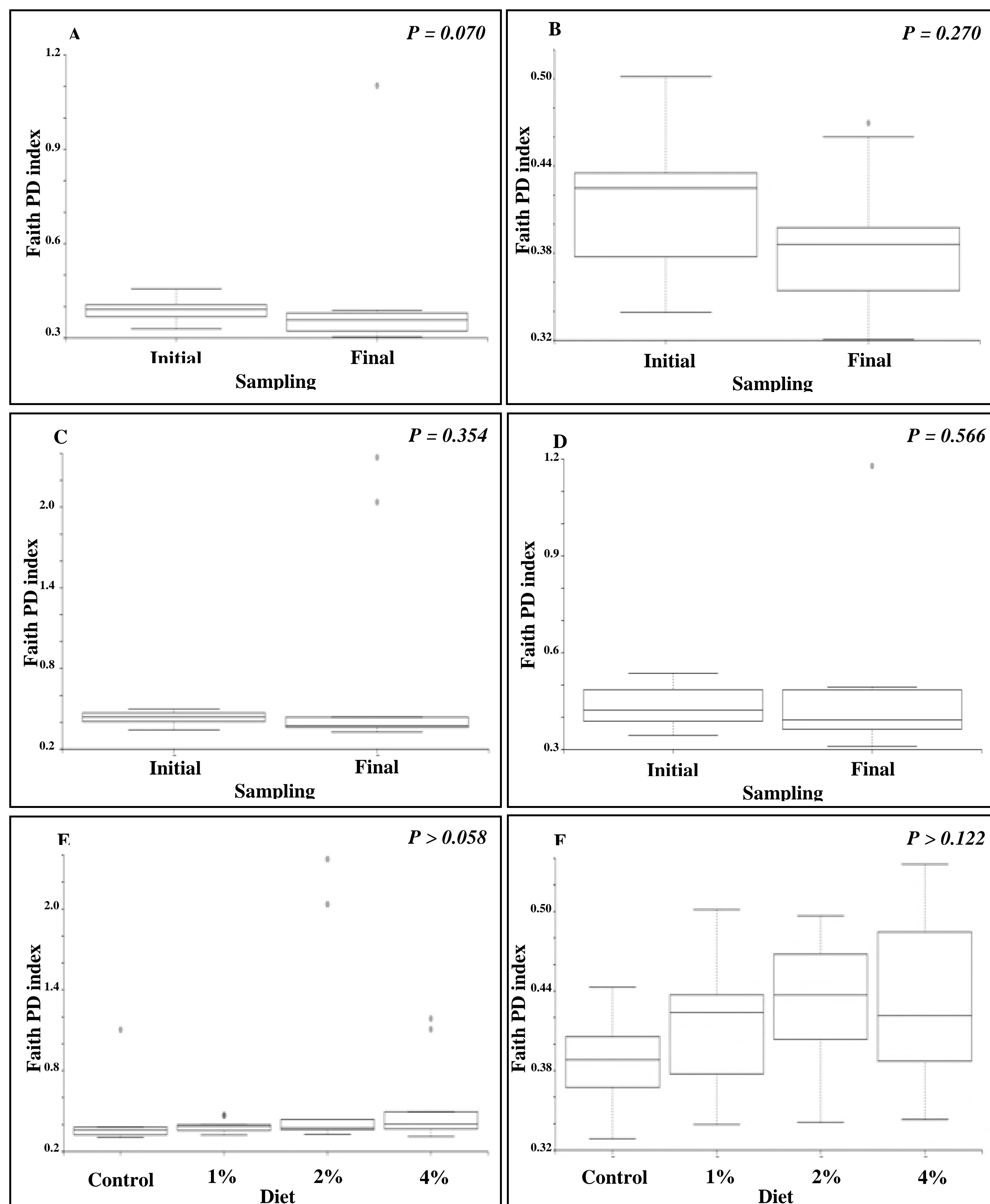


Figure 3.2. Archaea alpha diversity calculated by Faith's Phylogenetic Diversity index, contrast between initial and final sampling on (A) control, (B) 1%, (C) 2%, and (D) 4%, and contrast between treatments at (E) initial and (F) final sampling.



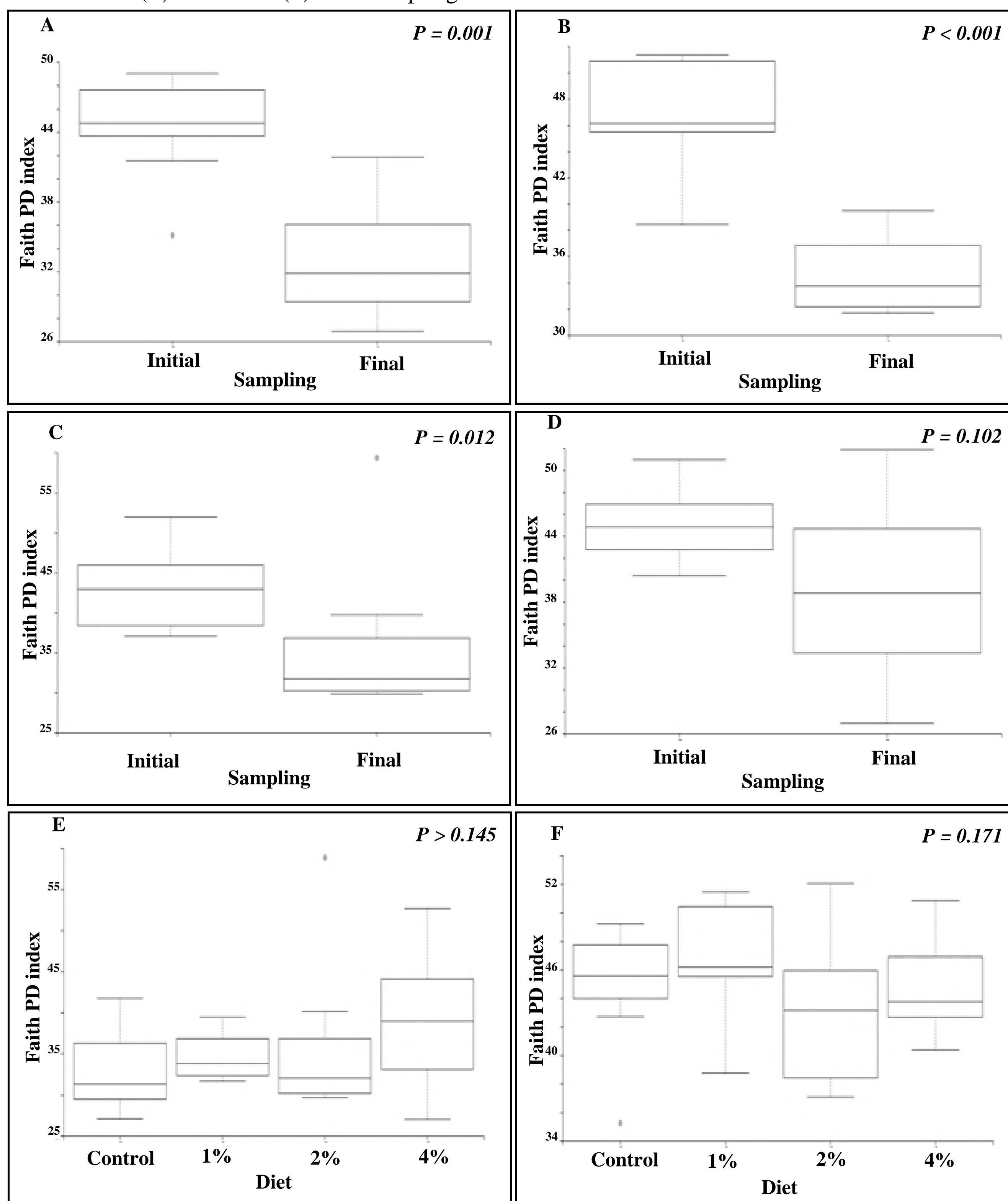
Source: own authorship

Figure 3.3. Protozoa alpha diversity calculated by Faith's Phylogenetic Diversity index, contrast between initial and final sampling on (A) control, (B) 1%, (C) 2%, and (D) 4%, and contrast between treatments at (E) initial and (F) final sampling.



Source: own authorship

Figure 3.4. Bacteria alpha diversity calculated by Faith's Phylogenetic Diversity index, contrast between initial and final sampling on (A) control, (B) 1%, (C) 2%, and (D) 4%, and contrast between treatments at (E) initial and (F) final sampling.



Source: own authorship

DISCUSSIONS

Apparent digestibility coefficient

The results presented above shows an increase on a general nutrient intake, that increased response is probably due to the hypothesis that moderates quantities of dietary inclusion of yerba-mate extract can stimulate animal's feed ingestion, explained by Po et al. (2012), in their study using growing lambs fed with addiction of 2.5% of yerba-mate extract increase dry matter intake. However, results presented by Zawadziki et al. (2017), shows that inclusions of yerba-mate extract on steers diet up to 1.5% do not change dry matter intake.

In the study published by Po, Xu, and Celi (2012), dorper ewes were fed with 2.5% of yerba-mate extract, and comparing to the control, yerba-mate extract decrease ingestion few weeks after partum, it indicates that yerba-mate extract can act differently according to the physiological status of the animal. In additional, important differences is observed by different extractions methods and also by the plant anatomic structure, physiological status, and other variables inherent to the plant used as substrate on extract production, and it must have different effects on animal nutrition.

The apparent digestibility coefficient from the nutrients presented above shows a great general digestibility, according to the literature (NKOSI et al., 2011; KHAN et al., 2014; MALEKKHAHI et al., 2014; KHAN et al., 2011; RAZAEI et al., 2014, SCHULZ et al., 2018, HABELO et al., 2018).

The apparent digestibility of the fat components were decreased by the inclusion of the yerba-mate extract up to 2%, its characteristic could be explained by the literature (DICKEL et al., 2007; BRACESCO et al., 2011). Those authors attribute this effect to the caffeine and saponin (most abundant components of the yerba-mate extract) on the delay of the intestinal absorption of the fat. It also corroborates with blood parameters

that had a decrease on triglycerides and very low-density lipoprotein on lambs fed up to 2% of yerba-mate extract (data not presented).

Now looking to the protein metabolism, as is knew, the protein from high quality feed in ruminants nutrition is solubilized during the mastication processes, consequently, a large quantity of soluble protein is degraded by rumen microorganisms (PATRA and SAXENA, 2011). On this study, no effect of the treatment were observed on total tract apparent digestibility of the protein, that could be explained by the fact that yerba-mate extract can reduce feed protein degradability in the rumen, hypothesis tested and proved by Hartemink et al. (2015). The yerba mate extract is rich on hydrolysable tannins (ZAWADZIKI et al., 2017), and these compounds were tested by Tabacco et al. (2006), and a positive effect on reduction of rumen protein degradability were reported. However, no improvement on absorption of the protein in the intestine were observed, in the other hand, more high quality protein reach the intestine and could be absorbed by the animal.

Fiber (TF and NDF) digestibility shows increase with animals fed up to 2% of yerba-mate extract. Fiber digestibility is carried out by the fibrolitic microorganisms in the rumen, that class of organisms colonize plant cell walls and produce fibrolitic enzymes. The host animal cannot produce that enzyme, thus the NDF digestion is carried out in its majority on the rumen (NOZIÈRE et al., 2010).

Dry matter intake and fiber digestion have a negative correlation, when the animal have a great intake, that means a great rate of passage is observed, in this case, particles stay less time in the rumen and fiber digestion decrease (SNIFFEN et al., 1992). In our case, increasing on dry matter intake and also increase on total fiber and NDF digestion could be observed, that response could be explained by the antimicrobial activity of the yerba-mate extract (MARTIN et al, 2013; BURRIS et al., 2011; TSAI et al., 2010). Maybe, yerba mate extract cannot changes the alpha-diversity of the ruminal microbiome,

however, it can inhibit partially the growth of lactating-producing bacteria. But, deeper studies must be carried out to clarify the antimicrobial activity of the yerba-mate.

Ruminal metabolism

A strong difference between sampling type were observed. That response is explained by the time of the sampling, the first collection by orogastric tube were carried out 2h after morning feed, responses must be a low pH and ammoniac nitrogen, and a high concentration of short-chain fatty acids, because of the high fermentation ratio. In the other hand, the second sampling type were carried out after 16h of fasting, without feed flow, parameters should be the opposite of the first sampling, according to the results obtained by Devant et al. (2000) and Queiroz et al. (2012).

In general, no differences were observed by the treatment on ruminal metabolism, except on absolute and relative quantity of butyrate and absolute quantity of isovalerate. That iso-acid is a marker for branched-chain amino acids fermentation, in this case, a reduction on isovalerate indicates a reduction on deamination of the aminoacids (VARGAS et al., 2001). That result support the hypothesis that yerba-mate can reduce protein degradation in the rumen, and protein with high quality reach the real stomach and could be absorbed (hypothesis explained in the previous section).

Methane parameters

Daily methane emission corroborates with the literature (SAVIAN et al., 2014; SUN, et al., 2012; HAMMOND et al., 2011; ULYATT et al., 2005; PINARES-PATIÑO et al., 2003; ULYATT et al, 2002; PELCHEN, PETERS, 1998), daily emission could range from 12.2 to 37.3g CH4 per animal per day. Moreover, emission by animals fed yerba-mate extract up to 2% had an increased response, these results could be explained by the increased dry matter intake and increased digestibility of the nutrients.

A contradiction by the results comparing to the literature is observed, because most studies that have objective to compare the relationship between feed intake and methane emission, shows that increasing on feed intake must reduce methane emission per unit of feed eaten (PINARES-PATIÑO et al., 2003; HART et al., 2009; YAN et al., 2010). However, in this study, we could observe an increasing on daily methane emission, but, no differences for methane yield per unit of feed intake or per unit of gain on live weight.

Microbiome analysis

First observation for this subject is a reduction on bacterial diversity between the beginning and end of the period, before start the experiment, all animals were placed into a pasture and bring to the experimental building, 3 days after the initial sampling were carried out. The results shows, at that point a higher bacterial alpha diversity could be determined. At the end of the period, a reduction on bacterial alpha diversity is presented. This response could be explained by the adaptation period, the animals were fed with the control diet only 3 days, and it suggest not be enough, a reduction on alpha diversity by increasing the concentration of the concentrate is a normal response, according to the literature (LIU et al., 2019; GRILLI et al., 2016; PETRI et al., 2012).

No diversity differences were observed by group of treatments, even with the proved antimicrobial activity of the yerba-mate (MARTIN et al, 2013; BURRIS et al., 2011; TSAI et al., 2010). Those results could be explained by the idea that yerba-mate have no power to eliminate some types of microorganisms from the microbiome, but they decrease its growth rates and consequently its action in the feed substrates. However, more deep bioinformatics analyses are need to confirm that hypothesis.

CONCLUSIONS

Including yerba-mate extract up to 2% of the dry matter intake must increase intake of the nutrients by lambs, and increase digestibility of dry matter, total fiber, non-nitrogen extract, and NDF. The extract could reduce ether extract digestibility, probably because of the inhibition of the absorption of fat compounds in the gut. Reduction on deamination of amino acids were decreased by the yerba-mate extract, and no effects on methanogenesis and general ruminal fermentation were caused by the extract. No differences on alpha diversity were observed by the treatments, however, more analysis are needed to clarify the effect of the yerba-mate extract on microbiome composition.

REFERENCES

PELCHEN, A.; PETERS, K.J. Methane emissions from sheep. **Small Ruminant Research**, v. 27, p. 137-150, 1998.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis**. 14th ed. 4 rev. Arlington, VA: The Association, 1990. 2 v.

BACH, A.; CALSAMIGLIA, S.; STERN, M.D. Nitrogen metabolism in the rumen. **Journal of Dairy Science**, v. 88, p. E9–21, 2005.

BASTOS, D.H.M. et al. Yerba Mate: Pharmacological Properties, Research and Biotechnology. **Medicinal and Aromatic Plant Science Biotechnology**, v. 1, p. 37–46, 2007.

BERNDT, A. et al. **Guidelines for use of Sulphur hexafluoride (SF6) tracer technique to measure enteric methane emissions from ruminants.** Wellington: Ministry for Primary Industries, 166 p. v. 1, 2014.

BERTÉ, K.A.S. et al. Chemical composition and antioxidant activity of yerba-mate (Ilex paraguariensis A. St.-Hil., Aquifoliaceae) extract as obtained by spray drying**. Journal of Agricultural and Food Chemistry**, v. 59, p. 5523-5527, 2011.

BOLYEN E. et al. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. **PeerJ Preprints**, 6:e27295v2, 2018.

BONANNO, A. et al. Intake and milk production of goats grazing Sulla forage under diferentes stocking rates. **Italian Journal of Animal Science**, v. 6, p. 605-607, 2007b.

BONANNO, A., et al. **Grazing sulla and/or ryegrass forage for 8 or 24 hours daily. Effects on ewes feeding behaviour. Permanent and temporary grassland: plant,**

environment and economy. In: Proceedings of the 14th Symposium of the European Grassland Federation. P. 208-211, 2007a.

BRACESCO, N. et al. Recent advances on Ilex paraguariensis research: minireview. **Journal of Ethnopharcology**, v. 136, p. 378-384, 2011.

BURRIS, K.P. et al., Antimicrobial activity of yerba mate (Ilex paraguariensis) aqueous extract against Escherichia coli O157:H7 and Staphylococcus aureus. **Journal of Food Science**, v. 76, 2011.

PINARES-PATIÑO, C.S. et al. Persistence of differences between sheep in methane emission under generous conditions. **Journal of Agricultural Science**, v. 140, p. 227-233, 2003.

CALLAHAN, B.J. et al. DADA2: High-resolution sample inference from Illumina amplicon data. **Nature Methods**. v. 13, p. 581–583, 2016.

CLIFFORD, M.N.; RAMIREZ-MARTINEZ, J.R. Chlorogenic acids and purine alkaloids contents on maté (Ilex paraguaiensis) leaf and beverage. **Food Chemistry**, v.35, p. 13-21, 1990.

COELHO G.C. et al. Quantification of Saponins in Extractive Solution of Mate Leaves (Ilex paraguariensis A. St. Hil.). **Journal of Medicinal Food**, v. 13, p. 439–443, 2010.

DE MEJÍA, E.G. et al.Yerba mate tea (Ilex paraguariensis): Phenolics, antioxidant capacity and in vitro inhibition of colon cancer cell proliferation. **Journal of Functional Foods**, v. 2, p. 23-34, 2010.

DEVANT, M. et al. Effects of protein concentration and degradability on performance, ruminal fermentation, and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100 to 230 kg body weight. **Journal of Animal Science**, v.78, p.1667-1676, 2000.

DICKEL, M.L.; RATES, S.M.K.; RITTER, M.R. Plant popularly used for losing weight purposes in Porto Alegre, South Brazil. **Journal of Ethnopharcology**, v. 109, p. 60-71, 2007.

FAITH, D.F. Conservation evaluation and phylogenetic diversity. **Biological Conservation**, v. 61, pp. 1-10, 1992.

FOLDAGER, J. **Protein requirement and non protein nitrogen for high producing cow in early lactation.** 1977. Tese (Doutorado em Ciência Animal) – Michigan State University, East Lasing, MI. 1977.

FRIEDMAN, J.; ALM, E.J. Inferring Correlation Networks from Genomic Survey Data. **PLoS Computational Biology,** v. 8, p. 1–11, 2012.

GETACHEW, G. G. et al. Laboratory variation of 24h in vitro gas production and estimated metabolizable energy values of ruminant feeds. **Animal Feed Science and Technology**, v. 102, p. 169-180, 2002.

GNOATTO, S.C.B.; SCHENKEL, E.P.; BASSANI, V.L. HPLC method to assay total saponins in Ilex paraguariensis aqueous extrac. **Journal of the Brazilian Chemical Society**, v. 16, p. 723-725, 2005.

GOSMANN, G. et al. Phenolic Compounds from Maté (Ilex paraguariensis) Inhibit Adipogenesis in 3T3-L1 Preadipocytes. **Plant Foods for Human Nutrition**, v. 67, p. 156-161, 2012.

GRILLI, D.J. et al. Analysis of the rumen bacterial diversity of goats during shift from forage to concentrate diet. **Anaerobe**, v. 42, p. 17-26, 2016.

HABELO, C.H.S. et al. Performance of finissing beef cattle fed diets containing maize silage inoculated with lactic-acid bacteria and Bacilus subtilis. **Animal Production Science**, v. 59, p. 266-276, 2018.

HARTEMINK, E. et al. The effect of Yerba Mate (Ilex Paraguariensis) suplementation nutriente degradability in dairy caws: Na In sacco and In vitro study. **Asian-Australasian Journal Animal Science**, v. 28, p. 1606-1613, 2015.

HARTEMINK, E. et al. The effect of yerba mate (Ilex Paraguariensis) supplementation on nutrient degradability in dairy cows: an in saco and in vitro study. **Asian-Australian Journal of Animal Science**, v. 28, p. 1606-1613, 2015.

JOHNSON, K. et al. Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique. **Environmental Science & Technology**, v. 28, n. 2, p. 359–362, 1994.

HAMMOND, K.J. et al. Effects of feeding fresh white clover (Trifolium repens) or perennial ryegrass (Lolium perenne) on enteric methane emissions from sheep. **Animal Feed Science and Technology**, v. 166, p. 398-404, 2011.

HART, K.J. et al. Effect of sward dry matter digestibility on methane production, ruminal fermentation, and microbial populations of zero-grazed beef cattle. **Journal of Animal Science**, v. 87, p. 3342-3350, 2009.

KEARL, L. C. **Nutrient requirements of ruminants in developing countries.** Logan: International Feedstuffs Institute, pp. 381, 1982.

KHAN, S.H. et al. Nutrient intake, digestibility, nitrogen balance and growth performance of sheep fed diferente silages with or without concentrate. **Tropical Animal Health and Production**, v. 43, p. 795-801, 2011.

KITTELMANN, S. et al. Phylogeny of Intestinal Ciliates, Including Charonina ventriculi, and Comparison of Microscopy and 18S rRNA Gene Pyrosequencing for Rumen Ciliate Community Structure Analysis. **Applied Environmental Microbiology**, v. 81, p. 2433-2444, 2015.

KITTELMANN, S. et al. Simultaneous amplicon sequencing to explore co-ocorrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. **Plos One**, v. 8, 2013.

KLINDWORTH, A. et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. **Nucleic Acids Research**, v. 41, 2013.

KRAN, N.A. et al. Nutritive value of maize silage in relation to dairy cow performance and milk quality. **Journal of the Science of Food and Agriculture**, v. 95, 2014.

LIU, H. et al. Effect of dietary concentration to forage ratio on growth performance, rumen fermentation and bacterial diversity of Tibetan sheep under barn feeding on Qinghai-Tibetan plateau. **Peerj**, v. 7, 2019.

ULYATT, M.J. et al. Methane emission from sheep grazing four pastures in late summer in New Zealand. **New Zealand Journal of Agriculture Research**, v. 48, p. 385-390, 2005.

ULYATT, M.J. et al. Methane emission from dairy cows and wether sheep fed subtropical grass-dominant pastures in midsummer in New Zealand. **New Zealand Journal of Agriculture Research**, v. 45, p. 227-234, 2002.

MALEKKHAHI, M. et al. Effect of essential oils, yeast culture and malate on rumen fermentation, blood metabolites, growth performance and nutrient digestibility of Baluchi lambs fed high-concentrate diets. **Journal of Animal Physiology and Animal Nutrition**, v. 99, 2014.

MARTIN, J.G.P. et al. Antimicrobial activity of yerba mate (Ilex paraguariensis St. Hil.) against food pathogens. **Revista Argentina de microbiología**, v. 45, p. 93-98, 2013.

MCCANN, J.C.; ELOLIMY, A.A.; LOOR, J.J. Rumen microbiome, probiotics, and fermentation additives. **Veterinary Clinics: Food Animal Practice**, v. 33, p. 539-553, 2017.

MERTENS, D.R. Gravimetric determination of amylase treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. **Journal of AOAC International**, v. 85, p.1217-1240, 2002.

NKOSI, B.D. et al. Effects of bactéria silage inoculants on whole-crop maize silage fermentation and silage digestibility in rams. **South African Journal of Animal Sciences**, v. 41, p. 350-360, 2011.

NOZIÈRE, P. et al. Carbohydrate quantitative digestion and absortion in ruminants: from feed starch and fiber to nutrients available for tissues. **Animal**, v. 4, p. 1057-1074, 2010.

NRC – National Research Council. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. **The National Academies Press**, Washington DC, 384 p., 2007.

PATRA, A.K. Enteric methane mitigation Technologies for ruminant livestock: a synthesis of current research and future directions. **Environmental Monitoring and Assessment**, v. 184, p. 1929-1952, 2012.

PATRA, A.K.; SAXENA, J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. **Journal of Food and Agriculture**, v. 91, p. 24-37, 2011.

PETRI, R.M. et al. Characterization of rumen bacterial diversity and fermentation parameters in concentrate fed cattle with and without forage. **Journal of Applied Microbiology**, v. 112, 2012.

PO, E. et al. Yerba Mate (Ilex paraguariensis) as a novel feed supplement for growing lambs. **Small Ruminant Research**, v. 106, p. 131-136, 2012.

PO, E.; XU, Z.; CELI, P. The effect of Yerba Mate (Ilex paraguarensis) supplementation on the productive performance of Dorper Ewes and their progeny. **Asian-Australasian Journal Animal Science**, v. 25, p. 945-949, 2012.

QUEIROZ, M.F.S. et al. Metabolism and ruminal parameters of Holstein x Gir heifers fed sugarcane and increasing levels of crude protein. **Revista Brasileira de Zootecnia**, v. 41, 2012.

GRUNINGER, R.J. et al. Anaerobic fungi (phylum Neocallimastigomycota): advances in understanding their taxonomy, life cycle, ecology, role and biotechnological potential. **FEMS Microbiology Ecology**, v. 90, p. 1-17, 2014.

REZAEI, J. et al. Effect of substituting amaranth silage for corn silage on intake, growth performance, diet digestibility, microbial protein, nitrogen retention and ruminal fermentation in fattening lambs. **Animal Feed Science and Technology**, v. 192, p. 29-38, 2014.

RUSSELL, J.B.; RYCHLIK, J.L. Factors that alter rumen microbial ecology. **Science**, v. 292, p. 1119-1122, 2001.

SALDANA, M.D.A. et al. Extraction of purine alkaloids from maté (Ilex paraguariensis) using supercritical CO2. **Journal of Agricultural and Food Chemistry**, v. 47, p. 3804-3808, 1999.

SAVIAN, J.V. et al. Grazing intensity and stocking methods on animal production and methane emission by grazing sheep: Implications for integrated crop–livestock system. **Agriculture, Ecosystems & Environment**, v. 190, p. 112-119, 2014.

SCHENKEL, E.P. et al. Saponins from maté (Ilex paraguariensis) and other South American Ilex species: ten years research on Ilex saponins. **Ciência e Cultura**, v. 49, p. 359-363, 1997.

SCHULZ, F. et al. Replacing maize silage plus soybean meal with red clover silage plus wheat in diets for lactating dairy cows. **Journal of Dairy Science**, v. 101, p. 1216-1226, 2018.

SEEDORF, H. et al. RIM-DB: a taxonomic framework for community structure analysis of methanogenic archaea from the rumen and other intestinal environments. **PeerJ**., v. 2, 2014.

SNIFFEN, C.J. et al., A net carbohydrate and protein system for evaluating cattle diets. **Journal of Animal Science**, v. 70, p. 3562-3577, 1992.

YAN, T. et al. Mitigation of enteric methane emissions through improving efficiency of energy utilization and productivity in lactating dairy cows. **Journal of Dairy Science**, v. 93, p. 2630-2638, 2010.

TABACCO, E. et al. Effect of Chestnut Tannin on fermentation quality, proteolysis, and protein remen degradability of alfalfa silage. **Journal of Dairy Science**, v. 89, p. 4736-4746, 2006.

TSAI, T.H. et al. In vitro antimicrobial and anti-inflamatory effects of herbs against Propionibacterium acnes. **Food Chemistry**, v. 119, p. 964-968, 2010.

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergente fiber, and non-starch polysaccharides in relation to animal nutrition. **Journal Dairy Science**, v.74, p.3583-3597, 1991.

VARGAS, L.H. et al. Influencia de Rumensin, óleo de soja e níveis de concentrado sobre o consumo e os parâmetros fermentativos ruminais em bovinos. **Revista Brasileira de Zootecnia**, v. 30, p. 1650-1658, 2001.

WESTBERG, H.H. et al. **A SF6 tracer technique: methane measurement from ruminants.** Pullman: Washington State University, 1998. 40p.

SUN, X.Z. et al. Sheep fed forage chicory (Cichorium intybus) or perennial ryegrass (Lolium perenne) have similar methane emissions. **Animal Feed Science and Technology**, v. 172, p. 217-225, 2012.

ZAWADZKI, A. et al. Mate extract as feed additive for improvement of beef quality. **Food Research International**, v. 99, p. 336-347, 2017.

ZHONG, R.Z., et al. Effects of dietary supplementation with green tea polyphenols on digestion and meat quality in lambs infected with Haemonchus contortus. **Meat Science**. p. 1-7, 2015.

CHAPTER 4 - Effect of levels of dietary inclusion of yerba-mate extract on physicochemical properties of lamb meat

RESUMO

O uso de aditivos nutricional com o intuito de modular características dos produtos de origem animal é uma pratica bastante utilizada, no entanto, nos últimos anos uma busca por aditivos naturais e que evitem contaminações químicas e deletérias ao meio ambiente vem movimentando a pesquisa e indústria da carne. Com este intuito, o presente trabalho tem o objetivo de avaliar o potencial uso do extrato de erva-mate como um modulador dos parâmetros de qualidade de carne e sua possível utilização para produção de carnes para um o nicho de mercado de carnes funcionais. Foram utilizados 36 cordeiros machos e não castrados, que foram divididos em 4 grupos, sendo o primeiro alimentado com uma dieta controle, ou seja, sem adição de extrato, e os outros 3 grupos foram alimentados com dietas contendo inclusões crescentes de extrato de erva-mate (1, 2 e 4%). Os animais permaneceram por 53 dias em confinamento e foram abatidos. Logo após o abate, uma amostra do musculo *longíssimos thoracis* foi coletado e armazenado em freezer biológico para análise de metabolômica. As carcaças foram então armazenadas à 4°C e 24h depois foram desossadas, e o corte cárneo proveniente da região do musculo *longíssimos thoracis* foi coletado para as análises de qualidade de carne (composição centesimal, pH, cor, maciez, capacidade de retenção de agua, perdas por cocção, etc.). As análises dos parâmetros de qualidade da carne foram avaliados utilizando o software SAS, e analisados pelo procedimento GLM. Os dados de metabolômica foram analisados pela plataforma MetaboAnalyst 3.0. Os resultados dos parâmetros de qualidade de carne mostram que a composição centesimal teve uma resposta quadrática, na maioria dos aspectos, indicando que animais alimentados com dietas com 1 e 2% de extrato apresentam uma menor quantidade de agua e consequentemente uma maior concentração de materiais orgânicos (como gordura e proteína). Já os parâmetros de qualidade físico-química da carne,

animais que foram alimentados com dietas contendo 1 e 2% de extrato de erva-mate apresentaram-se mais macia e clara (parâmetro L* maior), no entanto, com o acréscimo na inclusão do extrato as perdas pela cocção foram aumentadas. As analises de metabolômica indicaram que existe diferença entre o perfil de metabólitos do músculo de animais que foram alimentados com dietas contendo 1 e 2% de extrato versus animais alimentados com a dieta controle e 4% de extrato, sendo que alguns metabólitos foram aumentados e são indicadores de melhorias na qualidade físico-química da carne e auxiliam na prevenção de doenças nos consumidores. Portando, podemos concluir que inclusão de extrato de erva-mate à níveis de até 2% podem ser indicados, porém mais estudos devem ser feitos para desvendar qual o mecanismo de ação desse extrato no organismo animal e seus efeitos na dietas dos consumidores.

Palavras-chave: alimento funcional; extrato de planta; qualidade de carne.

ABSTRACT

The use of nutritional additives in order to modulate characteristics of animal products is a widely used practice, however, in recent years a search for natural additives that avoid chemical and harmful contamination to the environment has been driving the meat research and industry. To this end, the present work aims to evaluate the potential use of yerba-mate extract as a modulator of meat quality parameters and its possible use for meat production for a functional meat market niche. Thirty-six male and non-castrated lambs were divided into 4 groups, the first fed a control diet, ie without the addition of extract, and the other 3 groups were fed diets containing increasing inclusions of yerba-mate extract (1, 2 and 4%). The animals were kept for 53 days in confinement and were slaughtered. Shortly after slaughter, a sample of the very long thoracis muscle was collected and stored in a biological freezer for metabolomics analysis. The carcasses were then stored at 4 ° C and 24h later were boned, and the meat cut from the *longíssimus*

thoracis muscle region was collected for meat quality analysis (centesimal composition, pH, color, tenderness, water hold capacity, cooking losses, etc.). Analyzes of meat quality parameters were evaluated using SAS software and analyzed by the GLM procedure. Metabolomics data were analyzed by the MetaboAnalyst 3.0 platform. The results of the meat quality parameters show that the centesimal composition had a quadratic response, in most aspects, indicating that animals fed diets with 1 and 2% of extract presented a smaller amount of water and consequently a higher concentration of organic materials. (such as fat and protein). The parameters of physical and chemical quality of meat, animals that were fed diets containing 1 and 2% yerba-mate extract were more tender and clear (parameter L *), however, with the addition of inclusion of the extract the cooking losses were increased. Metabolomics analyzes indicated that there is a difference between the muscle metabolite profile of animals fed diets containing 1 and 2% extract *versus* animals fed control diet and 4% extract, and some metabolites were increased and are indicators of improvements in the physicochemical quality of meat and assist in the prevention of disease in consumers. Therefore, we can conclude that inclusion of yerba mate extract at levels up to 2% may be indicated, but further studies should be done to understand the mechanism of action of this extract in the animal organism and its effects on the diets of consumers.

Key-words: functional food; meat quality; plant extract.

INTRODUCTION

Yerba mate (or only mate) is a kind of tea, made from an infusion of the dried and grounded leaves of *Ilex paraguariensis* plant. Its consumption is increasing around of the world and yerba mate is sold as individual tea bags and extract/concentrate for use as ingredient in the pharmaceutics, food or dietary supplement industries in many countries.

Different phytochemical compounds were found in the profile of composition of yerba mate. The two most important components of the mate, according to Pomilio et al. (2002) and Zaporozhets et al. (2004), are polyphenols and xanthine, followed by alkaloids, flavonoids, amino acids, minerals (P, Fe, and Ca), and vitamins (C, thiamine, and riboflavin). Kawakami and Kovayashi (1991) mention that mate has amount of phenolic compounds (*p*-coumaric and ferulic acid) and flavonoids in about 10% on the dry matter. Those biocompounds described in yerba mate have an important role in human health, including activity as an anticarcinogenics, antiobesity, antioxidant, antitumor, antibacterial, antidiabetic, diuretic, antialergic, analgesic, etc. (DUKE, 1992). On the last years, the use of those secondary compounds from plants in animal nutrition has increased, like an effort to promote those plants as tools to improve animal performance, quality of the products (milk and meat; VASTA; LUCIANO, 2011) and health of animals (FRANKIC et al., 2009) and consumers.

The World Cancer Research Fundation (WCRF) and the American Institute for Cancer Research (AICR) mention that intake of red meat is related to cancer risk (WCRF/AICR, 2007). Many mechanisms were proposed as a promoting effect of red meat on the cancer induction, including action of free radical generation due to the heme iron, damage of the DNA by N compounds, mutagens generation by grilling (CROSS et al., 2010) and consumption of sialic acid (N-glycolylneuraminic acid) produced by nonhuman mammals (SAMRAJ et al., 2015).

In the other hand, that food have an important dietary role on the human nutrition, because meat is a valuable source of nutrients, mainly cobalamin, zinc, iron and protein (WCRF/AICR, 2007), and then study strategies to decrease that risk from meat became a goal for many research groups.

The hypothesis of the study was that yerba mate extract can positively change instrumental parameters of acceptability (color, tenderness, centesimal composition, and weight and nutrient losses) of lamb meat and produce a functional meat, that decrease exposure of the consumers to some diseases related to red meat consumption. Objective was to evaluate the meat quality, composition, metabolic and lipid profile of the lamb meat fed with increased levels of inclusion of Yerba mate (*Ilex paraguariensis* St. Hilaire) extract.

MATERIAL AND METHODS

All the procedures using animal were evaluated and approved by the Ethic Committee on Animal Use of the College of Animal Science and Food Engineering – University of Sao Paulo (Protocol number CEUA 3497040618).

Location, animals and experimental design

The animals were born and raised in a commercial farm at Uberaba city at State of Minas Gerais, Brazil, and had access to pasture and feed supplementation on a creep-feeding system. After weaning they were transported to Pirassununga city, where the study was conducted at College of Animal Science and Food Engineering (FZEA), University of Sao Paulo (USP).

Thirty-six male lambs (crossbreed *Texel* x *Dorper* x *Santa Inês*) weighting 23.8±3.7 kg were used on this trial. The animals were weighted and assigned into nine groups, each group had 4 animals with similar weight and those animals was randomly assigned into one of experimental treatments, according to a randomized block design statement. Each animal was placed into a tie stall with free and individually access to water and feed.

Diet and experimental treatment

The animals were fed *ad libtum* twice a day at 8 am and 4 pm. Diets were calculated following the nutritional requirements for finishing lamb according to NRC (2007). Experimental treatments were one control diet and three diets with increased levels of inclusion of yerba mate extract (1, 2 and 4%) as described at Table 2.1.

The extract was produced by *Centro Flora Company* at Botucatu city at State of Sao Paulo, Brazil, from fresh leaves of *Ilex paraguariensis* A. St-Hil. Plant. Accordingly to *Centro Flora Company* the extracts were prepared using water:ethanol 75:25 v/v 90 °C, containing the total phenolic concentration of 21.7 g GAE/100 g extract and 6% (w/w) of caffeine, composition and characterization of this extract was well described on Zawadzki et al. (2017).

Slaughter and sample collection

The animals were raised per 53 days until obtain weight around 40 kg. Before slaughter, animals were submitted to 16h of solid feed fasting. The slaughter was carried out at the Experimental Slaughterhouse at FZEA/USP, for stunning was used a pneumatic pistol, the animals were hang up and bleeding procedure were carried out by the jugular vein. The skin, paws and head was removed from the carcasses and evisceration was made. After those processes the carcasses was weight and a muscle sample was collected from the right side of the carcass on the *Longissimus thoracis* (LT) region. All subcutaneous fat was removed from the sample and the muscles were quickly stored at liquid nitrogen and then at freezer (-80ºC) for metabolomics analysis. They were 72 samples of animals muscles in total, representing 36 animals collected in duplicate of the four groups of animals in analysis (mate extract levels 0%, 1%, 2%, and 4% w/w) containing 9 animals each. Then, carcasses were kept into a cold storage (2ºC) during around 24h. On the day after, a part of carcasses was used to collect steak samples from

LT region for meat quality and centesimal analysis, those samples were vacuum-packed and stored in a freezer (-20ºC).

Physicochemical Analysis

Meat was exposed for 30 minutes to oxygen, and then color (lightness – L*, redness – a*, and yellowness – b*) were measured in three different places, according the guidelines of American Meat Science Association (AMSA, 2012), using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc, Osaka, Japan). Some calculations were carried out: ratio of a*:b* were calculated as (a*/b*), chroma were calculated $(a^{*2}+b^{*2})^{1/2}$, and hue angle were calculated as arctangent(b*/a*). The pH of meat was measured in duplicate using a Testo pHmeter (205, Testo Inc., Sparta, NJ, USA) with a penetration electrode inserted into the steak sample.

The water holding capacity (WHC) was analyzed in duplicates according to Hamm (1960), 2 grams of meat sample were placed in a folded paper filter and between two acrylic plates, and subjected to 10 kg of pressure for 5 minutes, and weighted again. The cooking weight loss (CWL) was measured in samples with 2.5 cm of thickness; those samples were weighted and placed into a preheated grill covered with aluminum foil. The internal temperature of the meat sample was measured by individual thermocouples (Flyever Ltda., São Carlos, São Paulo, Brazil) placed into each sample, until internal temperature reach 71 °C, leave cooling and weighted again, according to Wheeler et al. (2005). The result from WHC and CWL shows the difference between the initial and final weight, the unit used was w/w.

From the cooked samples in the CWL analysis, at the minimum of three cubic subsamples was collected and cut with length of 110 mm. The subsamples were placed with the fibers perpendicular to the Warner-Bratzler shear device, connected to a TA-

XT2i texture analyzer (Stable Micro Systems, LTD., Godalming, UK) to measure shear force (SF) (WHEELER et al., 2005), the unit used was Newton (N).

Another steak sample was processed to remove the subcutaneous fat and that meat were lyophilized to obtain homogeneous and reduced-moisture samples, and the centesimal composition was determined according to AOAC (1990). Dry matter (DM, method 934.01), ash (ASH, method 923.03), and total fat (TF, method 920.85) were measured, and crude protein (CP, method 920.87) were analysed by Kjeldahl method. Moisture content (MC) were calculated subtracting DM from 100, as well organic matter (OM) were calculated subtracting ASH from 100, the ratio between OM:CP were calculated as (CP/OM), and the ratio between OM:TF were calculated as (TF/OM).

Metabolites extraction

Approximately 0.3 g of lamb muscle were homogenized with 1.2 ml of a solvent mixture (methanol:chloroform:water 2:2:1 v/v/v) (BLIGH; DYER, 1959) using a commercial cell disruptor (FastPrep®, MP Biomedicals) for 1 min . Homogenates were centrifuged for 10 min at 10,000 g at 10 °C. The chloroform phase was carefully separated from the hydroalcoholic phase (polar metabolites), and reserved. To the hydroalcoholic phase chloroform was added (0.3 ml), then agitated for 1 min (vortex), centrifuged at the previously condition, and after the separation of the solvent phases, the chloroform phase was added together to the reserved phase. The hydroalcoholic phase (polar metabolites) had the solvent evaporated in a centrifugal concentrator during 12h (Speed-Vac, Thermo Savant). The extracts were measured in an analytical balance.

NMR analysis

The dried extract containing the polar metabolites was re-suspended in 600µl of deuterium oxide phosphate buffer (0.10 M, pD = 7.3) containing 0.05%

w/w of sodium 3-trimethylsilyl-2,2,3,3-d4-propionate (TMSP-d4, from Cambridge Isotopes, Leicestershire, UK). 550µl were transferred to a 5 mm NMR tube and analyze.

Metabolomics NMR analysis were conducted at 298 K on a 14 T Bruker Avance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 5 mm PABBO probe head with gradients, automated tuning and matching accessory (ATMATM), BCU-I for regulation of temperature, and a Sample-Xpress sample changer. First a protocol was acquired (calibrated 90° pulse and irradiation at the water frequency), and then this protocol was performed in fully automatic mode using Bruker routines (load, automatic tuning, locking, phase, shimming, acquisition, process) by ICON-NMR interface for each sample. The spectra were acquired for proton NMR using 90° pulse (zg sequence), 64 K data points, with a spectral width of 20.0276 ppm, an acquisition time of 2.726 s, were acquired a receiver gain of 2.25, a recycle delay of 1 s, dummy scans of 0, an accumulation of 16 transients. The water suppression was achieved using the NOESY-presaturation pulse sequence (Bruker 1D noesygppr1d pulse sequence) using the same data points, spectral width and acquisition time of proton experiments, with irradiation at the water frequency (O1 around 2821.30 Hz depending of QC) with a receiver gain of 128, a recycle delay of 4 s, dummy scans of 4, an accumulation of 256 transients, and a mixing time of 0.005s. FIDs were multiplied by a 0.3 Hz exponential multiplication function prior to Fourier transformation, only a zero order phase correction was allowed, and the TMSP-d4 signal was calibrated at δ 0.00. The 1D spectra were assigned using the databases software Chenomx NMR Suite (professional version 8.1), literature values (ZAWADZKI et al., 2017;

CERIBELI et al., 2018), and the 2D NMR experiments JRES, ^{1}H-^{13}C HSQC, and ^{1}H-^{1}H COSY on selected samples.

Data processing

The experiment was structured following the random block designer, all statistics analysis from the physicochemical parameters were analyzed by SAS Studio. Normality of the Residues and influencer point analyses were estimated by MIXED procedures. Statistics analysis were carried out using GLM procedure, and the model were composed by the treatments (control, 1, 2, and 4% of inclusion) and blocks (1 to 9), as fixed effects, as well error were included into the model. Treatments were analyzed by orthogonal contrast (linear, quadratic, and cubic).

The obtained ^{1}H-NMR spectra data had the binning of 0.04 ppm applied and then were transformed into a data matrix, using *MNova software*. The spectra data was used from 0 to 7 ppm once the peaks signals in the aromatic region were discarded because of the presence of variations of chemical shift due to slightly differences in pH and ionic strength. Data were then analyzed in MetaboAnalyst 3.0 platform (http://www.metaboanalyst.ca/faces/home.xhtml), using principal component analysis (PCA). Data preprocessing enrolled data filtering interquantile range (IQR), no sample normalization, no data transformation, and Pareto scaling (mean-centered and divided by the square root of standard deviation of each variable). Five principal components were used for discrimination of the analyzed metabolites samples.

RESULTS

Physicochemical parameters

The results for centesimal composition of the lamb meat were showed on table 4.1. All parameters analyzed shows quadratic effect of the treatments

(*P<0.05*), except moisture content (average value = 63.00 g $100g^{-1}$ meat), ratio CP:OM (average value = 0.74 g of CP per g of OM), ratio TF:OM (average value = 0.08 g of TF per g of OM) , and polar metabolites content (average value = 31.07 g $100g^{-1}$ meat).

Table 4.1. Centesimal composition of the meat from lamb raised with increased levels of Yerba-mate extract into the diet.

Parameters[1] (g $100g^{-1}$ meat)	**Level of inclusion (%)**					**P value**[3]		
	0	**1**	**2**	**4**	**MSE**[2]	**Lin**	**Quad**	**Cub**
MC	65.51	60.46	60.55	65.48	1.75	0.638	0.112	0.569
ASH	1.56	1.70	1.96	1.60	0.09	0.804	0.005	0.227
OM	32.92	37.71	37.50	32.92	1.66	0.624	0.012	0.540
CP	24.25	27.13	28.09	24.79	1.16	0.996	0.011	0.965
TF	2.60	3.21	3.52	2.97	0.30	0.488	0.041	0.937
Ratio CP:OM	0.74	0.72	0.75	0.76	0,013	0.162	0,629	0,180
Ratio TF:OM	0.077	0.082	0.094	0.079	0.007	0,785	0,117	0,389
PM	3.05	3.19	3.12	3.07	0.06	0.813	0.163	0.233

[1]MC – moisture content; ASH – ash content; OM – organic matter content; CP – crude protein; TF – total fat content; PM – polar metabolites.
[2]MSE – mean square error;
[3]Values from orthogonal contrast (linear, quadratic, and cubic) are significantly different if P<0.05 and tendency if 0.05<P<0.10.

Source: own authorship

Meat quality parameters results are showed on table 4.2, pH (average value = 5.38), hold water capacity (average value = 51.77%), and parameters a* (average value = 14.09), b* (average value = 4.10), ratio a*:b* (average value = 3.54), chroma (average value = 14.71), and hue angle (average value = 0.28) from color analysis shows no statistics differences (P>0.05). However, cooking weight loss had a linear increasing (P=0.050) by the treatment, shear force and L* parameter of the color had a tendency to a quadratic effect (P=0.067 and P=0.094, respectively). Meat from lambs fed yerba-mate extract, compared to control animals, had an increasing of 24.6%, 12.2%, and 8.0% on tenderness, by 1, 2, and 4% of extract inclusion, respectively.

Table 4.2. Meat quality parameters of lamb meat from animals fed or not with increased levels of yerba-mate extract.

Parameter[1]	Unit	Level of inclusion (%)					P value[3]		
		0	1	2	4	MSE[2]	Lin	Quad	Cub
pH		5.37	5.37	5.39	5.39	0.01	0.134	0.674	0.396
CWL	%	26.26	24.32	27.24	29.16	1.33	0.050	0.395	0.191
WHC	%	49.73	52.31	32.98	52.20	1.75	0.412	0.279	0.844
SF	N	32.21	24.28	28.28	29.63	2.12	0.895	0.067	0.068
Color									
L*		38.18	38.19	37.96	41.00	0.72	0.006	0.094	0.581
a*		14.03	14.34	13.69	14.30	0.40	0.802	0.574	0.261
b*		3.63	3.99	4.49	4.27	0.37	0.222	0.278	0.678
Ratio a*:b*		3.69	3.77	3.19	3.48	0.25	0.396	0.406	0.194
Chroma		14.51	14.91	14.47	14.94	0.43	0.610	0.869	0.407
Hue angle		0.272	0.269	0.290	0.289	0.016	0.400	0.779	0.470

[1] pH- potential of hydrogen; CWL – cooking weight loss; WHC - water holding capacity; SF – shear force; L* – lightness; a* – redness; b* – yellowness.
[2]MSE – mean square error;
[3]Values from orthogonal contrast (linear, quadratic, and cubic) are significantly different if $P<0.05$ and tendency if $0.05<P<0.10$.

Source: own authorship

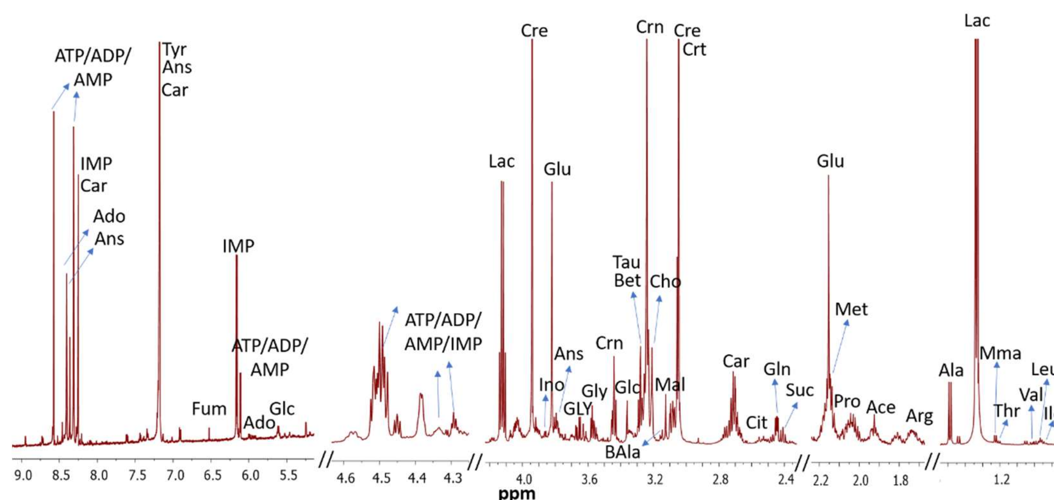
Metabolites

A total of 36 metabolites were find in the *longissimus thoracis* muscle of lambs, an example of a spectrum from a meat from lambs fed 2% of yerba-mate extract is presented in figure 4.1.

The analysis of principal components (PC) is a statistical procedure that uses an orthogonal transformation to convert an amount of data into a set of values of linearly uncorrelated variables called PC. The results from PC analysis from lamb meat metabolomics fed increased levels of yerba-mate is presented in figure 2. The first image presented in figure 2A is a 2D graphic in which 71.3% of the chemical shift information were explained (69.1% in PC1 and 2.2% in PC2). Two contrasting groups of metabolomics profiles were clustered in component 1 (PC1). One group was represented by metabolomics profile correspond to animals fed control diet and animals fed 4% of

yerba-mate versus the second group which includes animals fed 1 and 2% of yerba-mate extract.

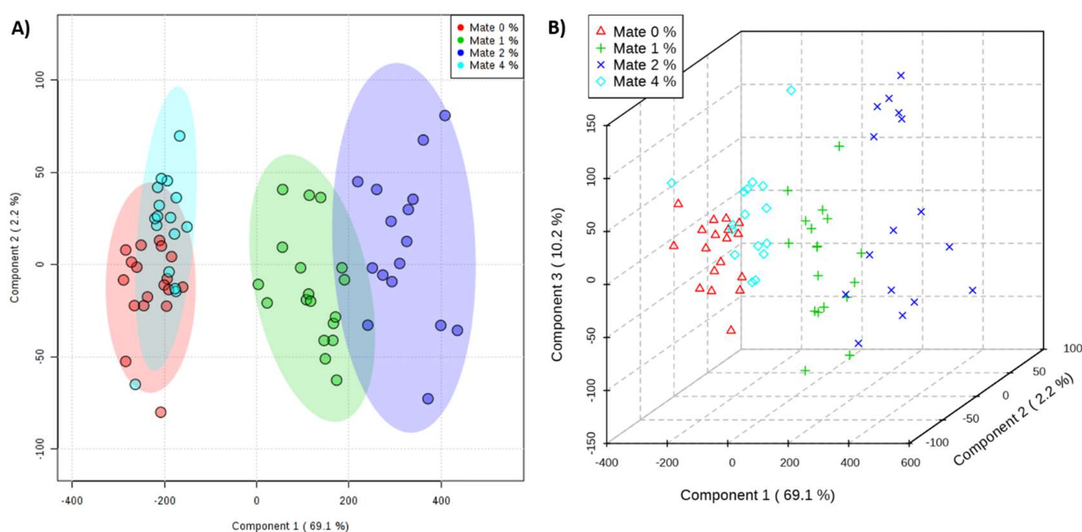
Figure 4.1. ^{1}H NMR spectra obtained from the metabolites extracted of lambs muscle animals fed 2% of yerba-mate extract.



Ile: L-Isoleucine; **Leu:** L-Leucine; **Val:** L-Valine; **Thr:** L-Threonine; **Mma:** Methylmalonate; **Lac:** lactate; **Ala:** L-Alanine; **Arg:** L-Arginine; **Ace:** Acetate; **Pro:** Proline; **Met:** Methionine; **Glu:** Glutathione; **Suc:** Succinate; **Gln:** L-Glutamine; **Cit:** Citrate; **Car:** Carnosine; **Cre:** Creatine; **Crt:** Creatinine; **Mal:** Malonate; **BAla:** β-Alanine; **Cho:** Choline; **Crn:** L-carnitine; **Tau:** Taurine; **Bet:** Betaine; **Glc:** Glucose-6-phosphate; **Gly:** Glycine; **GLY:** Glycerol; **Ans:** Anserine; **Ino:** Inosine; **IMP:** Inosine monophosphate; **ATP/ADP/AMP:** Adenosine tri-, di-, or mono-phosphate; **Ado:** Adenosine; **Fum:** Fumarate; **Tyr:** Tyrosine.

Source: own authorship

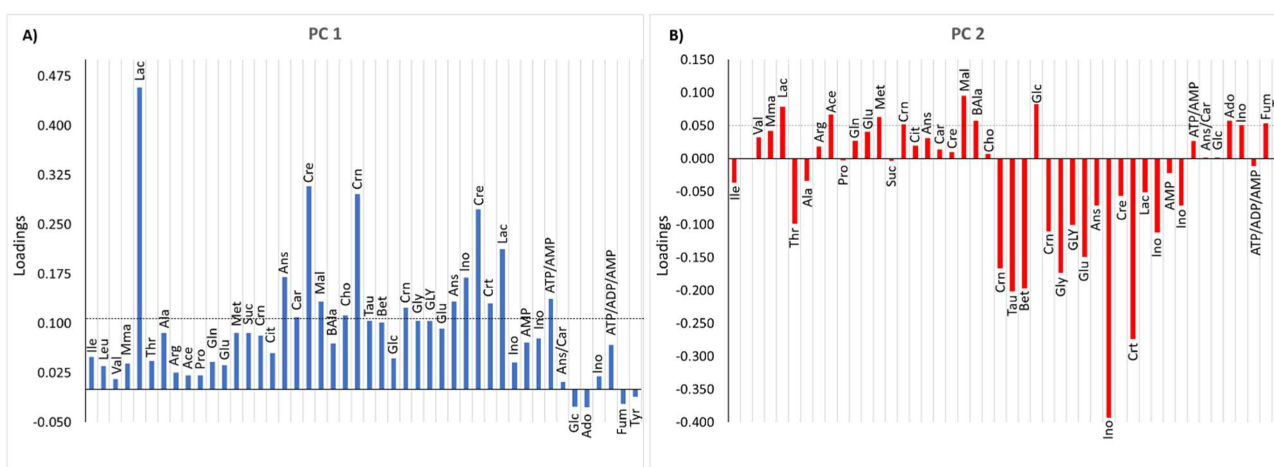
Figure 4.2. PLS-DA scores of metabolites of meat animals: A) PC1xPC2, B) PC1xPC2xPC3



Source: own authorship

A tendency to separate the metabolomics profile between animals fed control diet and animals fed 4% yerba-mate extract was presented when component 2 was observed. Indeed, this tendency group is clarifying in figure 2B which shows a 3D graphic, comprising component 3 explaining more 10.2% lead to an 81.5% of total contribution variation on data. Furthermore, animals fed 2% of yerba-mate extract are most different from animals fed control diets as seen in both PCA projections scores (Figure 2).

Figure 4.3. PLS-DA loadings of metabolites of meat animals at A) PC1 and B) PC2.



Ile: L-Isoleucine; **Leu:** L-Leucine; **Val:** L-Valine; **Mma:** Methylmalonate; **Lac:** lactate; **Thr:** L-Threonine; **Ala:** L-Alanine; **Arg:** L-Arginine; **Ace:** Acetate; **Pro:** Proline; **Gln:** L-Glutamine; **Glu:** Glutathione; **Met:** Methionine; **Suc:** Succinate; **Crn:** L-carnitine; **Cit:** Citrate; **Car:** Carnosine; **Ans:** Anserine; **Cre:** Creatine; **Mal:** Malonate; **BAla:** β-Alanine; **Cho:** Choline; **Tau:** Taurine; **Bet:** Betaine; **Glc:** Glucose-6-phosphate; **Gly:** Glycine; **GLY:** Glycerol; **Ino:** Inosine; **Crt:** Creatinine; **ATP/ADP/AMP:** Adenosine tri-, di-, or mono-phosphate; **Ado:** Adenosine; **Fum:** Fumarate; **Tyr:** Tyrosine; **Phe:** Phenylalanine; **Nia**: Niacinamide; **Hyp:** Hypoxanthine.

Source: own authorship

The metabolites profile responsible for the differentiation between the degree of yerba Mate which the lambs have been fed must be presented by the loadings charts showed figure 3, which had a threshold determined as 0.1 and 0.05 for PC1 and PC2, respectively. These thresholds demark the high intense metabolites.

PC1 (figure 4.3A) have highlighted the metabolites: lactate, anserine, creatine, malonate, L-carnitine, inosine, creatinine, and adenosine tri- and mono-phosphate as the

most important in the group with animals fed 1 and 2% of yerba-mate extract against control and animals fed 4% of yerba-mate extract. While, in PC2 (Figure 3B), animals fed 4% of yerba-mate have high intensity of the metabolites lactate, acetate, methionine, malonate, glucose-6-phosphate, adenosine, and tyrosine, when compared to animals fed with control diet.

DISCUSSION

Meat quality analysis

The pH parameter from lamb meat in this experiment shows values lower than the literature, normal values are described between 5.5 to 5.8 (ABREU et al., 2019; STEART et al., 2018; BEZERRA et al., 2016; JANDASEK, MILERSKI, LICHOVNIKOVA, 2014). Low pH after 24h of the slaughter indicates that the animals had some kind of stress just before slaughter, that stress use muscle glycogen and produce high quantities of lactic acid, that acid helps decrease fast the pH and have several impacts on meat quality. However, no differences between treatments were observed, than no effect of the treatments on pH maintenance were noted, and consequently all treatments had the same effect of pH on the other parameters.

The results from pH analysis must interfere in almost all other parameters of physic-chemical analysis. The moisture content shows lower them the observed for lamb's *Longissimus* muscle in the literature (ABLIKIM et al., 2016; BEZERRA et al., 2016; HAJJI et al., 2016). That could be explained by the literature (ORDÓÑEZ, 2005), low pH can induce precipitation of the sarcoplasmatic protein and, consequently, reduction on water retention during the storage and trawing process.

The protein content of the fresh meat of lamb is a little bit higher than the results find in the literature (BABIKER; EL KHIDER; SHAFIE, 1990; ESENBUGA; YANAR; DAYIOGLU, 2001; SEN; SANTRA; KARIM, 2004; WILLIAMS, 2007; BONANNO et

al., 2012), which could be explained by the low moisture content. The other nutritional components (fat and ash) are on the normal range for lamb meat according to the literature (BABIKER; EL KHIDER; SHAFIE, 1990; ESENBUGA; YANAR; DAYIOGLU, 2001; SEN; SANTRA; KARIM, 2004; WILLIAMS, 2007; BONANNO et al., 2012).

No differences on moisture content were noted by the treatment; however, numerically we can observe a difference between control and 4% *versus* 1 and 2%, that numerical difference must have influenced the composition content of the fresh meat and explain the treatment effect on nutritional components.

Parameters of meat quality, as cook weight loss, water hold capacity, shear force, and color parameters (L*, a*, b*, chroma, and angle hue) are in the normal range according to the literature for lamb meat (ABREU et al., 2019, BEZERRA et al., 2016; MONACO et al., 2014; ESENBUGA; YANAR; DAYIOGLU, 2001; BONANNO et al., 2012; BABIKER; EL KHIDER; SHAFIE, 1990; SEN; SANTRA; KARIM, 2004). Animals fed 1% of yerba-mate extract had the smallest cook weight loss and biggest tenderness, that results could be explained by the literature (ZAWADZKI et al., 2017; LUND et al., 2011; HUFF-LONERGAN; LONERGAN, 2005), cross-linking of the myofibrillar protein caused by protein oxidation could affect tenderness, and the yerba-mate compounds could change the metabolic profile of the muscle, and consequently quality of the fresh meat. It could prevent protein oxidation and increase tenderness and reduce cook weight loss. The luminosity (L*) parameter of the color of the meat had higher values with the use of yerba-mate extract in the animals diet, this results agree with Zawadzki et al. (2017).

Metabolomic analysis of the lamb muscle

All metabolites presented in this study corroborates with the literature for ruminant muscle metabolite profile (ZAWADZKI et al., 2017; OSORIO et al., 2012;

BROGNA et al., 2014). The only metabolite that differ in the muscle of lambs fed control diet versus all other three treatments with increased levels of yerba-mate extract (1, 2, and 4%) is the lactate, we can see that on analysis of PC 1 and 2. For a long time, lactic acid was considered a dead-end product from the glycolysis pathway, however, recent studies reported new functions, as immune tolerance, memory formation, wound healing, energy regulation, ischemic tissue injury, and grow and metastasis of the cancer (SUN et al., 2017).

In the skeletal muscle after slaughter, lactate is a product from the glycolysis when animals had pré-slaughter stress. In our case, no stress signal where observed from the meat quality parameters, the color, tenderness, and hold water capacity are in the normal range, and the metabolomics profile shows an increase on ATP metabolite in muscle from animals fed 1 and 2% of yerba-mate extract, however, in stress scenarios a lower ATP concentration is observed. Indicating that increase on lactate is from the yerba-mate treatment and it could be playing a different role in the muscle, however more studies need to be done to better understand it.

Dietary treatments affected positively the levels of histidine-containing dipeptides as anserine and carnosine, on animals fed 1 and 2% of yerba-mate extract. Carnosine and anserine is a molecule well studied on the last decades, and was proved its properties as antioxidant and its function on inhibition of autoxidation of fatty acids (WU et al., 2003; KOUTSIDIS et al, 2008) and consequently reduction of formation of off-flavors. It could indicate a better meat quality from animals fed 1 or 2% of yerba-mate extract.

Also, increase those metabolites in meat is desired regarding the human nutrition and health. High levels of antioxidants in human food could lower the tendency to induce radical formation in gastrointestinal tract during the digestion following meat

consumption, and it must decrease the risk of colorectal cancer (ZAWADZKI et al., 2017).

In addition, increase anserine in food is also desirable, due to its function on human neural disorders. Recent studies have showed that increased dietary anserine could preserve prefrontal brain blood flow and prevent elderly people with the APOE4 allele (gene that indicates predisposition to Alzheimer's disease) and prevent that disorder (DING et al., 2018), or help to reduce the decline in cognitive function to peoples that are in advanced stage of the disease (KANEKO et al., 2017).

Creatine is a natural molecule found in meat and fish, and can be synthetized by the liver and pancreas (WALKER, 1979) from arginine, glycine, and methionine (Bloch and Schoenheimer, 1941), and in the skeletal muscle plays an important role in the energetics production; the usage of creatine release creatinine. Both, creatine and creatinine are in high intensity on metabolomics profile of animals fed 1 and 2% of yerba-mate extract.

Some studies have reported that creatine are involved in the muscle hypertrophy by increase free-fat mass (INGWALL et al., 1974; PARISE et al., 2001; FORBES et al., 2019), that idea corroborates with our previous results, where animals fed 1 and 2% of yerba mate extract could reach a higher body weight and decrease carcass fat thickness. In addiction, supplementation of dietary creatine in human food is also desirable, due to its properties on increase free-fat mass in the people body with tendency to obesity (FORBES et al., 2019).

However, more studies need to be done using yerba-mate extract to consolidate its beneficial usage in the animal nutrition industry and to better understand its effects on animal products as meat, milk, and eggs, and if its usage is economically viable for the industrial livestock production.

CONCLUSION

In conclusion, yerba mate extract fed up to 2% of the dry matter intake on lamb's diet is beneficial to produce meat with desirable characteristics, as tenderness, composition, and visual aspects. A great composition of metabolites with high intensity of some endogenous antioxidants could preserve the meat and its characteristics for a better time, and its possible effects on human nutrition could classify the meat from animals fed yerba-mate extract like functional food.

REFERENCES

ABLIKIM, B. et al. Effect of breed, muscle type, and frozen storage on physic-chemical characteristic of lamb meat and its relationship with tenderness. **CyTA – Journal of Food**, v. 14, p. 109-116, 2016.

ABREU, K.S.F., et al. Quality of meat from sheep fed diets containing spineless cactus (*Nopalea cochenillifera Salm Dyck*). **Meat Science**, v. 148, p. 229-235, 2019.

AMSA – American Meat Science Association. **Meat color measurement guidelines**, American Meat Science Association, pp. 124, 2012.

AOAC – Association of Official Analytical Chemists, 1990. **Official Methods of Analysis.** 14 ed., 4 rev., 2v.

BABIKER, S.A.; EL KHIDER, I.A.; SHAFIE, S.A. Chemical composition and quality attributes of goat meat and lamb. **Meat Science**, v. 28, p. 273-277, 1990.

BEZERRA, L.S. et al. Meat quality of lambs fed diets with peanut cake. **Meat Science**, v. 121, p. 88-95, 2016.

BLIGH, E.G.; DYER, W.J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**, v. 37, p. 911-917, 1959.

BLOCH, K.; SCHOENHEIMER, R. The biological precursors of creatine. **Journal of Biological Chemistry,** v. 138, p. 167–194, 1941.

BONANNO, A. et al. Effect of legume grains as a source of dietary protein on the quality of organic lamb meat. **Journal of the Science of Food and Agriculture**, v. 92, p. 2870-2875, 2012.

BROGNA, D.M.R. et al. The quality of meat from sheep treated with tannin- and saponin-based remedies as a natural strategy for parasite control. **Meat Science**, v. 96, p. 744-749, 2014.

CERIBELI, C. et al. Mate as Dietary Supplement for Broiler Chickens: Effect on the Metabolic Profle and Redox Chemistry of Meat. **Journal of the Brazilian Chemical Society**, v. 29, p. 2266-2277, 2018.

CROSS, A.J. et al. A large prospective study of meat consumption and colorectal cancer risk: An investigation of potential mechanisms underlying this association. **Cancer Research**, v. 70, p. 2406–2414, 2010.

DING, Q. et al. Anserine/Carnosine supplementation preserves blood flow in the prefrontal bfrain of elderly people carrying APOE e4. **Aging and Disiase**, v. 9, p. 334-345, 2018.

DUKE, J.A. **Handbook of phytochemical constituents of GRAS herbs and other economic plants**. Boca Raton , Fla. : CRC Press, 1992.

ESENBUGA, N.; YANAR, M.; DAYIOGLU, H. Physical, chemical and organoleptic properties of ram lamb carcasses from four fat-tailed genotypes. **Small Ruminant Research**, v. 39, p. 99-105, 2001.

FORBES, S.C. et al. Changes in fat mass following creatine supplementation and resistance training in adults ≥50 years of age: A meta-analysis. **Journal of Functional Morphology and Kinesiology**, v. 4, 2019.

FRANKIC, T. et al. Use of herbs and spices and their extracts in animal nutrition. **Acta Agriculturae Slovenica**, v. 94, p. 95-102, 2009.

HAJJI, H. et al. Meat physicochemical properties, fatty acid profile, lipid oxidation and sensory characteristics from three North African lamb breeds, as influenced by concentrate or pasture finishing diets. **Journal of Food Composition and Analysis**, v. 48, p. 102-110, 2016.

HAMM, R. Biochemistry of meat hydration. **Advances in Food Research**, v. 10, 335-443, 1960.

HUFF-LONERGAN, E.; LONERGAN, S. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. **Meat Science**, v. 71, p. 194-204, 2005.

INGWALL, J.S. et al. Specificity of creatine in the control of muscle protein synthesis. **The Journal of Cell Biology**, v. 63, p. 145-151, 1974.

JANDASEK, J.; MILERSKI, M.; LICHOVNIKOVA, M. Effect of sire breed on physico-chemical and sensory characteristics of lamb meat. **Meat Science**, v. 96, p. 88-93, 2014.

KANEKO, J. et al. Anserine (beta-alanyl-3-methyl-L-histidine) improves neurovascular-unit dysfunction and spatial memory in aged AβPPswe/PSEN1dE9 Alzheimer's-model mice. **Scientific Reports**, v. 7, 2017.

KAWAKAMI, M.; KOVAYASHI, A. Volatile constituents of green mate and roasted mate. **Journal of Agricultural and Food Chemistry**, v. 39, p. 1275-1279, 1991.

KOUTSIDIS, G. et al. Water-soluble precursors of beed flavor: I. Effect of diet and breed. **Meat Science**, v. 76, p. 124-130, 2008.

LUND, M.N. et al. Protein oxidation in muscle foods: A review. **Molecular Nutrition & Food Research**, v. 55, p. 83-95, 2011.

MONACO, C.A. et al. Eating quality of meat from six lamb breed types raised in Brazil. **Journal of the Science of Food and Agriculture**, v. 95, p. 1747-1752, 2014.

NRC – National Research Council. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. **The National Academies Press**, Washington DC, 384 p., 2007.

ORDÓÑEZ, J.A. **Tecnologia de Alimentos: Componentes dos Alimentos e Processos**. Porto Alegre: Artmed; 2005.

OSORIO, M.T.; et al. Authentication of beef production systems using a metabolomic-based approach. **Animal**, v. 6, p. 167-172, 2012.

PARISE, G. et al. Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. **Journal of Applied Physiology**, v. 91, p. 1041-1047, 2001.

POMILIO, A.B.; TRAJTEMBERG, S.; VITALE, A.A. High-performance capillary electrophoresis analysis of Mate infusions prepared from stems and leaves of Ilex paraguariensis using automated micellar electrokinetic capillary chromatography. **Phytochemical Analysis**, v. 13, p. 235–241, 2002.

SAMRAJ, A.N. et al. **A red meat-derived glycan promotes inflammation and cancer progression**. Proceedings of the National Academy of Sciences of the United States of America, v. 112, p. 542-547, 2015.

SEN, A.R.; SANTRA, A.; KARIM, S.A. Carcass yield, composition and meat quality attributes of sheep and goat under semiarid conditions. **Meat Science**, v. 66, p. 757-763, 2004.

STEWART, S.M. et al. Association between loin ultimate pH and plasma indicators of pre-slaughter stressors in Australian lamb. **Meat and Muscle Biology**, v. 2, p. 254-264, 2018.

SUN, S. et al. Lactic acid: no longer an inert and end-product of glycolysis. **Physiology**, v. 32, p. 453-463, 2017.

VASTA, V.; LUCIANO, G. The effects of dietary consumption of plants secondary compounds on small ruminants’ production quality. **Small Ruminant Research**, v. 101, p. 150-159, 2011.

WALKER, J.B. Creatine: biosynthesis, regulation, and function. **Advances in Enzymology and Related Areas of Molecular Biology**, v. 50, p. 177-242, 1979.

WCRF/AICR, World Cancer Research Fund and American Institute for Cancer Research . **Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective.** American Institute for Cancer Research (AICR); Arlington, VA, USA: 2007.

WHEELER, T.L. et al. **Shear Force Procedures For Meat Tenderness Measurement.** United States Department of Agriculture (USDA), Clay Center, NE, (A. R. Service, Trans.). p. 7, 2005.

WILLIAMS, P. Nutritional composition of red meat. **Journal of the Dietitians Association of Australia**, v. 64, p. 113-119, 2007.

WU, H. et al. Antioxidant activities of carnosine, anserine, some free amino acids and their combination. **Journal of Food and Drug Analysis**, v. 11, p. 148-153, 2003.

ZAPOROZHETS, O.A. et al. A new test method for the evaluation of total antioxidant activity of herbal products. **Journal of Agriculture and Food Chemistry**, v. 52, p. 21–25, 2004.

ZAWADZKI, A. et al. Mate extract as feed additive for improvement of beef quality. **Food Research International**, v. 99, p. 336–347, 2017.