UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE AGRONOMIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

QUALIDADE E TRAÇABILIDADE DO PRODUTO CÁRNEO GERADO EM SISTEMAS PASTORIS COM BAIXO APORTE DE INSUMOS

THAIS DEVINCENZI

Engenheira Agrônoma, Mestre em Zootecnia - UFRGS

Tese apresentada como um dos requisitos à obtenção do Grau de Doutor em Zootecnia

Área de Concentração Plantas Forrageiras

Porto Alegre (RS), Brasil

Abril, 2015.

THAIS DEVINCENZI
Engenheira Agrônoma

TESE

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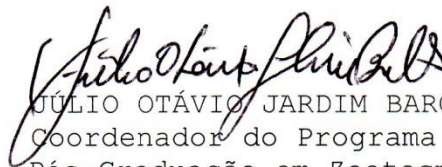
para obtenção do Grau de

DOUTOR EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia

Faculdade de Agronomia

Universidade Federal do Rio Grande do Sul

Porto Alegre (RS), Brasil

Aprovado em: 13.04.2015
Pela Banca Examinadora

Homologado em: 17.06.2015
Por

CARLOS NABINGER
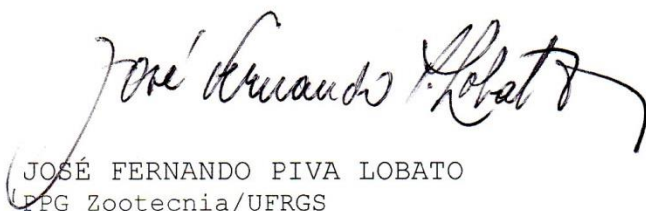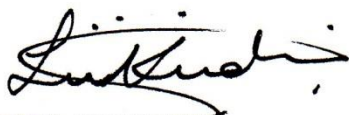PPG Zootecnia/UFRGS
Orientador

JÚLIO OTÁVIO JARDIM BARCELLOS
Coordenador do Programa de
Pós-Graduação em Zootecnia

JOSÉ FERNANDO PIVA LOBATO
PPG Zootecnia/UFRGS

LIRIS KINDLEIN
Faculdade de Veterinária/UFRGS

SÉRGIO OLIVEIRA JUCHEM
Embrapa Pecuária Sul

PEDRO ALBERTO SELBACH
Diretor da Faculdade de Agronomia

À memória dos meus avós Maria e Sidney e Nina...que foram o início de tudo.

AGRADECIMENTOS

Aos efeitos aleatórios.

Aos meus pais, Rui e Lélia, pela base firme que me proporcinaram e ao meu irmão Daniel.

Ao professor Nabinger, pela confiança, convivência e pelo exemplo do que é ser um mestre.

Aos CNPq e a Capes pela concessão das bolsas.

Um agradecimento especial à Sophie Prache, que além da formação científica me brindou com a sua amizade. Je te remercie Sophie...

Ao pessoal de campo e Laboratórios do INRA Theix, Sandrine Faure, Fréderic Anglard, Lionel Lavelle, Pascal Payard, Bruno, et les petits agneaux de m....e.

À Maguy Eugène pour les conseilles et pour le bons vins.

Á Néna Echasseux e seus filhos por serem minha família durante o período Clermont Ferrand e tornarem essa experiência inesquecível.

Aos colegas forrageiros Juliana, Fedrigo, Pablo, Lucas, João, Fábio e Martin pela agradável convivência.

A todos mencionados e aos não citados, meu reconhecimento e agradecimento.

QUALIDADE E TRAÇABILIDADE DO PRODUTO CÁRNEO GERADO EM SISTEMAS PASTORIS COM BAIXO APORTE DE INSUMOS [1]

Autora: Thais Devincenzi
Orientador: Carlos Nabinger
Co-orientador: Sophie Prache

Resumo – Resumo – A produção de carne de ruminantes em sistemas forrageiros de baixos aporte de insumos (*Low Input*) engloba atributos extrínsecos valorizados pelo consumidor, porém esse tipo de produção não deve comprometer a qualidade intrínseca desses produtos. Nesse contexto, estudou-se os aspectos de qualidade intrínseca e a traçabilidade do produto cárneo em dois dispositivos experimentais de produção *Low Input*. Primeiramente, avaliaram-se as características sensoriais, a concentração de compostos aromáticos e de ácidos graxos na carne e tecido adiposo de cordeiros em pastejo de gramíneas suplementados com níveis de alfafa fresca: (U-sem suplementação; L-baixa; M- intermediária e H- alta) representando 0, 25, 50 e 75% desta leguminosa, respectivamente. Utilizou-se quatro grupos de 9 cordeiros distribuídos em quatro parcelas de *Dactilys glomerata*. Também avaliou-se o valor $\delta^{15}N$ visando autenticar sistemas de produção ricos em leguminosas. O teor escatol na gordura perirenal foi superior nos animais que consumiram alfafa ($P < 0,05$), aumentando a partir do nível L e sendo associado ao *flavour animal* da carne. A intensidade deste atributo apresentou o mesmo comportamento que o teor de escatol na gordura perirenal, sugerindo que a partir de uma concentração 0,26-0,34 µg de escatol/g de gordura líquida ocorre estabilização da intensidade de percepção dos odores e *flavours* relacionados à este composto. O incremento de alfafa na dieta proporcionou aumento do teor C18:3 *n*-3 e também de C16:0. O valor $\delta^{15}N$ classificou corretamente 85,3% das amostras quando comparados animais U com os que receberam alfafa, demonstrando ser eficiente em autenticar sistemas de produção com leguminosas. No segundo dispositivo experimental avaliou-se o perfil de ácidos graxos da carne de bovinos Aberdeen angus em três sistemas de produção no Rio Grande do Sul utilizando diferentes níveis de insumos (NG=Pastagem Natural n=16; ING= Pastagem Natural Melhorada, n=18 e SP= Pastagem de Sorgo, n=8), resultando em distintas diversidades florísticas na dieta dos animais. Menor teor de C14:0 e maiores teores de ácidos graxos *n*-3 foram obtidos nas carnes de NG e de ING do que na carne de SP. Apesar de apresentarem maior idade ao abate, a carne de ING apresentou menor SFA que a carne SP. Além disto, a relação *n*-6/*n*-3 foi menor na carne de ING. Desta forma, a carne de bovinos terminados em pastagem natural melhorada mostrou um perfil de ácidos graxos mais benéfico à saúde humana.

Palavras chave: pastagens naturais, leguminosas, análise sensorial, ácidos graxos, certificação

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QUALITY AND TRACEABILITY OF MEAT PRODUCT FROM LOW INPUT SYSTEM [1]

Author: Thais Devincenzi
Advisor: Carlos Nabinger
Co-advisor: Sophie Prache

Abstract - Ruminant meat production on *Low-Input* herbage systems embody extrinsics attributes that consumers value, however this way of production must conciliate the intrinsic quality of this products. On the light of this, we investigated the intrinsic quality aspects of meat products in two experimental contexts of Low-Input livestock system. Firstly,we evaluated the sensory characteristics, the concentration of aromatic compounds, the fatty acid composition of meat and adipose tissue of lambs grazing a cocksfoot pastures and receiving a supplemented with different levels of fresh alfalfa forage to obtain four dietary proportions of alfalfa: U-unsuplemented; L-Low; M- Medium e H- high, corresponding respectively to 0%, 25%, 50% and 75% of dietary alfalfa. It was used four groups of nine lambs distributed on four padocks of cocksfoot. We also evaluated the hability of $\delta^{15}N$ value to authenticate legume rich production systems. Skatole concentration on perirenal fat was higher on lambs consuming alfalfa than for those that did not (P<0.05). Perirenal fat skatole concentration increased as soon as the dietary proportion of alfalfa reached 25%, being the skatole associated to *animal flavour* on meat, and presenting the same behaviour as skatole concentration, suggesting that the threshold above which the off-flavour and off-odour related to skatole are perceived corresponds to a perirenal fat skatole concentration in the range 0.26–0.34 µg/g of liquid fat. The dietary alfalfa also increased C18:3n-3 and also C16:0 concentration. $\delta^{15}N$ value correct classified 85,3% of the samples (U x L, M, H), showing to be efficient in authenticate legume-rich production systems.
On the second Low Input experimental contex we evaluate the fatty acid profile of Aberdeen angus steers (n=42) on three production systems on Rio Grande do Sul which used different levels of input (NG=Natural Grassland, n=16, ING= Improved Natural Grassland, n=18 and SP= Sorghum pasture, n=8), which conduced to different floristic richness. Lower amounts of C14:0 and higher amounts of n-3 fatty acids were found on NG and ING than for SP meats. Besides presenting higher age at slaughter, steers finished on ING lower SFA than SP meats. Moreover, the n-6/n-3 ratio was lower for ING meats, therefore meats produced on ING systems presented a better fatty acid profile by humans health point of view.

Key words: natural grasslands, *leguminosea*, sensory analisys, fatty acids, certification.

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4. CAPÍTULO IV. Dose-dependent response of nitrogen stable isotope ratio to proportion of legumes in diet to authenticate lamb meat produced from legume-rich diets

LISTA DE ABREVIATURAS E SÍMBOLOS

µg	microgram
µm	micrometer
ABC	Agricultura de Baixo Carbono
ADG	Average daily gain
AGCR	Ácidos graxos de cadeia ramificada
AGMI	Ácidos graxos monoinsaturados
AGPI	Ácidos graxos poliinsaturados
AGS	Ácidos graxos saturados
AI	Atherogenic index
BW	Body weight
Ca	Calcium
CLA	Ácido linoleico conjugadoAltura
CP	Crude protein
DHA	Docosapentanoic acid
DIVMO	Digestibilidade *in vitro* da Matéria Orgânica
DM	Dry Matter
EPA	Eicosapentanoic acid
FA	Fatty acids
FAME	Fatty acid methyl esters
Fe	Ferro
FL	Fosfolipídios
FM	Forrage mass
GLC	Gas–liquid chromatography
GLM	General linear model
H	Hour
H	High supplemented
Há	hectares
HCl	Ácido clorírico
HDL	High density lipids
I	Iodo
ING	Improved natural grassland
K	Potássio
Kg	Kilogram
L	Low suplemented
LDL	Low density lipids
LM	*Longissimus* muscle
LTL	*Longissimus thoracis et lumborum*
LW	Live weight
M	Medium suplemented
Mg	Magnésio
Mn	Manganês
MUFA	Monounsaturated fatty acids
N	Nitrogen/Nitrogênio
n-3	Ômega 3
n-6	Ômega 6

Na	Sodium
NDF	Neutral detergent fiber
NG	Natural grassland
OM	Organic matter
PA	Proportion of alfalfa
PAC	Política agrícola comum
PRFS	Perirenal fat skatole concentration
PUFA	Polyunsaturated fatty acids
RPM	Rising plat meter
S.E.M	Standard Error Means
SD	Standard deviation
Se	Selênio
SFA	Saturated fatty acids
SIF	Sistema de Inspeção Federal
SP	Sorghum pasture
TG	Triglicerídios
U	Unsuplemented
UFV	Unité Fourragère viande
WBSF	Força de Cisalhamento de Warner-Bratzler
Zn	Zinco

CAPÍTULO I

1.1 INTRODUÇÃO GERAL

A presente tese insere-se num momento em que as discussões sobre os sistemas de produção de alimentos enfrentam o dilema da produção *versus* conservação dos recursos naturais. Certos países chegaram a tal grau de intensificação da produção agropecuária que colocaram em risco além da segurança ambiental, a saúde de sua população. Como exemplo, pode se citar a contaminação das águas subterrâneas com nitrato lixiviado pelo excesso de insumos nitrogenados, os casos de encefalopatia espongiforme bovina, (doença da vaca louca), e a própria epidemia de obesidade em alguns países, a qual gera despesas públicas impressionantes para o seu tratamento.

As reflexões sobre os sistemas de produção de alimentos são recorrentes. Sejam motivadas por demandas da sociedade ou por políticas públicas, novas formas de produção de alimentos têm sido discutidas, sendo denominadas por alguns autores como a Nova Equação Alimentar (Goodman, 2003; Morgan & Sonnino, 2010). Produzir carne de ruminantes sobre essa ótica pode pressupor a criação dos animais a pasto em sistemas de produção que utilizem baixo aporte de insumos (*Low Input*). Nessa situação, as principais fontes de nutrientes para esse sistema seriam o uso de pastagens consorciadas de gramíneas e leguminosas e/ ou pastagens permanentes bem manejadas.

Embora a produção de carne a pasto seja vista com bons olhos pelo consumidor moderno, visto que parece atender aspectos desejáveis aos mesmos, tais como o bem estar animal e conservação do ambiente, questões importantes acerca da qualidade sensorial e nutricional desses produtos são levantadas.

A Figura 1 representa um esquema ilustrativo de contextualização e as bases conceituais do estudo. Propõe-se que distintos sistemas de produção com baixo aporte de insumos são capazes de influenciar os atributos de qualidade da carne de ruminantes sendo possível autenticar tais sistemas de produção. Os conceitos e variáveis abordadas no presente trabalho são revisados no capítulo I (Revisão Bibliográfica). Os artigos científicos apresentados nos capítulos seguintes analisam aspectos relativos à qualidade do produto cárneo em sistemas de produção de baixo aporte de insumos (*Low Input*), em dois contextos, um europeu e outro brasileiro.

No contexto europeu (capítulos II, III e IV), estudou-se o efeito de níveis de leguminosas na dieta de ovinos sobre as variáveis de qualidade sensorial e nutricional da carne bem como o estudo da eficiência dos isótopos de nitrogênio para certificar sistemas de produção *Low Input*. Partiu-se do pressuposto de que além do increx'mento de nitrogênio ao solo, a utilização de pastagens consorciadas de gramíneas e leguminosas contribui para melhoria

da qualidade nutricional das pastagens através do aporte de maior teor de proteínas para a dieta dos animais. Contudo, o elevado teor de proteínas altamente degradáveis parece ter impacto negativo sobre a qualidade sensorial da carne de cordeiros. A degradação das proteínas pelas bactérias do rúmem aumenta a disponibilidade do amino-ácido triptofano, precurssor do composto aromático denominado escatol, o qual é associado à aromas definidos como "fecal e animal" (YOUNG et al, 2003; SCHREURS et al, 2007). Os sistemas de produção em pastagens consorciadas de gramíneas e leguminosas poderiam ser certificados através da relação de isótopos estáveis do nitrogênio ($^{15}N/^{14}N$ ou $\delta^{15}N$) em função do enrriquecimento isotópico dos produtos que deverá ocorrer em função da proporção de leguminosas presentes no sistema.
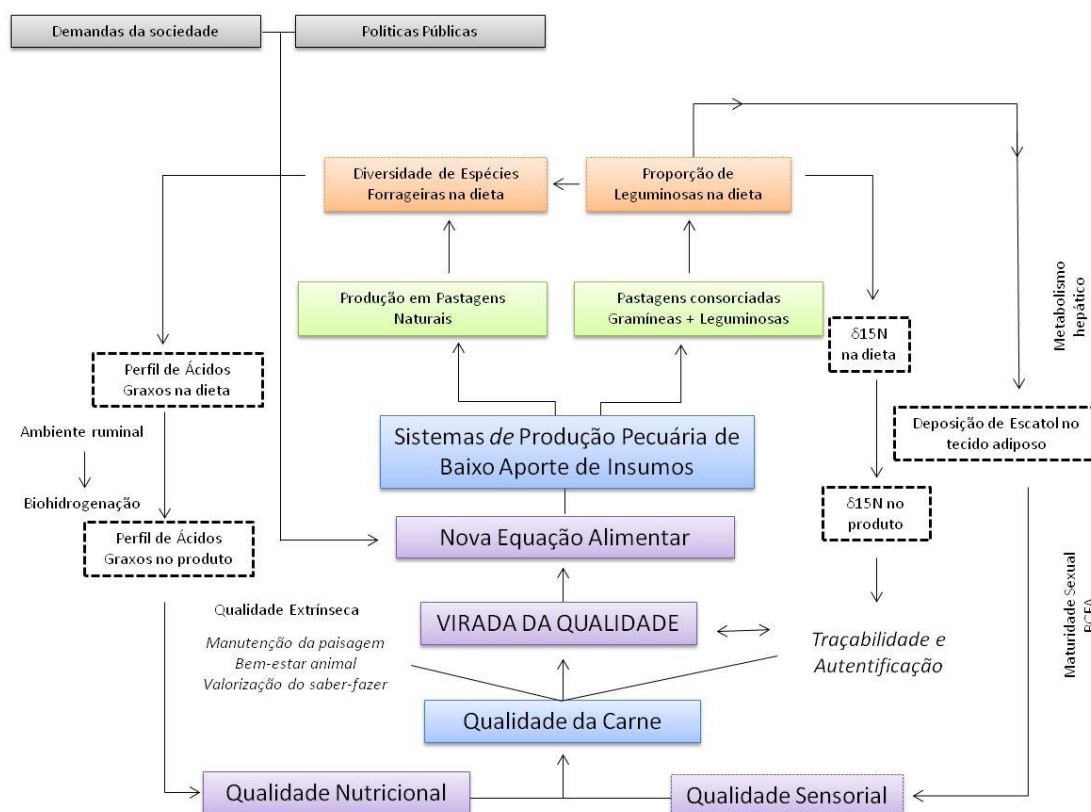
FIGURA1: Esquema ilustrativo de contextualização e bases conceituais do estudo.

No contexto brasileiro estudou-se o perfil lipídico da carne bovina produzida em sistemas de produção baseados em pastagens naturais. O artigo científico referente à este estudo é apresentado no capítulo V. Para este trabalho, partiu-se da premissa de que a diversidade de pastagens, resultado no nível de intensificação, é capaz de alterar a qualidade nutricional da carne através de possíveis modificações do perfil de ácidos graxos do produto (LOURENÇO et al., 2007).

Por fim, deve-se considerar que a consolidação dos atributos de qualidade do produto cárneo em função dos sistemas de produção *Low Input* se dá pela interação dos atributos de qualidade sensorial, qualidade nutricional

e também da qualidade extrínseca do produto a da possibilidade de autentificar tais sistemas de produção. Qualidade extrínseca nesse caso seriam os demais fatores proporcionados pelo sistema de produção mas que não podem ser verificados diretamente no produto, tais como, a eficiência do sistema de produção em manter as paisagens e os recursos naturais, em proporcionar bem estar-animal e em valorizar o saber-fazer do produto em questão. Todos esses fatores irão compor o que alguns autores propõem como a "Virada da Qualidade" dos alimentos (GOODMAN, 2003), a qual está vinculada aos valores da Nova Equação Alimentar no sentido da seguinte questão: Quais os benefícios e o ônus, relativos à qualidade do produto, de se produzir carne em sistemas de baixo aporte de insumos?

1.2 Sistemas de Produção *Low Input* e a Nova Equação Alimentar

Novas formas de produção de alimentos têm sido discutidas como alternativas ao modelo de produção predominante baseado em produções intensivas de commodities. Esse novo pensar, proposto por alguns autores como a Nova Equação Alimentar (GOODMAN, 2003, MORGAN & SONINO, 2010) além de discutir as formas de produção de alimentos, sugere ideias como as de redes alimentares alternativas, relocalização dos alimentos (FONTE, 2008) e virada da qualidade (GOODMAN, 2003).

O modelo hegemônico de produção de alimentos, o qual vem dominando os modelos de produção desde a revolução industrial é, segundo alguns autores como Goodman et al., (2012), responsável por insegurança alimentar e má-nutrição, crise ecológica e relacionado aos modos de vida e escassez de recursos globais. Essa crise justifica-se pelo fato de que os sistemas de produção convencionais são altamente dependentes de combustíveis fósseis e outros insumos como, por exemplo, os defensivos característicos dos monocultivos intensivos (LAL & PIECE, 1991). Além disso, o produto oriundo desse sistema de produção tem como destino a produção de alimentos processados industrialmente, os quais estão vinculados à doenças associadas a dietas ricas em açúcar, carboidratos, sódio e gorduras vegetais hidrogenadas. Esse modelo de produção, muitas vezes baseado em processos dissipativos de energia, acaba por levar o sistema a um empobrecimento generalizado, tornando-o insustentável ao longo do tempo (ADDISCOTT, 1995).

Nesse sentido, o conceito de sistemas de produção *Low Input* (ou sistemas de produção com baixo aporte de insumos) pode agregar à Nova Equação Alimentar no que se refere às discussões ligadas ao modo de produção dos alimentos.

O termo sistemas de produção *Low Input* ainda não apresenta uma definição oficial. Uma definição abordando o conceito de *Low Input* foi proposta por Parr et al., (1990). Os autores definem que *Low Input* são aqueles sistemas de produção que buscam otimizar a gestão e utilização de insumos internos à propriedade e minimizar o uso de insumos externos à propriedade, para, sempre que possível, reduzir os custos de produção, evitar poluição das áreas de exploração e dos cursos da água, reduzir resíduos de pesticidas em alimentos, diminuindo o risco do produtor tanto no curto quanto no longo prazo,

aumentando a rentabilidade da propriedade rural.

Para Elbersen & Andersen (2007), na Europa, existem muitas formas de sistemas de produção *Low Input* (tais como, orgânico, *High Nature Value*, *Low Input*, Biodinâmico). Em comum, eles utilizam nenhum ou relativamente poucos insumos externos, como agroquímicos (*i.e.* herbicidas, pesticidas), fertilizantes artificiais, irrigação e alimentos concentrados. A diminuição do aporte de insumos nesses sistemas de produção é geralmente acompanhada de menores produtividades (produção por área), no entanto, os produtos gerados sob essa perspectiva possuem um maior valor em termos de ambiente (água, ar, solo e clima), biodiversidade e manutenção da paisagem. Frequentemente, na Europa, a menor produtividade é compensada financeiramente ao produtor, pela possibilidade de valor agregado ao produto, e, portanto, produtos comercializados a um maior preço.

A definição de sistemas de produção *Low Input* é relativamente vaga e bastante generalizada. Um ponto de vista interessante a ser abordado é a discussão das clássicas curvas de produção em função das doses de fertilizante aplicada a uma cultura hipotética (Figura 2).
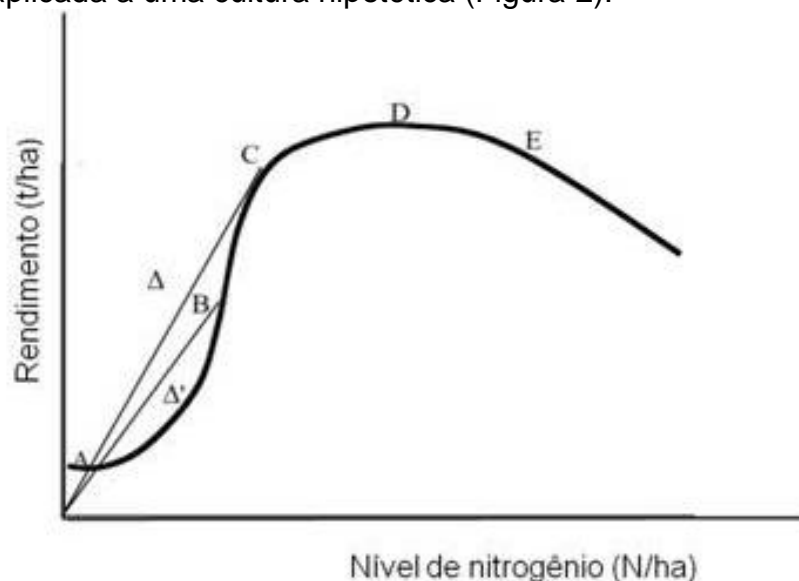
FIGURA 2. Rendimento hipotético de uma cultura em função do nível de nitrogênio aplicado. Os pontos A, B, C, D e E representam diferentes tipos de sistemas de produção na curva teórica de rendimento.

Conforme a análise de Shortel et al., (2001), os sistemas de produção A, B, C e D, poderiam ser considerados *Low Input*, quando comparados ao sistema E. Contudo, as estratégias de utilização de insumos são diferentes entre os sistemas de produção A, B, C e D, sendo que o significado de *Low Input* não é o mesmo entre os pontos. A situação D está minimizando a perda de N na tentativa de atingir o máximo rendimento de colheita. Esta estratégia poderia ser a típica de um produtor engajado na "agriculture raisonnée" (Termo regulamentado na França pelo Ministério da Agricultura e Ecologia que considera práticas de proteção ao meio ambiente, saúde e bem-estar animal e que poderia ser análogo à Produção Integrada no

Brasil). Nesse sentido o sistema de produção C, pelo fato de estar minimizando as perdas de N, ainda é uma situação de *Low Input*, no entanto, é também uma situação de produção com alto aporte de insumos e em um padrão de produção convencional. Já a situação C está maximizando a eficiência do uso do nitrogênio ($\Delta > \Delta$') e, assim, poderia ser uma alternativa e uma melhor estratégia ser utilizada na "agriculture raisonnée". Na situação A, com o menor aporte de insumos nitrogenados, percebe-se que o nitrogênio é o fator limitante da produção.

O nível de insumos poderá variar entre os sistemas *Low Input*, e isso provavelmente determinará diferentes benesses ambientais. Dessa forma, os sistemas *Low Input* devem ser analisados a partir de uma visão holística, considerando os fluxos de nutrientes em todos os níveis da paisagem que permitem ao produtor utilizar mais ou menos insumos (ELBERSEN & ANDERSEN, 2007).

Fundamentalmente os numerosos fluxos de nutrientes entre os compartimentos do sistema de produção implicam na necessidade de nutrientes de origem natural na escala da propriedade rural. Duas fontes principais de nutrientes nessa escala são consideradas: (i) As plantas leguminosas, as quais são plantas fixadoras de nitrogênio e podem integrar rotações de culturas ou mesmo utilizadas em consórcio com gramíneas em pastagens e (ii) Cultivos permanentes, tais como pastagens naturais, as quais, se corretamente manejadas, retiram nutrientes do solo em um nível que não altera a sustentabilidade do ciclo de nutrientes.

Na Europa, a substituição dos sistemas de produção tradicionais (*Low Input* até os anos 1950) por sistemas de produção intensivos tem sido apontada como responsável pelos principais problemas ambientais enfrentados atualmente tais como poluição dos recursos hídricos, queda na biodiversidade, aumento da erosão (BALDOCK et al., 2002, BUCKWELL & ARMSTRONG-BROWN, 2004), dessa forma, voltar às formas tradicionais de produção de alimentos tem sido sugerido como um dos mecanismos para diminuição dos danos ambientais (POUX, 2007). Outro argumento para utilização dos sistemas *Low Input* no continente europeu, é também a questão relativa aos problemas de saúde pública. Os pesticidas utilizados em larga escala nos sistemas de produção intensivos são fonte de preocupação para a população como um todo, pois têm sido associados a doenças como o câncer e a problemas de fertilidade, as quais têm sido chamadas de "doenças do ambiente". Os custos de combate a essas doenças na Europa são crescentes, e esses valores acabam por incentivar o desenvolvimento dos sistemas de produção *Low Input* (POUX, 2007).

No passado recente, a Europa experimentou grandes safras como resultado da intensificação dos sistemas de produção. Em 1992, a reforma da Política Agrícola Comum (Nova PAC), com o intuito de reduzir os excedentes, criou a política das cotas de produção de leite e a criação da PAC para limitar a produção de cereais. Nesse contexto, após um período de grande abundância de alimentos (segurança alimentar) e de preços favoráveis, os sistemas de produção *Low Input* são desejáveis, e defensáveis (no caso do apelo por "produção limpa", no sentido de preservação do meio ambiente), no entanto, em situações nas quais a Europa e o mundo se mostram em situação de

insegurança alimentar, os sistemas *Low Input* parecem ser vulneráveis nesse sentido.

Percebe-se que áreas de produção *Low Input* encontram-se predominantemente em regiões da Suécia, Reino Unido, França, Itália, Espanha e Portugal, sobretudo nas regiões do Valle d´Aosta, Madrid, Extremadura, Alentejo-Algarve e Sardegna, onde as despesas médias em insumos eram menos de 33 Euros por hectare (ELBERSEN & ANDERSEN, 2007).

Uma hipótese que pode ser colocada é a de que as regiões supracitadas, em função das suas características fisiográficas, pois normalmente são regiões montanhosas ou regiões áridas não apresentam aptidão para a exploração agrícola intensiva sendo, portanto, os sistemas *Low Input*, os mais passíveis de serem explorados nessas regiões (Figura 3).
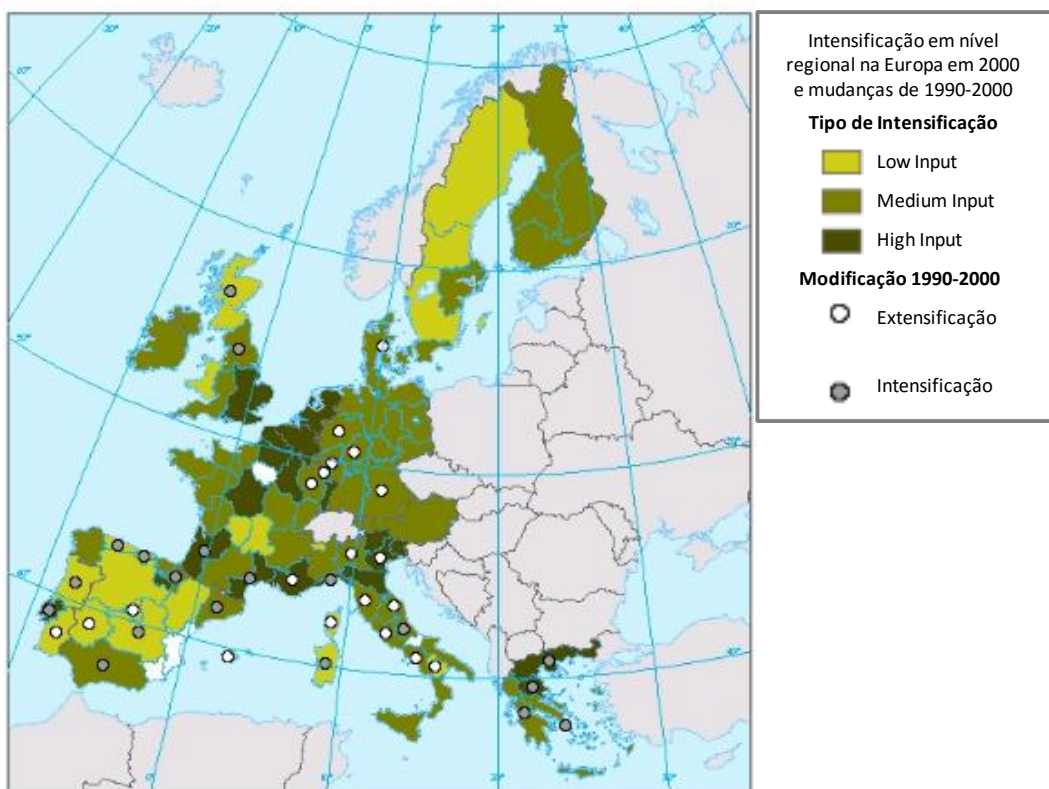
FIGURA 3. Importância regional de sistemas de produção agropecuários *Low Input, Medium Input and High Input na Europa* no período de 1990-2000. (ELBERSEN & ANDERSEN, 2007). *Nota: Extensificação = um decréscimo superior a 15% da despesa média regional por ha em terras agrícolas em insumos; Intensificação= um acréscimo superior a 15% da despesa média regional por ha em terras agrícolas em insumos.*

Aqui um paralelo pode ser traçado com algumas regiões do estado do Rio Grande do Sul. Cerca de 63% da área do estado do Rio Grande do Sul faz parte do Bioma Pampa. Atualmente, da vegetação original deste bioma neste território, 31,4% ainda possuem vegetação original. Para Cordeiro &

Hasenack (2009), a maior integridade dessas formações vegetais campestres em relação às formações vegetais florestais mostra que o uso tradicional dado a estas áreas (pecuária extensiva em campo nativo) tem sido mais sustentável do ponto de vista da conservação da paisagem do que aqueles levados a efeitos em áreas originais de floresta.

No caso específico dos campos sulinos existe também um grande tônus sobre o aspecto da territorialidade. Para Lyns (2004), território significa colocar em primeiro plano a territorialidade, a qual é percebida como conjugação de ativos específicos, dificilmente encontrados com as mesmas características em outros locais. Os ativos específicos seriam aqueles recursos essenciais disponíveis no território, para as atividades produtivas, os quais podem ser caracterizados através do conhecimento implícito difundido no arranjo local, da identidade sociocultural e das instituições presentes.

Sob essa análise, o Estado do Rio Grande do Sul parece possuir potencial para produzir carne sob a ótica de sistemas de produção *Low Input,* uma vez que essa proposta, não acarretaria modificaçãoes nas formas tradicionais de produzir. Ao mesmo tempo, percebe-se que o processo da globalização e o modelo hegemônico de produção também têm demonstrado consequências negativas na pecuária do Rio Grande do Sul sobre diversos aspectos. Além da dificuldade de competir economicamente com os estados do centro-oeste do Brasil, a pressão para aumentar a competitividade do sistema de produção tem causado erosão genética dos recursos naturais, como bem representada na charge de Santiago (2010) (Figura 4). Também deve-se ressaltar a erosão cultural e do saber fazer, afinal, o *Gaúcho*, manejador das pastagens naturais, passa a não mais existir com o esgotamento desse recurso.

FIGURA 4. Charge do cartunista Santiago sobre a modificação do ambiente no Bioma Pampa (Fonte: Zero Hora, 2010).

1.3 Efeito da dieta na qualidade da carne de ruminantes

A qualidade da carne é resultado de uma interação multifatorial, no entanto, esses fatores podem ser resumidamente, divididos em duas categorias: fatores intrínsecos ao animal (raça, idade, sexo, etc) e fatores do ambiente (alimentação, clima, procedimentos de abate, etc). No entanto, o efeito da alimentação dos animais sobre a qualidade da carne não é simples de avaliar (PRIOLO et al., 2001). Assim, cuidados devem ser tomados para que não exista confundimento sobre se os efeitos ocorrem em função dos componentes intrínsecos da dieta ou se a dieta influenciou na taxa de crescimento dos animais e na composição corporal dos mesmos (MUIR et al., 1998). Em muitos experimentos, ao avaliar o efeito de dietas na qualidade da carne é comum encontrar as seguintes situações: i) Os animais são abatidos ao mesmo peso, mas com idades diferentes, portanto taxas de crescimento diferentes, ii) os animais são abatidos com pesos diferentes e mesma idade portanto, taxas de crescimento distintas. Essas dissimilaridades levam a variados estágios de acabamento dos animais, o que pode acarretar diferentes taxas de resfriamento na carcaça e alterando, portanto, a cor da carne e a maciez, entre outras características. Ajustar as ofertas de matéria seca das dietas para a obtenção da mesma taxa de crescimento nos animais é uma das alternativas, no entanto, quando se avaliam pastagens, nem sempre esse ajuste é obtido. Em condições de produção a pasto, além da variabilidade entre os animais com relação ao seu comportamento ingestivo, também existe grande heterogeneidade do consumo de forragem, tanto espacial quanto na escala de tempo (PRACHE & PEYRAUD, 2001). Dessa forma, utilizar o termo sistema de produção, por incluir de forma mais abrangente os fatores extrínsecos à dieta, pode ser mais adequado do que tratar apenas os efeitos da alimentação em casos específicos, *e.g.* pastagens de diferentes espécies.

Também, na avaliação da qualidade do produto cárneo em diferentes sistemas de produção é importante que o abate dos animais ocorra no mesmo estado de maturidade fisiológica (mesmo nível de cobertura de gordura subcutânea) sendo esse, o critério que permitirá a comparação desses sistemas

1.4 Dilema *Low-Input* e Qualidade Sensorial da carne

Os sistemas de produção *Low Input* apresentam reflexos variados na qualidade sensorial dos produtos cárneos

1.4.1 Cor

A cor da carne é um dos primeiros atributos percebidos pelo consumidor na hora da compra e um dos que mais influencia na decisão em adquirir ou não o produto (MANCINI & HUNT, 2005). A cor da carne é resultado da concentração do pigmento mioglobina, uma cromoproteína que estoca oxigênio para o metabolismo aeróbico do músculo (LAWRIE, 2005). Uma vez que os músculos diferem com relação às suas demandas de atividade, diferentes concentrações de mioglobina serão encontradas nos diferentes músculos. A concentração desse pigmento também é variável entre as

espécies animais, explicando a ocorrência de carnes mais escuras em equinos do que em bovinos ou ovinos. Além disso, o estado químico da mioglobina também responde por grande parte da variação da cor, sendo altamente dependente do estado do íon Ferro localizado na porção não proteica da mioglobina. O sistema de produção tem efeito sobre a cor da carne. Priolo et al., (2001), ao revisarem 35 trabalhos que compararam terminação com concentrado *versus* terminação a pasto, concluíram que irrefutavelmente a carne de animais terminados a pasto é mais escura que a de animais terminado com concentrado. Contudo, não existe um efeito direto entre dieta e concentração de mioglobina e possíveis diferenças devem ser influenciadas por pH final, idade do animal, peso da carcaça, conteúdo de gordura intramuscular e até mesmo pela atividade física (VESTERGAARD et al., 2000; DUNNE et al., 2011).

1.4.2 Maciez

A maciez da carne, juntamente com o sabor e aroma, são as características que determinam a aceitação do produto pelo consumidor (FELÍCIO, 1998).

A maciez pode ser medida de diversas formas. Uma das mais comuns é pela força de cisalhamento de Warner-Bratzler (WBSF), que indica a força máxima necessária para romper uma amostra de carne cozida, sendo essa medida expressa em kgf/cm² e bem correlacionada com o parâmetro dureza em painéis sensoriais, sobretudo para o músculo *Longissimus* (SHACKELFORD et al., 1995).

Os principais fatores que influenciam a maciez da carne são a idade do animal e fatores genéticos.

É comum nos sistemas de terminação a pasto, sobretudo em sistemas mais extensivos, que os animais sejam abatidos com idade superior a de animais confinados, verificando-se, em geral, carnes de menor maciez (MOLONEY et al., 2011). O avanço da idade dos animais ocasiona a formação de ligações cruzadas termoestáveis de colágeno entre os feixes de fibras de muscular, formando uma rede tridimensional com alta força tensional (LAWRIE, 2005), incrementando a firmeza dos tecidos e determinando maiores forças de cisalhamento. Assim, Moloney et al., 2011 verificaram maior força de cisalhamento para animais terminados a pasto do que animais terminados em confinamento, sendo os de confinamento cerca de cinco meses mais jovens que os animais terminados a pasto.

Por outro lado, o desenvolvimento de carnes macias em animais com idade mais avançada pode ocorrer em função de ganhos compensatórios pré-abate, conforme relatado por Devincenzi et al.,(2012). Situações de ganho compensatório são muito comuns em sistemas de baixo aporte de insumos nos quais os animais encontram-se em condições onde há sazonalidade na produção de forragem. Aparentemente, o crescimento compensatório promove um *turn-over* proteico formando novas estruturas de tecido conjuntivo com colágeno mais solúvel, apresentando relativos incrementos na proteólise *post-mortem* e consequentemente na maciez da carne (HARPER, 1999; ANDERSEN et al., 2005). O efeito do ganho compensatório na maciez da carne foi estudado por Alligham et al., (1998). O autor encontrou que as

medidas relativas à resistência do tecido conjuntivo (compreesão e coesão) foram menores no contrafilé de bovinos que sofreram restrição alimentar e depois foram realimentados do que em animais que apresentavam ganhos constantes. O ganho médio diário no período de terminação parece ser correlacionado com a atividade calpaína, enzima que atua na proteólise *post-mortem*, incrementando a maciez da carne (THOMSON et al., 1999; PERRY &THOMPSON, 2005). As tecnologias utilizadas na indústria frigorífica podem melhorar significativamente os defeitos de baixa maciez da carne (LOBATO et al., 2014). RIBEIRO et al., (2002) relataram grande eficiência da maturação em embalagem a vácuo na redução da força de cisalhamento na carne de bovinos. MOLONEY et al., 2011 também obtiveram resultados similares, sendo que após 14 dias de maturação não verificaram diferenças para força de cisalhamento na carne de bovinos terminados em confinamento e da carne de bovinos terminados em pastagens.

É importante ressaltar ainda que, apesar de apresentar correlação significativa, a godura intramuscular representa pouco efeito sobre a força de cisalhamento (entre 2 a 10%)(WHEELER, 1994; KIM, et al., 2007).

1.4.3 Sabor-aroma

O sabor-aroma, ou *flavour* também tem grande relevância como aspecto de aceitação da carne, sobretudo para a carne ovina, onde esse parâmetro tem maior importância para a satisfação do consumidor (PETHICK,et al. 2006). Sabor–aroma refere-se aos componentes dos alimentos responsáveis por causar estímulos sensoriais. As informações de sabor-aroma são integradas a informações de textura, e visuais para formar uma assinatura sensorial única.

Os compostos precursores do sabor-aroma são em sua maioria lipídios ou compostos hidrofílicos que sofrem reações termo-induzidas durante o processo de cocção. As duas principais reações para a formação do sabor-aroma da carne são: reações de Maillard entre os aminoácidos e açúcares, e reações de oxidação dos componentes lipídicos. (CALKINS & HODGEN, 2007).

Embora exista a percepção que a carne produzida em sistemas intensivos seja mais saborosa (KEANE & ALLEN, 1999), os trabalhos com preferência de consumidores mostram que existe uma tendência de que os mesmos prefiram a carne produzida localmente (OLIVER et al., 2006; REALINI et al., 2013) já que as preferências também são definidas por hábitos culturais, considerando, portanto, sistemas de produção particulares de cada país (FONT-I-FOURNOLS, 2014).

Estima-se que mais de uma centena de compostos sejam responsáveis pelo sabor e aroma da carne (CALKINS & HODGEN, 2007), sendo que alguns desses compostos podem ser influenciados por constituintes da dieta (VASTA & PRIOLO, 2006) e outros são especifícos para cada espécie animal e estágio fisiológico. A interação da dieta e espécie animal também é importante para a percepção sensorial de alguns compostos, portanto, alguns desses apresentam um efeito diferenciado de sabor-aroma dependendo da espécie animal (PRIOLO et al., 2001). Vários autores relatam que dietas a pasto produzem carnes de sabor-aroma diferenciado, em geral mais intenso, e

descritos como pastoral, *grassy* (referente à grama), *barnyard* (referente a celeiros) ou mesmo fecal, na carne de ruminantes (ROUSSET-AKRIM et al., 1997; PRIOLO et al., 2001; YOUNG et al., 2003).

O uso de pastagens consorciadas (leguminosas + gramíneas) é uma opção para sistemas de produção *Low Input* como forma a diminuir o aporte de nitrogênio no sistema via fixação biológica pelas leguminosas e tem sido recomendada como prática em diversos programas de produção sustentável (*e.g:* Programa ABC do Ministério de Agricultura Pecuária e Abastecimento, Brasil). Contudo, pastagens consorciadas com leguminosas parecem ter impacto sobre as características sensoriais da carne, sobretudo da carne ovina. Prache et al., (2011), ao compararem a carne de cordeiros produzidas em sistema de produção orgânico *versus* convencional (adubação com 100kgN/ha) através de painel sensorial, verificaram maior intensidade de flavour anormal para os animais terminados em sistema orgânico, e sugeriram que esse efeito fosse devido à maior participação de trevo branco nas pastagens orgânicas. Muitos estudos apontam que a causa de descriptores desagradáveis de sabor-aroma nesses sistemas de produção de carne ovina é o composto aromático denominado escatol (DESLANDES et al., 2001, YOUNG et al., 2003, SCHREURS et al., 2007a).

1.4.4 Efeitos do escatol na produção animal

O escatol ou 3-metilindol é um composto volátil, lipossolúvel, sendo produto da metabolização bacteriana do aminoácido triptofano. O escatol é reportado como sendo tóxico especialmente aos tecidos pulmonares dos organismos, sendo o agente etiológico de doenças como enfisema e edema pulmonar bovino (DESLANDES et al., 2001). Em suínos, a interação do escatol com o esteróide sexual androstenona é altamente associada com o *boar taint* ou odor de macho inteiro, acarretando prejuínos na indústria cárnea, já em ruminantes, está associado ao sabor-aroma característico de pasto ou *pastoral,* ou animal (ROUSSET-AKRIM et al., 1997)*.*

Além do escatol, o metabolismo anaeróbico do triptofano leva a formação de indol, outro composto aromático lipossolúvel, porém de menor efeito sobre as características sensoriais da carne. Ambos compostos são produtos finais da degradação microbiana do triptofano, e são produzidos no rumem, nos animais ruminantes e no ceco e cólon nos monogástricos. O triptofano é normalmente originário da dieta. Leguminosas como trevo-branco, alfafa, ou mesmo gramíneas como o azevém em fase vegetativo são ricas em proteínas solúveis e provém uma fonte importante de triptofano para a fomação de escatol e de indol (SCHREURS et al., 2007). No rúmem, o triptofano sofre reações de desaminação desencadeadas por bactérias formando uma molécula de indolpiruvato. Em seguida o indolpiruvato é descarboxilado, formando indolacetato. Sucessivas reações de descarbolixação formarão as moléculas de indol e de escatol (3-metilindol) (Figura 5).

O rúmem e o intestino absorvem parte do escatol produzido e o restante é metabolizado no fígado. Quando a produção de escatol no rúmen ultrapassa a capacidade metabólica hepática, o escatol excedente é depositado nas células adiposas. Existe uma grande variabilidade individual em metabolizar escatol no fígado (VASTA & PRIOLO, 2006; SCHREURS, et al.,

2007b; ZAMARATSKAIA & SQUIRES, 2008) assim, a concentração desse composto nos tecidos adiposos também é bastante variável para o mesmo regime alimentar, tanto em bovinos quanto para ovinos (PRIOLO et al., 2001).
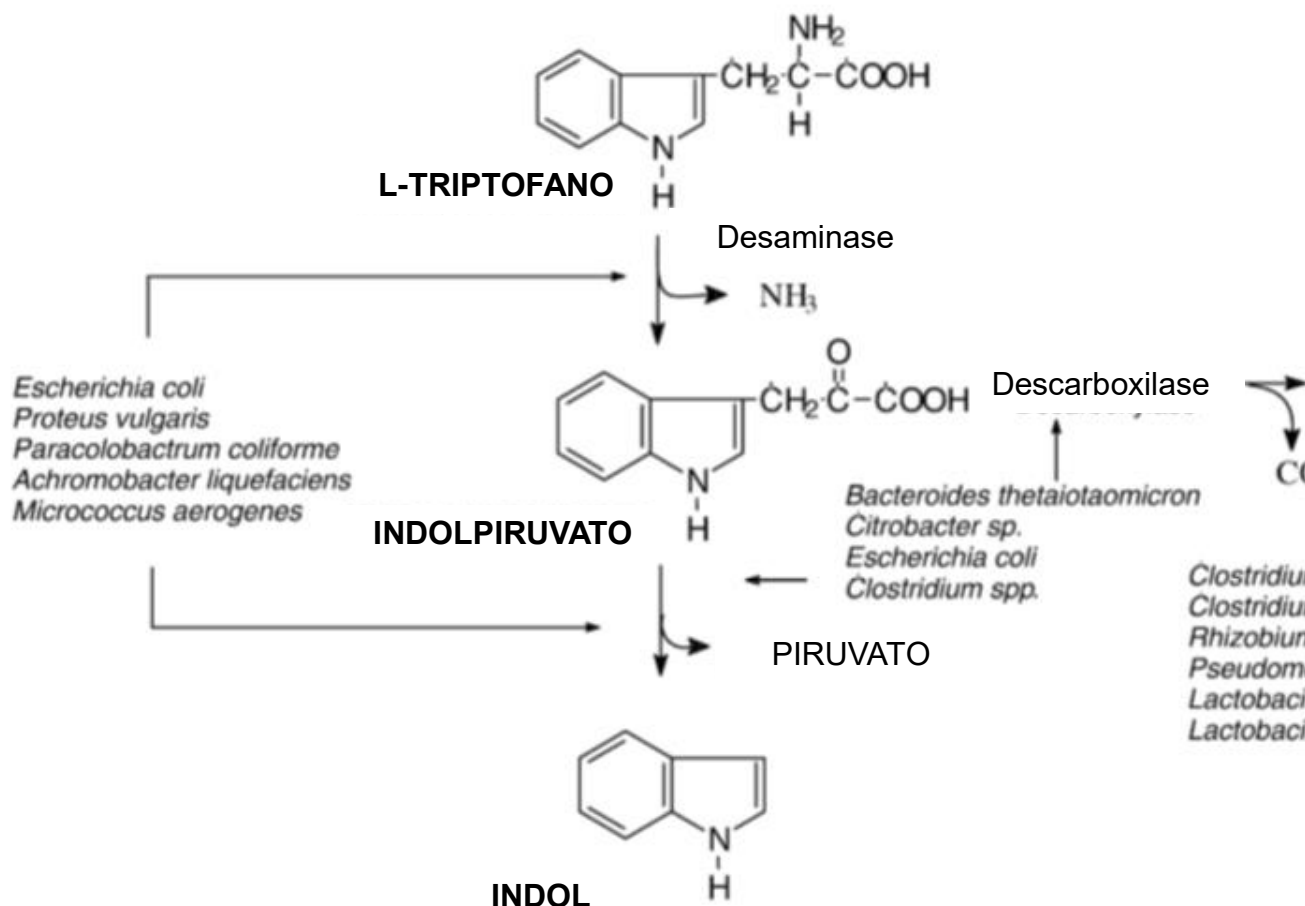
FIGURA 5. Fermentação do triptofano levando à formação de escatol (3-metilindol) e indol e principais bactérias envolvidas na reação (DESLANDES et al., 2001).

O efeito da concentração escatol sobre a intensidade de sabor-aroma indesejáveis parece ser mais significativo para a carne de cordeiros do que para a carne de bovinos. YOUNG et al., 1999 encontrou diferença na concentração de escatol na gordura da carne para bovinos alimentados a pasto e bovinos alimentados com concentrado, no entanto, o autor não observou diferença sensorial entre essas carnes. Na carne de ovinos, a ocorrência de sabor-aroma desagradáveis em função da presença de escatol é agravada pela presença de outros compostos voláteis: os ácidos graxos de cadeia ramificada (AGCR). Os AGCR, especialmente os ácidos 4-metiloctanóico e 4-metilnonanóico, são responsáveis pelo sabor-aroma característico de carne ovina. Os AGCR derivam do excesso de proprionato no rúmem. Assim, quando

a quantidade de propionato é maior que a capacidade do fígado em metabolizá-lo ocorre a formação de AGCR, sendo que em ovinos, o metabolismo do proprionato é diferente daquele dos bovinos (WONG et al., 1975, HA & LINDSEY, 1991). Apesar de que para ovinos as dietas ricas em concentrados produzam mais AGCR, observa-se que em sistemas de produção a pasto a presença de escatol pode aumentar a percepção do sabor característico de ovino causado pelos AGCR, mesmo este último estando presente em níveis mais baixos nos tecidos (YOUNG et al., 1999).

Estratégias vêm sendo estudadas com o intuito de minimizar os riscos da ocorrência de sabor-aroma desagradáveis na carne produzidas em sistemas de produção que utilizam leguminosas. O uso de espécies ricas em taninos, como o cornichão (*Lotus corniculatus*), Sulla *(Hedysarum coronarium L.)* e o quebracho (*Schinopsis loretzii*) pode diminuir a degradabilidade das proteínas, possibilitando menor produção de escatol (SCHREURS et al., 2007a; SCHREUS et al., 2007b), no entanto esses autores concluíram que a redução na produção de indol e escatol induzida por essas leguminosas não foram suficientes para afetar o odor da gordura derretida.

1.5 A carne de ruminantes como alimento funcional

Na nutrição humana a carne ocupa um lugar singular. O consumo de carne ultrapassa, mais do que qualquer alimento, a função nutricional. A carne é um alimento, mas é também rica em símbolos: símbolos de força, de potência (devido ao privilégio da caça) e de riqueza (carne é um alimento festivo). Por seus valores simbólicos, a carne ocupa uma parte central nas ideologias, nos mitos e nas crenças. Também as lógicas de proximidade com certos animais (animais selvagens, domésticos e familiares) com o homem, determinam a exclusão de alguns da categoria de comestíveis (PATOU-MATHIS, 2009).

Do ponto de vista fisiológico são as demandas energéticas que comandam a escolha dos alimentos. Contudo, para os homens, o paladar é essencial na escolha dos mesmos. A espécie humana sempre comeu carne. Um estudo citado por VARELLA (2011) e realizado com arcadas dentárias concluiu que nossos antepassados prefeririam a carne a outros alimentos e que a carne cozida teve papel fundamental para o desenvolvimento da cognição do homem.

No Brasil, a Secretaria da Vigilância Sanitária do Ministério da Saúde, define através da Portaria n°398 30/04/99 que alimento funcional é todo aquele alimento ou ingrediente que, além das funções nutricionais básicas, quando consumido na dieta usual, produz efeitos metabólicos e/ou fisiológicos e/ou benefícios à saúde (BRASIL, 1999). A carne pode ser considerada um alimento funcional sem nenhum processo adicional (FERGUSON, 2010 e ARIHARA, 2004), sendo fonte de compostos saudáveis da dieta (BINNIE et al., 2014). A carne, especialmente a carne de ruminantes, é rica em proteínas (tais como isoleucina, lisina, leucina, triptofano, treonina, metionina, fenilanina, valina, histidina), em vitaminas do complexo B (niacina, tiamina, riboflavina ácido pantotênico), em minerais (Fe_{heme}, Zn, K, P, Mg e Se) e ácidos graxos essenciais.

Após a o início do período industrial percebeu-se uma grande

modificação dos hábitos alimentares da população ocidental. Houve um significativo incremento da participação de gorduras tanto saturadas quanto do tipo trans e um decréscimo importante na participação de ácido ascórbico, vitamina E e ácidos graxos da família ômega-3 (SIMOPOULOS, 1991) (Figura 6).

Após a segunda guerra mundial descreveu-se uma epidemia de ataques cardíacos em homens de 50 anos e mulheres na menopausa. A população começou a se perguntar qual seriam as causas desses novos problemas de saúde. Interessante a análise de Varella (2011) sobre como a suspeita sobre as causas dessa epidemia recaiu sobre a carne bovina:

"Habituados a interpretar fenômenos biológicos com lógica religiosa, os homens associaram o prazer ao pecado. Sexo e paladar, os maiores prazeres conhecidos, são os principais suspeitos de qualquer doença. Como no caso dos infartos não parecia razoável culpar o sexo, praticado à larga pelo homem desde tempos ancestrais, a suspeita caiu sobre a alimentação. Estávamos nos anos 60, era da contracultura, da valorização da vida campestre em oposição à sociedade industrial. Era moda acreditar na alimentação vegetariana produzida sem fertilizantes químicos como condição de saúde. A suspeita, então, caiu em cheio sobre a carne vermelha, o alimento preferido pela maioria das pessoas. Afinal, gostamos de peixe, mas precisa ser bem feito; e de frango, dependendo do tempero; mas carne vermelha, de qualquer jeito é bom. Não é preciso ciência no preparo. Basta pôr na brasa e jogar sal grosso. O cheiro de peixe na panela faz perder o apetite, o de frango é neutro, mas o de carne junta saliva na boca. É reflexo ancestral"
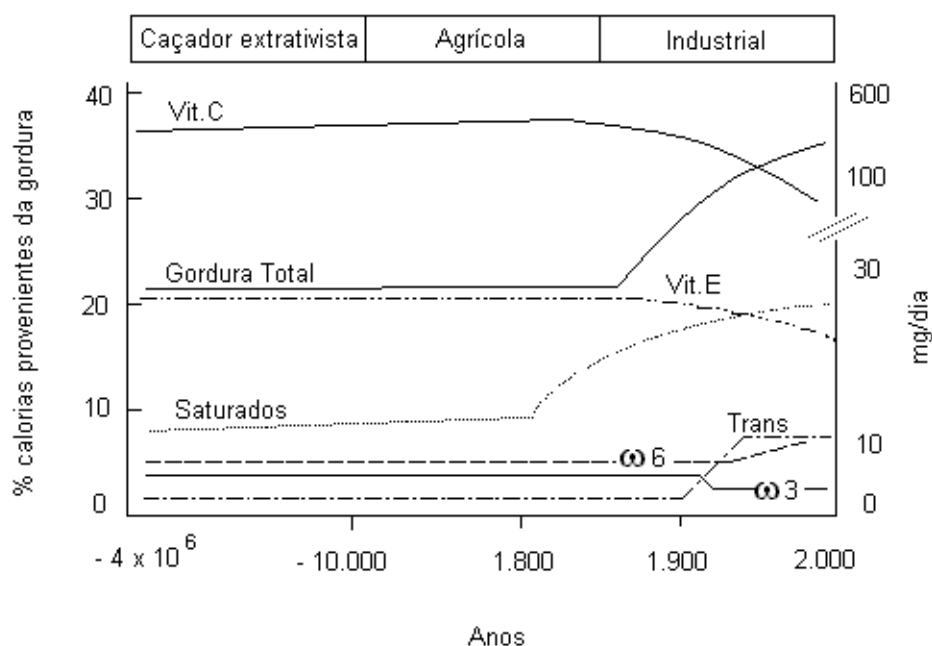

FIGURA 6. Evolução da participação dos componentes da dieta humana ao longo dos períodos históricos (Adaptada de Leaf et al., 1987).

Percebe-se que frequentemente a carne vermelha é alvo da mídia no que tange seus reflexos para a saúde humana. A recomendação habitual de médicos e nutricionistas para uma dieta saudável consiste na redução drástica da ingestão de carnes vermelhas usando as carnes ditas "magras", tais como peixes e frango e leguminosas como fonte de proteínas. Inclusive, a nova pirâmide alimentar proposta por Skerrett & Willet, 2010, sugere que carnes vermelhas devem ser consumidas com menos frequência que o álcool.

De fato, muitas revisões tem evidenciado os riscos e benefícios em relação ao consumo de carne vermelha sobre doenças cardíacas (MC AFEE et al, 2010, MC NEIL & VAN ELSWYK, 2012, WYNESS et al., 2011) risco de câncer (BIESALSKI, 2005, CORPET, 2011, DEMEYER HONIKEL & DE SMET, 2008, FERGUSON, 2010, MC AFEE et al., 2010, MC NEIL &VAN ELSWYK, 2012 e WYNESS et al., 2011), risco de obesidade (MC NEIL &VAN ELSWYK, 2012, SCHÖENFELDT & GIBSON, 2008, WYNESS et al., 2011) e risco de diabetes tipo 2 (WYNESS et al., 2011).

Por outro lado, os trabalhos mais recentes de meta análise e revisões sobre o assunto concluíram que não existe associação entre consumo de carne vermelha, mortalidade por câncer e doenças cardiovasculares (KAPPELER et al., 2013). O consumo de 100g/dia de carne vermelha não processada não foi associada ao risco de doenças cardiovasculares (Micha et al., 2010). Além disso, o trabalho de Kappeler et al., (2013) verificou que existe associação positiva entre o consumo de carne vermelha e o consumo de vegetais. Com relação ao consumo de carne e câncer, o câncer colo-retal é o principal tipo de câncer associado ao consumo de carne vermelha, no entanto, a evidência mais convincente é a da associação de carne processada com os diversos cânceres (FERGUSON, 2010).

Nos últimos anos muito enfoque tem sido dada à qualidade da fração lipídica da carne como um dos indicadores da saudabilidade desse produto (RIBEIRO et al., 2010). Sabe-se que a composição lipídica, da fração comestível de carnes vermelha é dependente de diversos fatores a dieta dos animais, raça, sexo, idade, tipo de músculo, bem como a interação entre fatores (DE LA TORRE et al., 2006, JUÁREZ et al., 2008), sendo que raros estudos epidemiológicos descrevem tais esses detalhes.

1.5.1 Caracterização do perfil lipídico da carne de ruminantes e lipídios bioativos

Nos ruminantes, os lipídios são majoritariamente sintetizados nos adipócitos à partir dos ácidos graxos voláteis (principalmente o acetato), provenientes da fermentação ruminal, sendo uma parte minoritária de origem alimentar. De fato, o teor de lipídios da dieta dos ruminantes alimentados com volumosos corresponde a cerca de 2 a 5% da matéria seca e são basicamente galactolipídios e fosfolipídios. Os principais ácidos graxos presentes nos lipídios da dieta habitual dos ruminantes são o C18:3 *n*-3, nos pastos verdes e fenos e 18:2 *n*-6 nos alimentos concentrados (farelos, grãos, e rações).

Diferentemente dos monogástricos, nos ruminantes, os lipídios provenientes dos alimentos são hidrolizados e depois hidrogenados pela flora microbiana do rúmen. Esse processo conduz ao desaparecimento de 70% a 90% dos ácidos graxos poliinsaturados (AGPI), os quais são transformados em

ácidos graxos saturados (AGS) ou ácidos graxos monoinsaturados (AGMI) *trans* (BONNET et al., 2010).

Nos enterócitos (células presentes na camada superficial do intestino), os ácidos graxos são reesterificados em triglicerídeos (TG) e fosfolipídios (FL) e ésteres de colesterol, e em seguida são absorvidos via linfa, principalmente na forma de proteínas de muito baixa densidade (VLDL para *very low density lipoproteins*) onde os ácidos graxos absorvidos são basicamente os AGS e algumas vezes na forma de quilomicrons, no caso do uso de lipídios protegidos ricos em AGPI (BAUCHART, 1993). A composição química e a taxa de secreção das lipoproteínas são os principais fatores que controlam a utilização de TG do plasma e por consequência, sua distribuição entre músculo, fígado e tecido adiposo (BONNET et al., 2010).

A fração lipídica da porção comestível das carnes vermelhas é em geral composta por 46,2% de AGS, 37,2% de AGMI e 14 % de AGPI (Tabela 1, Média de compilação de dados). Destaca-se a grande participação de AGS nesse alimento, o que poderia ser um problema do ponto de vista à saúde humana, visto de em geral, os AGS tem efeito colesterolêmico e aterogênico (ULBRITCH & SOUTHGATE, 1991). Entretanto, grande parte do total de AGS (aproximadamente 40%) é composta por ácido esteárico (C18:0), que tem efeito neutro quanto a sua aterogenicidade (KRIS-ETHERTHON & YU, 1997) .

TABELA 1. Efeito de raças, regimes alimentares, tempo de permanência e horário de pastejo na composição centesimal do músculo *Longissimusi* em ruminantes (Compilação de dados da literatura).

Estudo	Tratamentos	AGS	AGMI	AGPI	n-6	n-3	n-6/n-3	CLA
Freitas et al., 2014	Bovinos (Regime alimentar)							
Local: Brasil Sul	Concentrado	48,84	41,11	9,98	8,18	1,45	5,82 a	0,26
	Pasto	45,66	43,86	10,31	7,66	2,14	3,64 b	0,28
Rossato et al., 2010	Bovinos (Raças)							
Local: Brasil Central	Angus	49,17	42,79	8,05 b	4,33	2,31 b	1,88 a	0,59 b
	Nelores	47,87	42,41	9,72 a	4,93	3,11 a	1,58 b	0,66 a
Vasta et al., 2012	Cordeiros							
	Tempo e período de permanência na pastagem							
Local: Itália	Pasto 8h	38,08 b	34,5 ab	24,41 b	.	.	3,01	1,85 b
	Pasto Manhã 4h	39,76 a	33,71 b	26,53 b	.	.	3,21	1,45 c
	Pasto Tarde 4h	34,85 c	34,86 a	30,29 a	.	.	3,04	2,39 a
Muchenje et al., 2009	*Bovinos (Raças)*							
Local:África do Sul	Nguni	43,8	34,19	21,92	13,95	7,97	1,83	0,34
	Bonsmara	43,89	34,1	21,82	14,9	7,92	1,78	0,31
	Angus	44,75	35,85	19,31	11,93	7,39	1,63	0,33
De Menezes et al, 2008	Bovinos (Regime alimentar)							
Local: Brasil Sul	Confinamento	48,94 ab	38,28 a	7,38	4,64	1,19 b	4,36 b	0,21 b
	Pastagem temperada (Azevém)	46,6 b	40,8 a	8,47	3,72	2,13 a	1,77 a	0,62 a
	Pastagem tropical (Milheto e Papuã)	50,69 a	34,24 b	9,08	4,47	1,94 ab	2,38 a	0,41 ab
Lourenço et al., 2007	Cordeiros (Tipos de pastagens)							
Local: França	Pastagem >Azevém perene	53,4	35,9	4,55 b	.	.	.	1,01 b
	Pastagem >Leguminosas	50,3	35,3	7,78 a	.	.	.	0,68 a
	Pastagem biodiversa	55,7	33,2	4,63 b	.	.	.	1,32 a
Realini et al., 2004	Bovinos (Regime alimentar)							
Local: Uruguai	Pastagem	49,08	40,96 a	9,96 a	.	.	1,44 a	0,41 a
	Concentrado	47,72	46,36 b	6,02 b	.	.	3,0 b	0,23 b

Médias seguidas por letras diferentes na coluna apresentam

diferença estatística conforme o nível de significância adotado em cada estudo

Os principais lipídios bioativos funcionais encontrados na carne vermelha são os ácidos graxos da família ômega-3, representados pelo ácido linolênico C18:3 *n*-3, o ácido eicosapentaonóico (C20:5 *n*-3) e docosahexaenóico (C22:6 *n*-3) e os isômeros do Ácido Linoléico Conjugado (CLA).

Os ácidos graxos da família ômega-3 (*n*-3) representam aproximadamente 5% do total de ácidos graxos da porção comestível da carne vermelha (Tabela 1). Os ômega-3 são ácidos graxos essenciais e possuem reconhecida importância por compor os lipídios das membranas celulares e por manter as funções cerebrais e na transmissão de impulsos nervosos. Esses ácidos graxos também atuam na transferência do oxigênio atmosférico para o plasma sangüíneo, na síntese da hemoglobina e na divisão celular (MARTIN et al., 2006). Conforme a revisão de Martin *et* al., (2006), diversos estudos demonstram a capacidade dos ácidos graxos da família ômega-3 em reduzir significativamente o colesterol total plasmático LDL, HDL e triglicerídeos totais. Em estudos com células de câncer de mama humana, camundongos que recebiam ácidos graxos ômega-3 na dieta apresentaram menor incidência de metástases pulmonares (FERNANDES & VENKATRAMAN, 1991). Também são relatados os efeitos benéficos dos ômega-3 no combate à doenças inflamatórias e auto-imunes como artrites e psoríase (SIMOPOULOS, 1991).

Uma vez que não é sintetizado pelo organismo animal, o C18:3 *n*-3 presente nos tecidos de ruminantes é proveniente da dieta consumida pelo mesmo. Esse ácido graxo só está presente nos tecidos porque "escapou" do processo de biohidrogenação. Esse escape pode ser decorrente da alta taxa de passagem da dieta, no caso de forragens de alta digestibilidade, ou mesmo pelo uso de C18:3 *n*-3 protegido em certas rações. Já a presença de C20:5 *n*-3 e de C22:6 *n*-3 na carne é resultado de processos enzimáticos e dessaturação e elongação do C18:3 *n*-3 que ocorrem no próprio tecido animal (Figura 7).

C18:3 Ω-3
Ác. Linolênico

Δ-6-dessaturase

C18:4 Ω-3

Elongase

C20:4 Ω-3

Δ-5-dessaturase

C20:5 Ω-3
Eicosapentanóico

Elongase

C22:5 Ω-3

Δ-4-dessaturase

C22:6 Ω-3
Ac.docosahexanóico
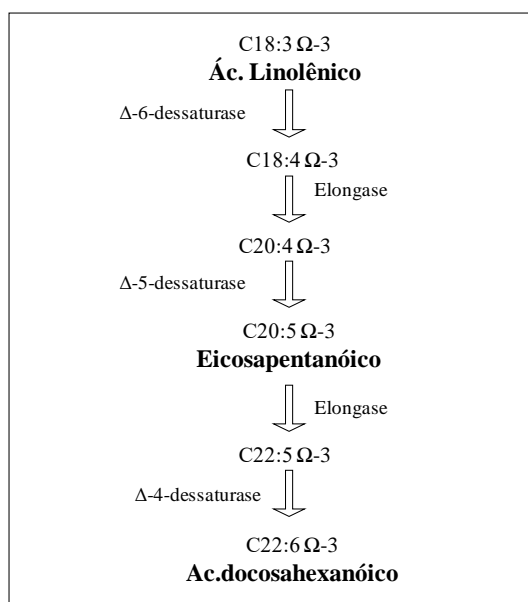
FIGURA 7. Representação esquemática da biossíntese dos ácidos graxos de cadeia longa da

família ômega- 3 nos tecidos (Adaptado de Palmquist e Mattos, 2006).

Os diversos isômeros do ácido linoleico (CLA) encontrados nos produtos de ruminantes são produtos intermediários do processo de hidrogenação realizado pelas bactérias ruminais (GRIINARI & BAUMAN, 1999). Ao total foram identificados 24 isômeros de CLA (CRUZ –HERNANDEZ et al., 2004), sendo que o principal isômero presente nos produtos de ruminantes é o cis-9, trans-11 C18:2 *n*-6, denomimando ácido rumênico. A bactéria *Butyrivibrio fibrisolvens* é a que possui maior capacidade de biohidrogenação (citação), no entanto, a biohidrogenação completa dos AGPI é dependente da ação sinérgica de outras espécies de bactérias, como a *Fusocillus sp.* Ainda que se suspeite que seja um mecanismo de detoxificação (os AGPI são tóxicos para bactérias ruminais), a função do processo de biohidrogenação ainda não está claramente elucidada. Resumidamente a biohidrogenação consiste na atividade de isomerases e redutases que transformam o C18:2 e C18:3 a C18:0 (Figura 8).
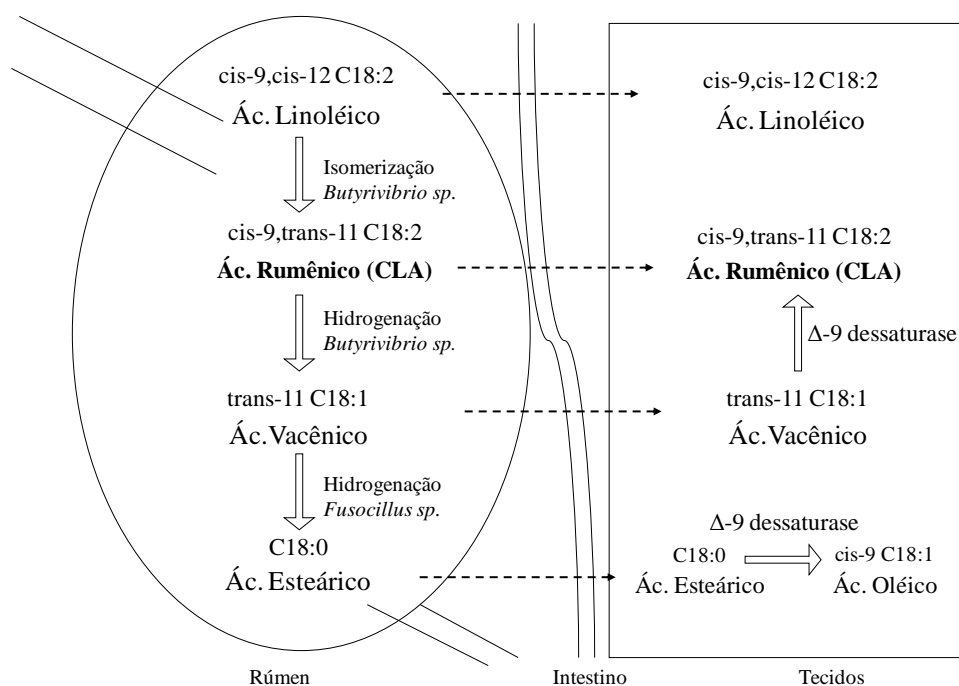
FIGURA 8. Representação esquemática da biohidrogenação e consequente biossíntese do Ácido Rumênico (CLA *cis9, trans 11* C18:2 *n-6*) (Adaptado de Griinari & Baumman, 1999).

No final da década de 80 ocorreram as primeiras descobertas sobre as propriedades do CLA na luta contra o câncer (HA et al., 1987). Atualmente é reconhecido como o único ácido graxo anti-cancerígeno e anti-carcinogênico, sendo portanto capaz de evitar o aparecimento do câncer e de combatê-lo após instalado. Em modelos com camundongos mostrou ainda ser auxiliar no controle da diabetes, reduzir a arterogênese (PARIZA et al., 2000). Os mecanismos de ação do CLA ainda não são totalmente conhecidos. Suspeita-se que essas moléculas devem influenciar a progressão e o desenvolvimento

do câncer basicamente de três maneiras: i. Afetando diretamente o processo de carcinogênese, ii. Reduzindo o acúmulo de gordura corporal, o que indiretamente influencia o risco de câncer e iii. Reduzindo a caquexia, a qual é a associada a estágios avançados de câncer e à algumas estratégias de tratamento *e.g.* quimioterapia (PARIZA et al, 2000).

        1.5.2. Modificação da participação de lipídios funcionais na carne de ruminantes através de fatores de produção

        Na carne, a quantidade de ácidos graxos da família ômega 3 é pouco influenciada pelo genótipo do animal. (MUCHENJE et al., 2009), sendo o sistema de produção, e consequentemente o tipo de dieta os fatores mais importantes para a determinação dos teores desses ácidos graxos (SAÑUDO et al., 2000; PONNAMPALAM et al., 2012). Embora sejam modificados pelo metabolismo ruminal, o perfil de ácidos graxos na dieta dos animais está correlacionada com o perfil de ácidos graxos na carne (RAES et al., 2004). As maiores fontes de ômega-3 são as forragens verdes, óleos de peixes e de linhaça.

        Os pastos frescos ou até mesmo certas forragens conservadas tais como a silagem de azevém, são ricos em C18:3 *n*-3 , diferentemente dos cereais utilizados usualmente nos alimentos concentrados (Tabela 2). Esses ácidos graxos, dependendo da abundância com que estão presentes em cada tipo de forragem e também dependendo da taxa de passagem da forragem pelo rúmen, ficam mais ou menos suscetíveis ao processo de biohidrogenação. Se a ingestão de ácidos graxos insaturados é muito grande, a capacidade dos microorganismos do rúmen em biohidrogenar pode ser excedida, ocorrendo uma maior absorção intestinal dos ácidos graxos insaturados (RULE & BEITZ, 1986).

TABELA 2. Composição centesimal em C18:3 *n*-3 (Ácido Linolênico) de alimentos para ruminantes (Compilação de dados da literatura).

Alimento	Teor de Ácido Linolênico C18:3 (g/100g de AG)	
Cereais		
Cevada	4,3	Palmquist &Mattos, 2006
Milho	4,2	Palmquist &Mattos, 2006
Sorgo	2	Palmquist &Mattos, 2006
Aveia	2,1	Palmquist &Mattos, 2006
Trigo	4,5	Palmquist &Mattos, 2006
Forrageiras frescas		
Azevém perene	61,0 ±7,32	Glasser et al., 2013
Gramínea temperada	49,9 ± 3,94	Glasser et al., 2013
Pastagem tropical	22,74	De Menezes., 2008
Pastagem natural do Rio Grande do Sul	39,6	Freitas et al., 2011
Alfafa	41,7 ± 5,81	
Trevo vermelho	49 ± 9,11	Glasser et al., 2013
Trevo branco	58,0 ±5,51	Glasser et al., 2013
Forragens conservadas		
Feno de alfafa	22,6 ± 8,19	Glasser et al., 2013
Feno de trevo vermelho	46,9 ± 8,85	Glasser et al., 2013
Silagem de azevém	53,4 ± 6,12	Glasser et al., 2013
Silagem de milho	5,04 ± 2,42	Glasser et al., 2013
Óleos		
Caroço de algodão	0,1	Palmquist &Mattos., 2006
Colza	8,2	Palmquist &Mattos., 2006
Soja	6,8	Palmquist &Mattos., 2006
Girassol	0,45	Zambiazi et al., 2007
Linhaça	54,24	Zambiazi et al., 2007

É importante ressaltar que a digestibilidade dos pastos e o teor de C18:3 *n*-3 estão diretamente relacionados (GLASSER et al., 2013). Pastos hibernais (de ciclo fotossintético C3) tem suas membranas celulares mais ricas em C18:3 *n*-3 como estratégia para proporcionar maior fluidez na membrana em ambientes frios. Da mesma forma, seu aparato fotossintético realiza a fixação de CO_2 nas células do mesófilo, o que diminui significativamente o teor de lignina das folhas quando comparadas a plantas estivais (de ciclo fotossintético C4), nas quais a fixação do dióxido de carbono é realizada nas células da bainha vascular, as quais são ricas em lignina (TAIZ & ZEIGER, 2004).

A adição de óleos ricos em C18:3 *n*-3 na ração concentrada, como o óleo de girassol e linhaça pode aumentar os teores de ácidos graxos da família ômega-3 na carne e essa estratégia tem se mostrado a mais eficiente em aumentar os teores de ácidos graxos essenciais e até o teor de CLA nos produtos de ruminantes (RIBEIRO et al, 2011). O uso de óleos protegidos também é bastante eficiente para produção de carne com perfil lipídico mais desejável no ponto de vista de saúde humana, mesmo em animais confinados. A saponificação dos ácidos graxos visando formar sais de cálcio, a reação de ácidos graxos com aminas primárias para produzir amino-acil graxos que resistem à biohidrogenação (JENKINS et al., 1996) e o uso de formaldeído (GULATI et al., 2000) são métodos eficientes de proteção de ácidos graxos,

contudo, deve-se observar a econimicidade do uso desses aditivos.

A concentração de CLA é variável em função da espécie de ruminante. Em geral, ovinos apresentam maior concentração de CLA que bovinos (SCHMID et al., 2006). Fatores intrínsecos ao animal, tais como, sexo, idade, genótipo e tipo de músculo estão envolvidos na modulação e produção dos isômeros de CLA (DE LA TORRE et al., 2006). Tais fatores estão relacionados com a deposição de gordura nos animais. De la Torre et al., 2006 verificaram que o conteúdo de CLA na carne bovina não foi relacionada ao estado de engorduramento final dos animais, mas sim pela taxa de deposição de gordura (diferença entre o escore de engorduramento final e o inicial). Os autores concluíram que a deposição de CLA no músculo é favorecida por baixas taxas de deposição de gordura. As diferenças na quantidade de gordura presente no animal são influenciadas pelo genótipo, sexo e idade (NURNBERG et al., 1998, LUCHIARI FILHO, 2000; RAES et al., 2004). De la Torre et al., 2006 encontrou maior concentração de CLA em touros (41% superior) do que em vacas maduras, ambos da raça charolesa, evidenciando o efeito do sexo. O sexo tem importante efeito no estado de engorduramento das carcaças, sendo que para um mesmo peso ao abate, tem-se carcaças mais engorduradas para fêmeas do que para machos inteiros.

No que toca ao genótipo, alguns autores reportam o efeito da raça para o teor de CLA (DE LA TORRE et al., 2006; GARCIA et al., 2008; ROSSATO et al., 2009; DANCE et al., 2009), sendo que nesses casos os maiores teores de CLA estão sempre relacionados à raças com menor teor de gordura intramuscular (Charolês, Cruzas Charolês x Angus e Holandês x Angus e Nelore). Por outro lado, outros autores não verificaram esse efeito entre raças bovinas europeias (Angus x Limosin) (WARD et al., 2010), nem ao comparar raças africanas com européias (Nguni x Bonsmara x Angus) (MUCHENJE et al., 2009).

O tipo de músculo e a localização anatômica influenciam na proporção de CLA. Esse composto está majoritariamente ligado à fração triacilglicerol, a qual é relacionada ao conteúdo de gordura dos tecidos. Assim, contrariamente ao verificado entre as raças, o CLA apresentou-se em maior proporção nos músculos com maior teor de gordura intramuscular (JIANG et al., 2010).

O efeito da dieta dos animais sobre a concentração de CLA na carne de ruminantes também tem sido bastante estudado. Embora os mecanismos que regem o padrão de deposição desses ácidos graxos sejam majoritariamente realizados via regulação enzimática, tem sido verificado que o conteúdo de CLA na carne de ruminantes possui uma relação linear e crescente com o conteúdo de pastos na dieta (FRENCH et al., 2000; BENOIT et al., 2010). Uma das hipóteses é a de que a dieta com base em pastos favoreça o crescimento das bactérias *Butyrivibrio fibrisolvens,* responsáveis pela isomerização do *cis9, cis 12* C18:2 *n*-6 no isômero *cis9, trans11* C18:2 *n*-6. Outra hipótese é a de que o aumento do CLA nos animais alimentados com pastagens é associada ao aumento de *trans 11,* C18:1 (Ácido Vacênico), o qual é o substrato da enzima Δ-9 dessaturase nos tecidos (Vide representação esquemática na Figura 7).

Os diferentes tipos de pastagens também têm reflexos no conteúdo

de CLA da carne. Lourenço, et al, (2007) encontraram maior conteúdo de CLA em cordeiros que foram terminados em pastagens com maior diversidade florística do que os animais que foram terminados em pastagens compostas por poucas espécies forrageiras. Ainda com relação ao tipo de pastagens, pastagens tropicais podem proporcionar carnes com perfil lipídico com menor participação de CLA do que pastagens temperadas (MEDEIROS et al., 2002).

Uma vez que é bastante conhecido o efeito deletério das dietas concentradas no que tange o teor de CLA nos produtos cárneos, muitos estudos vêm sendo desenvolvidos para melhorar esse perfil. Schmid et al., (2006) em uma revisão sobre ácido lineico conjugado na carne e em produtos cárneos motraram que estratégias eficientes para incrementar o CLA nos mesmos é através da adição de óleos ricos em AGPI , como óleo de girassol, óleo de peixe, óleo de linhaça. Da mesma forma, o uso de certas sementes oleaginosas também pode incrementar o teor de CLA no músculo.

1.6. Composição Isotópica como metodologia para traçar a origem dos alimentos

A preocupação em garantir a origem dos alimentos é cada vez mais recorrente seja na pesquisa ou entre os consumidores. Assim, o tema traçabilidade tem ganhado importância na área de produção de alimentos. A traçabilidade tem conceitos oriundos na indústria, e significa a capacidade de definir a origem de um produto. No caso de produção animal, pode-se se referir a identidade do animal, raça e origem geográfica, ou também a processos de produção, tais como dietas e sistemas de produção (PRACHE et al., 2007).

Diversos tipos de métodos tém sido estudados para assegurar a origem dos sistemas de produção e podem ser divididos em i) marcadores diretos, *i.e.,*que são provenientes diretamente da dieta, ii) marcadores indiretos, *i.e.* aqueles que sofrem transformações pelo metabolismo animal e iii) métodos espectrais, *i.e.* aqueles que realizam a caracterização espectral óptica dos tecidos (PRACHE, 2009).

Os isótopos são moléculas de um elemento químico que possuem mesmo número de prótons e um número variável de nêutrons. Na natureza, a composição relativa dos isótopos estáveis dos elementos é bastante variável. Esta variação é devida ao fracionamento isotópico provocado por diversos processos físicos, químicos e biológicos.

A composição isotópica, determinada pelo método de espectometria de massa de isótopos, tem sido utilizada com sucesso para autenticar regimes alimentares ou origem geográfica da carne (OLIVEIRA et al., 2014). Isso é possível porque todo animal ou planta possui sua própria composição isotópica natural, a qual só pode ser modificada por processos de *turn-over* metabólico, portanto, representando uma "impressão digital" ou assinatura isotópica nos tecidos (VINCI et al., 2012).

Na pesquisa em autenticação e traçabilidade de alimentos os principais isótopos utilizados são os isótopos de Oxigênio (relação $^{18}O/^{16}O$), de Hidrogênio ($^{2}H/^{1}H$), de Carbono ($^{13}C/^{12}C$), de Nitrogênio ($^{15}N/^{14}N$) e de Enxofre ($^{34}S/^{32}S$). Os valores dessas relações são usualmente expressos em delta por mil ($\delta/\text{‰}$), e são calculados relacionando a concentração dos isótopos em uma

amostra de referência com a da amostra a ser estudada, através da seguinte fórmula:

$$\delta\ (‰) = (R_{amostra} - R_{padrão})/R_{padrão} * 1000,$$

onde R é a relação do isótopo menos abundante e do mais abundante.

As relações de isótopos estáveis de Hidrogênio ($^2H/^1H$) e Oxigênio ($^{18}O/^{16}O$) têm se mostrado eficientes em determinar a origem geográfica dos produtos, tanto do leite (RENOU et al., 2004) quanto da carne de ovinos (PIASANTIER et al., 2003). Nas plantas, as relações de $^{18}O/^{16}O$ e $^2H/^1H$ dependem da quantidade água absovida pelo solo, no entanto nas folhas das plantas, a água é submetida a um processo de fracionamento isotópico devido à evapotranspiração. Nos animais, a água presente nos tecidos possui as mesmas carcterísticas da água ingerida pelo animal, seja via água disponível para consumo ou a água presente nos alimentos (SCHMIDT et al., 2001). Esses processos são altamente influenciados pela temperatura e umidade relativa do ar, levando ao aumento de isótopos mais pesados. O valor de $\delta^{18}O$ também já foi utilizado com êxito para diferenciar regimes alimentares de bovinos. A alimentação com forragem fresca aumenta o valor de $\delta^{18}O$ na porção de água do leite quando comparada a períodos nos quais os animais recebem dietas baseada em forragem conservada (ENGEL et al., 2007).

O valor de $\delta^{13}C$ nos tecidos animais tem sido fortemente correlacionado com a dieta dos mesmos. Isso ocorre porque existe diferente composição de isótopos de carbono para plantas com rotas fotossintéticas diferentes (plantas C3 e C4). Em produtos lácteos, vacas alimentadas com plantas C3 apresentaram menores valores de ^{13}C do que vacas alimentadas com plantas C4. No estudo de Schwertl et al., (2005), o conteúdo de milho da dieta, explicou 96% da variação total de ^{13}C no pêlo de bovinos. A composição de isótopos de C também teve seu uso validado para verificar a existência de açúcar de cana (planta C4) no mel que é principalmente produzido a partir do pólen de plantas C3 (VINCI et al., 2012).

O valor de $\delta^{15}N$ nos tecidos também é altamente relacionado com a dieta dos animais e é de especial importância para a certificação de sistemas de produção. Em frangos, o valor de $\delta^{15}N$ foi eficiente em diferenciar animais criados ao ar livre de animais confinados, uma vez que a dieta de animais ao ar livre é mais rica em fontes proteicas como insetos e minhocas, os quais possuem um maior valor de $\delta^{15}N$ (MORI et al., 2007). Bahar et al., (2008), estudando a traçabilidade da carne bovina adquirida em supermercados irlandeses, verificou que a carne produzida em sistemas orgânicos possuía menor valor de $\delta^{15}N$ que a carne convencional. Diversos trabalhos sugerem que elevados valores de $\delta^{15}N$ em carne bovina estão ligados à sistemas de produção convencionais, nos quais o uso de fertilizantes minerais resultam em um balanço positivo do isótopo ^{15}N nas plantas. Por outro lado, dietas ricas em leguminosas, em geral mais abundantes em sistemas de produção orgânicos de carne, parecem diminuir o valor $\delta^{15}N$ nos tecidos animais (ROSSMANN et al., 1998). O nitrogênio do ar é composto por 99.6337% do isótopo ^{14}N e

0.3663% de ^{15}N. À medida que o nitrogênio é transformado durante seu ciclo a relação de isótopos estáveis se modifica. Uma vez que as leguminosas captam nitrogenio do ar através da fixação simbiótica, muito poucas transformações são realizadas, fazendo que a composição isotópica das leguminosas seja mais rica em ^{14}N, e, portanto um valor de delta ^{15}N (δ^{15}N) menor.

A composição isotópica de enxofre é afetada pela origem geológica da área onde é produzido o alimento para o animal. O valor de δ^{34}S nos alimentos também é relacionado com a proximidade do mar, uma vez que a pulverização com água do mar é rica em sulfatos enriquecidos com ^{34}S (ROSSMANN et al, 1998).

O tecido amostrado para análise de isótopos pode influenciar as conclusões a respeito da dieta dos animais. A composição isotópica de um determinado tecido depende da taxa de assimilação (*turn-over*) do elemento no mesmo. Sabe-se que, em geral, tecidos mais ativos metabolicamente possuem maior taxa de turn over do que a de tecidos menos ativos. Assim, avaliações em distintos tecidos podem trazer informações sobre as mudanças na alimentação dos animais em diferentes períodos de tempo (MONAHAN et al., 2012). Desse modo, medições na composição isotópica em fezes ou sangue (tecidos de metabolismo rápido), são eficientes em indicar dietas recentes (MARTINS et al, 2012; MONAHAN et al., 2012), enquanto amostras de músculos (tecidos com baixa taxa de substituição) indicam dietas pretéritas. Já tecidos como pêlo e lã, podem fornecer informações integrada da dieta ao longo da vida do animal (SPONHEIMER et al., 2003; SCHWERTL et al., 2005; YANAGI et al., 2012)

A análise intergrada de múltiplos elementos (δ^{18}O, δ^{13}C, δ^{15}N, δ^{34}S) pode melhorar a confiabilidade da informação. Essa abordagem tem auxiliado na caracterização e discriminação de produtos com Denominação de Origem controlada e Indicação de Procedência tais como queijos (Parmigiano Reggiano e Grana Padano) e presuntos (Parma) e também ajudado a definir a procedência de carnes (VINCI et al., 2012).

TABELA 3: Síntese dos principais isótopos estáveis utilizados em pesquisas com autentificação de alimentos, abundância natural na atmosfera, forma de fracionamento e principal informação fornecida sobre o alimento (Adaptado de Vinci et al., 2012).

Relação Isotópica	Abudância natural	Fracionamento	Informação
^{2}H/^{1}H	99,985/0,015	Evaporação Condensação e Precipitação	Geográfica
^{13}C/^{12}C	98,892/1,108	Rota Fotossintética Plantas C3 e C4	Dieta Geográfica

$^{15}N/^{14}N$	99,6337/0,3663	Nível trófico, Ciclo biogeoquímico, Plantas marinhas e terrestres e Práticas culturais	Dieta Geográfica Sistemas de Produção
$^{18}O/^{16}O$	99,7587/0,2039	Evaporação Condensação e Precipitação	Geográfica
$^{34}S/^{32}S$	95,02/4,22	Bacteriano Geológico	Geográfica

Diante do exposto, compreende-se que qualidade de um produto cárneo é composta pela interação de múltiplos fatores. Com a expansão da consciência dos consumidores devido ao fenômeno de maior acesso à informação, percebe-se que cada vez mais certos atributos de qualidade são extrínsecos ao produto, como, o sistema produção. No entanto, atributos fundamentais relacionados à qualidade do produto, tais como sabor, maciez, saudabilidade e inocuidade, ainda são questões chave pra aceitação do mesmo. Portanto, elucidar os efeitos dos sistemas de produção sobre as variáveis clássicas de qualidade da carne e desenvolver métodos para autenticar tais sistemas ainda constituiem uma relevante questão de estudo.

1.7 HIPÓTESES DO TRABALHO

1. Diferentes níveis de alfafa na dieta de cordeiros em pastejo tem impacto i) nas concentrações de deposição de escatol no tecido adiposo, as quais são relacionadas com as características sensoriais da carne. ii) no perfil lipídico da carne e da gordura subcutânea dos animais ii) no enriquecimento isotópico da carne permitindo a discriminação dos níveis de alfafa na dieta pelo método de isótopos da relação de isótopos estáveis de nitrogênio.

2. Diferentes sistemas de produção de bovinos baseados em pastagens naturais com diferentes níveis de intensificação, e portanto diferentes níveis de diversidades de espécies forrageiras na dieta dos animais, ocasionarão diferenças no perfil de ácidos graxos da carne.

1.8 OBJETIVO

Avaliar as características físico- químicas e sensoriais e a traçabilidade da carne de ruminantes produzidos sob diferentes sistemas de produção forrageiros com baixo aporte de insumos

De forma mais específica, objetivou-se :

- Determinar a lei de resposta entre níveis de alfafa na dieta de cordeiros e a concentração de escatol nos tecidos dos animais.
- Determinar a lei de resposta entre níveis de alfafa na dieta de cordeiros e a ocorrência de *off-flavours* (sabores desagradáveis) na carne ovina.
- Analisar o efeito do nível de leguminosas na dieta de cordeiros sobre o perfil de ácidos graxos da carne ovina
- Avaliar a eficiência dos isótopos estáveis de nitrogênio como marcadores de sistemas de produção de cordeiros ricos em leguminosas.
- Avaliar o efeito de sistemas de produção em pastagens com diferentes composições florísticas sobre o perfil de ácidos graxos da carne de bovinos.

2. CAPÍTULO II

Influence of fresh alfalfa supplementation on fat skatole and indole concentration and chop odour and flavour in lambs grazing cocksfoot pasture [1]

Influence of fresh alfalfa supplementation on fat skatole and indole concentration and

chop odour and flavour in lambs grazing a cocksfoot pasture

Devincenzi T.[1, 4], Prunier A.[2], Meteau K.[3], Nabinger C.[4], Prache S.[1*]

[1] Institut National de la Recherche Agronomique (INRA), UMR1213 Herbivores, F-63122 St-Genès-Champanelle, France.

[2] Institut National de la Recherche Agronomique (INRA), UMR1348 Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Élevage, F-35590 St-Gilles, France.

[3] Institut National de la Recherche Agronomique (INRA), UE1206 Elevage Alternatif et Santé des Monogastriques, Le Magneraud, F-17700 St-Pierre-d´Amilly, France.

[4] Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 7712, CEP 91501-970, Porto Alegre-RS, Brazil.

* Correspondence to Sophie Prache

e-mail: sophie.prache@ clermont inra.fr

phone : +33 4 73 62 40 63

Introduction

Low-input and organic farming livestock systems embody features that consumers value, such as animal welfare, food healthiness and environmental acceptability (Montossi, Font-i-Furnols, Campo, San Julian, Brito & Sañudo, 2013). The presence of forage legumes in these systems is of major importance, because nitrogen-fixing plants improve pasture quality and reduce dependency on external inputs. However, the occurrence of off-flavours and off-odours in the meat has been shown to increase in lambs grazing legume-rich pastures (Young, Lane, Priolo & Fraser, 2003; Schreurs, McNabb, Tavendale, Lane, Barry, Cumming et al., 2007a; Prache, Gatellier, Thomas, Picard & Bauchart, 2011). Some legume species, such as white clover (*Trifolium repens*) or alfalfa (*Medicago sativa*), play a prominent role in the ruminal synthesis of indole and skatole, which are smelling volatile components (Young et al., 2003; Watkins, Frank, Singh, Young & Warner, 2013; Schreurs, Lane, Tavendale, Barry & McNabb, 2008). Flavours described as animal, pastoral and fecal have been related to the presence of indole and skatole in lamb meat (Young, Berdagué, Viallon, Rousset-Akrim & Theriez, 1997; Young et al., 2003; Watkins, Kearney, Rose, Allen Ball, Pethick & Warner, 2014). In ruminants, indole and skatole are formed in the rumen from the microbial deamination and decarboxylation of tryptophan (Deslandes, Gariépy & Houde, 2001; Schreurs et al., 2008). Their ruminal synthesis has been found to depend on diet (Carlson, Hammond, Breeze, Potchoiba & Heinemann, 1983), and to increase when the pasture is rich in legume species with high degradable protein content (Schreurs et al., 2007a and Schreurs, Tavendale, Lane, Barry, McNabb, Cummings, Fraser & Lopez-Villalobos, 2007b). However, although the proportion of legume in the animal's diet can vary widely according to its proportion in the pasture and sward

availability, there has not yet been any published information on the effect of the dietary legume level on the chop's sensory attributes. This study was therefore undertaken to evaluate the influence of different levels of alfalfa in the diet on fat indole and skatole concentrations and chop odour and flavour attributes.

2. Materials and Methods

The study was conducted at the Herbivore Research Unit at the INRA Clermont-Ferrand/Theix Research Centre, France. The animals were handled by specialized staff who ensured their welfare in accordance with European Union Directive No. 609/1986.

2.1. Experimental design, animals and diets

We compared four levels of fresh alfalfa (*Medicago sativa*) forage supplementation in lambs grazing a cocksfoot (*Dactylis glomerata*) pasture for at least 60 days before slaughter: no supplementation (U), low (L), medium (M) and high (H) level of supplementation. We used 36 non-castrated male Romane lambs from 9 rams and 28 dams. The supplementation level was calculated so that the alfalfa should represent 0%, 25%, 50% and 75% of the voluntary dry matter (DM) intake in U, L, M and H lambs respectively.

Two conterminal monocultures of alfalfa (1.0 ha) and cocksfoot (1.6 ha) were sown in October 2010. From 30 April 2012 onwards, both pastures were divided into four plots of similar area. In March 2012, both pastures received 67 kg P/ha and 100 kg K/ha. In April 2012, the cocksfoot pasture received 48 kg N/ha. Each week, one alfalfa plot was mown to ensure the provision of young forage throughout the experiment. The cocksfoot pasture was mown on 29 May to ensure a vegetative regrowth.

Lambs were born within 8 days (18 March-26 March 2012). Before weaning, the animals were managed uniformly and received no legumes in their diet (i.e. from the beginning of the gestation period for the dams). For approximately 50 days after lambing, the lambs and their dams were housed in a sheepfold, and the lambs were offered a commercial concentrate. From 04 May to 10 May, the lambs were offered freshly cut cocksfoot grass distributed indoors. Lambs and their dams were then turned out to the cocksfoot pasture for 18 days until weaning on 29 May (day 0, d0), at 69 days on average.

On d1, the 36 lambs were assigned to nine blocks of similar animals according to birth weight and average daily gain (ADG) between birth and d0. They were then randomly assigned from within the blocks to one of the four treatments. The treatments were thus applied to one lamb of each block. Mean lamb birth weight and ADG between birth and weaning were 4.2 (SD 0.71) kg and 301 (SD 49.6) g/d; lambs weighed 24.9 (SD 3.49) kg at weaning.

Pre-experimental adaptation period. During this 35-d pre-experimental period, the lambs were individually fed *ad libitum* indoors. From d1 to d8, all lambs were fed freshly cut cocksfoot. From d9 to d35, U, L, M and H lambs were fed a diet containing 0%:100%, 25%:75%, 50%:50% and 75%:25% alfalfa forage: cocksfoot forage (on a DM basis). The levels of fresh alfalfa and fresh cocksfoot offered were calculated on the basis of their assigned proportion in the diet and the estimation of their DM content. The two forages were offered in separate tubs.

Experimental period. From d36 until slaughter, each group of lambs was assigned to one of the four cocksfoot pasture plots and supplemented with freshly cut alfalfa using

racks. Both cocksfoot pasture quality and availability were assumed not to limit lamb voluntary intake. The amount of fresh alfalfa offered to each group was based on the assigned proportion of alfalfa in the diet and the estimation of its DM content. Care was taken to use sufficiently large racks to avoid between-animal competition for alfalfa.

In both pre-experimental and experimental periods, the mean voluntary lamb intake level was estimated at 78.1 g $DM/LW^{0.75}$, where LW was the average live weight of the group (Hassoun & Bocquier, 2007). Fresh forages were cut at 6 cm above the ground every morning at 8 a.m. and offered half in the morning at 9 a.m., and half in the afternoon at 4 p.m. after storage at 4 °C. Feed tubs and racks were emptied every morning before the distribution of the fresh forage, and the refusals were weighed, recorded and discarded. The estimation of the forage DM content was made daily (except on weekends) in duplicate using a microwave oven. Representative samples of offered and refused forages were collected daily for final DM measurements (made in duplicate). Water and salt blocks were always available. The salt blocks contained (g/kg, as-fed) 60 Ca, 20 P, 10 Mg, 280 Na, 17.5 Zn, 1.5 Fe, 5.5 Mn, 0.03 Co, 0.03 I, and 0.01 Se.

The cocksfoot pasture was grazed continuously during the experimental period, but the groups of lambs were changed weekly from one cocksfoot plot to another to avoid confounding effects of sward characteristics for cocksfoot forage intake level. All lambs received anthelmintic drenches monthly.

2.2. *Slaughter procedures*

The lambs were slaughtered at the INRA Clermont-Ferrand Centre experimental slaughterhouse, according to European Union welfare guidelines. Three, 2 and 4 blocks

of lambs were slaughtered on d97, d100 and d121. Blocks were selected for slaughter on the basis of mean LW, with the priority given to the heaviest blocks. Lambs were thus slaughtered at mean age 177 (SD 12) days, after an experimental period ranging from 61 to 85 days according to block. The lambs had access to food and water until approximately 30 min before slaughter, and were transported by truck to the slaughterhouse located close to the experimental pastures (< 500 m). Immediately on arrival, the lambs were electrically stunned and slaughtered by throat cutting. The carcasses were placed in a refrigerated room at 4 °C until 24 h *post mortem*.

2.3. Measurements

2.3.1. Animal body weight

Lambs were weighed once weekly before the alfalfa distribution in the morning using an electronic scales.

2.3.2. Pasture availability

Fifty measurements of the cocksfoot sward surface height per plot were made weekly using a sward stick. Herbage mass was estimated weekly with a rising-plate meter (RPM, weight 430 g, 0.30 m × 0.30 m) using a double-sampling technique (Prache, Duby & Froment, 1989). For each measurement date, a regression of herbage mass on RPM height was established on 12 quadrats (0.30 m × 0.30 m, three randomized quadrats per plot). At each location, the RPM height was measured, and the herbage in the quadrat was cut 1.5 cm above ground level with a "mini mower", and oven-dried at 60 °C for 72 h. Fifty measurements of the RPM height were then made on each

cocksfoot plot. Herbage mass was then estimated for each plot using the mean RPM height together with the regression of herbage mass on RPM height.

2.3.3. Carcass characteristics and meat and fat sampling

Carcass weight, perirenal fat weight and subcutaneous fat thickness over the last thoracic rib were measured after 24 h cooling. A sample of approximately 25 g of perirenal fat was taken for all lambs. The subcutaneous fat was taken from the posterior end of the loin when it was available in sufficient amount (at least 10 g), which was the case for 18 lambs (8 H, 7 M, 2 L and 1 U lambs). Fat samples were wrapped, vacuum-packed in sealable polyamide bags and frozen at −20°C until indole and skatole concentration analysis.

After chilling of the carcass for 24 h, a sample of the left *longissimus thoracis et lumborum* (LTL) muscle was taken from the last thoracic rib, vacuum packed and frozen at −20° C until N isotope ratio analysis. The value of the nitrogen stable isotope ratio ($^{15}N/^{14}N$) in LTL muscle largely depends on the dietary $^{15}N/^{14}N$ ratio which is lower in legume than in grass plant species due to the capacity of leguminous plants to fix atmospheric nitrogen (DeNiro & Epstein, 1981; Devincenzi, Delfosse, Andueza, Nabinger & Prache, 2014). Here, we used the $\delta^{15}N$ value of the LTL muscle as an indicator of the dietary proportion of alfalfa at the individual lamb level. The $\delta^{15}N$ value of the LTL muscle was calculated as ($[(^{15}N/^{14}N_{muscle}) − (^{15}N/^{14}N_{air})] / (^{15}N/^{14}N_{air})$) × 1000).

The saddles (muscle, fat and bones) were removed from the posterior end of the loin from the right side of the carcass, wrapped and vacuum-packed in sealable polyamide bags, and aged for 4 days at +4 °C. The saddles were then frozen at −20 °C until sensory evaluation.

2.3.4. Perirenal and subcutaneous fat indole and skatole concentrations

Indole and skatole concentrations in the fat were measured by HPLC according to the procedure described by Batorek, Škrlep, Prunier, Louveau, Noblet, Bonneau, et al. (2012). Concentrations were expressed in µg per gram of the lipid fraction from adipose tissue. The quantification limit was 0.03 µg/g of liquid fat.

2.3.5. Lamb chop sensory evaluation

Lamb chop sensory evaluation was performed by 12 trained panellists at INRA Magneraud Experimental Unit (UE1206 EASM), according to the rules set out in AFNOR NF ISO 8586-1 and ISO 8586-2. The panellists were all women, of an average age of 55 years. Before evaluation of the experimental chops, a preliminary session was held, using additional chops from one lamb with the highest skatole concentration in perirenal fat (0.82 µg/g of liquid fat), and one lamb with the lowest skatole concentration in perirenal fat (< 0.03 µg/g of liquid fat), for the panellists to experience perceptions linked to skatole and to agree on common criteria describing these perceptions. The criteria chosen by the panellists were 'animal' odour and 'animal' flavour. Two additional training sessions were held on additional randomly chosen lamb chops before evaluation of the experimental chops.

Lamb chop sensory evaluation was performed on eight chops per experimental lamb in nine panel sessions (one panel session per block). At each panel session, each panellist evaluated the four lambs from one block, with lambs from each group presented in randomized order (Table 1). Before each session, the 32 chops to be evaluated were thawed for 24 h at +4 °C. Each chop was individually put into an aluminum foil container covered with aluminum foil. Then, 2 chops per group were contact-grilled

'bone in' to an internal temperature of 75 °C, and served warm to the 12 panellists (2 chops per group being served to 3 panellists). The bone part was removed, then pieces (about 2 cm x 2 cm each) were cut from the lean part (LTL muscle) and pieces (about 1 cm x 2 cm each) were cut from the fat part (the rest of the chop) to provide a piece of both for all the panellists. The panellists were asked to taste each part separately and evaluate successively: the odour of the lean part, the flavour of the lean part, the odour of the fat part then the flavour of the fat part. They were asked to use 10 cm unstructured line scales (from 0 to 10) to evaluate the intensity of 'animal' odour and of 'animal' flavour in the fat and the lean parts of the chops. Assessments were subsequently scanned scored as the distance in cm from the left end of the line using the FIZZ ® software. Panellists were asked to drink water and eat toast and a piece of apple between assessments to ensure that each sample was assessed with a cleansed palate. This procedure was repeated four times, i.e. all 32 chops from one lamb block had been evaluated. Each panellist was asked to evaluate the 4 chops in a uniform manner. For the two final panel sessions, the panellists evaluated only three chops, because we lost one U lamb and one H lamb.

2.3.6. N isotope ratio analysis of LTL muscle

We measured the nitrogen stable isotope ratio ($^{15}N/^{14}N$) in LTL muscle for all lambs. Full details of the method used and of the response of the mean $\delta^{15}N$ value of the LTL muscle to the mean dietary proportion of alfalfa are given in Devincenzi et al. (2014).

2.4. Forage collection and analyses

During the pre-experimental period, representative samples of the forages offered were

taken weekly. During the experimental period, representative samples of the offered alfalfa were collected every 9 days on average. For the cocksfoot pasture, representative samples of the forage were taken every 9 days in each plot using the hand plucking technique (Cook, 1964). Briefly, approximately 250 g of fresh forage was collected, simulating the plant parts taken by the animal. This corresponded to 50% of the sward height (Prache & Peyraud, 2001). Bulked samples were then made, grouping the four plot samples. All the samples were dried for 72 h at 60 °C, milled in a 200 μm outlet mill, and then analyzed for crude protein (CP; AOAC, 1995) and neutral detergent fiber (NDF; Goering & Van Soest, 1970).

2.5. Data analysis

The data for animal performances, carcass characteristics and perirenal fat indole concentration underwent an ANOVA analysis using the GLM procedure (SAS Inst. Inc., Cary, NC) to examine the effect of the treatment. Beforehand, the variance of the data for perirenal fat indole concentration was stabilized using the natural logarithmic transformation.

We used the Bonferroni test for pairwise comparisons. As the variance for perirenal fat skatole concentration differed between treatment groups and was not stabilized using the natural logarithmic transformation, we used non-parametric statistics (Wilcoxon signed rank test) for pairwise comparisons between treatment groups and for comparing lambs that received alfalfa (L, M and H lambs) with those that did not (U lambs). A regression analysis was carried out using the GLM procedure of the SAS software package to examine the relationship between skatole concentration in subcutaneous fat and in perirenal fat.

The data for lamb chop sensory evaluation underwent an ANOVA analysis using a mixed model, with treatment and panel session as fixed factors and panellist as a random factor, and using the Bonferroni test for pairwise comparisons. Regression analyses were carried out using the GLM procedure of the SAS software package to examine whether the perirenal fat skatole concentration and the intensity of 'animal' odour and of 'animal' flavour in both the lean and the fat parts of the chops were linearly or curvilinearly (quadratic effect) related to the δ^{15}N value of LTL muscle.

Results

Pasture, intake and animal performance

During the study, one H lamb died from bloating and one U lamb died from causes unrelated to the experimental treatment.

Mean sward surface height over the whole cocksfoot pasture averaged 14.8 cm, ranging from 23.9 cm on d35 to 10.0 cm on d86. Herbage mass over the whole cocksfoot pasture averaged 1816 kg DM/ha, ranging from 1540 kg DM/ha on d59 to 2145 kg DM/ha on d123. Over the experiment, crude protein (CP) values averaged 163 mg/g of organic matter (OM) (ranging from 129 to 225 mg/g OM) for cocksfoot and 271 mg/g OM (ranging from 420 to 187 mg/g OM) for alfalfa. NDF values averaged 58.9% DM (ranging from 55.5% to 66.7% DM) for cocksfoot and 38.4% DM (ranging from 27.2% to 45.7% DM) for alfalfa (Figure 1).

During pre-experimental adaptation period, mean dietary proportion of alfalfa was 27.7 %, 52.2 % and 74.8 % for L, M and H lambs (Table 2). During experimental period, mean daily alfalfa intake was 269, 545 and 716 g DM for L, M and H lambs respectively, and refusals represented 3.7%, 3.7% and 15.7% of the alfalfa offered for

L, M and H lambs respectively. The temporal change in mean daily alfalfa intake throughout the experimental period is given in Figure 2.

Average daily gain during the experiment was higher for M and H lambs than for L and U lambs ($P < 0.001$) (Table 3). Liveweight at slaughter and cold carcass weight were therefore higher for M and H lambs than for L and U lambs ($P < 0.001$). Consequently, subcutaneous fat thickness and perirenal fat weight were higher for H and M lambs than for L and U lambs ($P < 0.001$).

$\delta^{15}N$ value of LTL muscle

The $\delta^{15}N$ value of the LTL muscle ranged between 5.1‰ and 6.3‰. It averaged 6.06‰ (5.8‰ to 6.3‰) for the U lambs, 5.77‰ (5.4‰ to 6.1‰) for the L lambs, 5.43‰ (5.1‰ to 5.8‰) for the M lambs and 5.44‰ (5.2‰ to 5.7 ‰) for the H lambs. Coefficient of variation of the $\delta^{15}N$ value of the LTL muscle was 3.41%, 4.21%, 5.11% and 3.70% for U, L, M and H lambs respectively. Full details of the effect of the treatment and the between-groups comparisons are given in Devincenzi et al. (2014).

Fat indole and skatole concentration

Perirenal fat indole concentration averaged 0.13 µg/g (0.02 to 0.51 µg/g), 0.11 µg/g (0.03 to 0.44 µg/g), 0.11 µg/g (0.02 to 0.29 µg/g) and 0.10 µg/g (0.04 to 0.19 µg/g) for U, L, M and H lambs respectively. It was not affected by the treatment ($P = 0.99$). Subcutaneous fat indole concentration was always below quantification limit. Perirenal fat skatole concentration averaged 0.16 µg/g (0 to 0.43 µg/g), 0.26 µg/g (0.11 to 0.51 µg/g), 0.34 µg/g (0.16 to 0.82 µg/g) and 0.24 µg/g (0.12 to 0.44 µg/g) for U, L, M and H lambs respectively (Figure 3). The between-animal variability in perirenal fat skatole

concentration was high, particularly for M lambs.

Perirenal fat skatole concentration (PFSC, µg/g) was higher in L lambs and M lambs than in U lambs ($P < 0.07$ and $P < 0.05$ respectively). The differences for the other pairwise comparisons were not significant. Calculated on all lambs consuming alfalfa, PFSC averaged 0.28 µg/g liquid fat (SD = 0.169) and was significantly higher than in U lambs ($P < 0.05$). It decreased linearly with $\delta^{15}N$ value of the LTL muscle ($P = 0.03$, $r^2 = 0.14$, Figure 4), with no quadratic effect ($P = 0.69$). Perirenal fat skatole concentration was linearly related to subcutaneous fat skatole concentration (SCFSC, µg/g) ($P < 0.0001$), the equation being:

PFSC = −0.007 (± 0.0449) + 1.664 (± 0.2217) SCFSC,

where $r^2 = 0.78$, RSD = 0.0888 and $n = 18$.

Lamb chop sensory evaluation

The intensity of the 'animal' odour in the lean part of the chops was affected by the treatment ($P < 0.05$) and varied between panel sessions ($P < 0.025$). The panellists found a higher intensity of 'animal' odour for L, M and H lambs than for U lambs ($P < 0.06$, $P < 0.05$ and $P < 0.01$ respectively; Figure 5). The other differences between treatment groups were not significant.

The intensity of the 'animal' odour in the fat part of the chops was affected by the treatment ($P < 0.05$) and varied between panel sessions ($P < 0.01$). The panellists found a higher intensity of 'animal' odour for M lambs than for U and L lambs ($P < 0.025$ for both, Figure 5). The other differences between treatment groups were not significant.

The intensity of the 'animal' flavour in the lean part of the chops was affected by the treatment ($P < 0.025$) and tended to vary between panel sessions ($P = 0.08$). The

panellists found a higher intensity of 'animal' flavour for L, M and H lambs than for U lambs ($P < 0.025$, $P < 0.01$ and $P < 0.025$ respectively; Figure 6). The other differences between feeding treatment groups were not significant.

The intensity of the 'animal' flavour in the fat part of the chops was affected by the treatment ($P < 0.025$) and varied between panel sessions ($P < 0.05$). The panellists found a higher intensity of 'animal' flavour for L, M and H lambs than for U lambs ($P < 0.01$, $P < 0.005$ and $P < 0.05$ respectively; Figure 6). The other differences between treatment groups were not significant.

The intensity of the 'animal' odour in the fat part of the chop tended to decrease linearly with the $\delta^{15}N$ value of the LTL muscle ($P = 0.089$), with no quadratic effect ($P = 0.46$). The intensity of the 'animal' flavour in the lean and the fat parts of the chop and the intensity of the 'animal' odour in the lean part of the chop did not vary significantly with the $\delta^{15}N$ value of the LTL muscle ($P = 0.73$, 0.53 and 0.92 respectively).

Discussion

The aim of this study was to investigate the influence of the level of fresh alfalfa supplementation on fat indole and skatole concentration and chop odour and flavour in lambs grazing a cocksfoot pasture. With a higher crude protein content and a lower fiber content facilitating the release of protein from plant cells into the rumen environment, alfalfa presented a higher propensity to form indole and skatole than cocksfoot.

The experimental design was chosen both to ensure that the range of alfalfa supplementation levels explored was large and to limit the risk of bloating. The highest level of alfalfa supplementation was calculated so that alfalfa should represent 75% of the voluntary DM intake, which mimicked the preferences of ruminants on fields

consisting of adjacent monocultures of grass and clover (Rutter, 2006).

During pre-experimental adaptation period, the proportion of alfalfa in the diet was close to the assigned value for each group. During experimental period, mean daily alfalfa intake varied greatly among treatments, reaching 269 g DM, 545 g DM and 716 g DM for L, M and H lambs respectively. On the assumption that lamb voluntary DM intake was reached, mean dietary proportion of alfalfa during experimental period was 27.7%, 52.2% and 62.4% for L, M and H lambs respectively. The dietary proportion of alfalfa was therefore close to the assigned value for L and M groups, but it was lower for H group. However, the level of alfalfa refusals demonstrated that this forage was consumed *ad libitum* by the H lambs.

The concentration of indole in the fat was lower than that of skatole in this study and it was not affected by the treatment. It is likely that indole was effectively metabolized by the animal so that the indole content in the fat was not different between treatment groups, as already observed by Schreurs et al. (2007a). Perirenal fat skatole concentration was higher for lambs that consumed alfalfa than for those that consumed only cocksfoot. This result is in line with those of previous studies showing a higher skatole concentration in the rumen of lambs fed white clover than in lambs fed ryegrass (Schreurs et al., 2007a and Schreurs et al., 2007c). However, although these authors observed that lambs fed white clover tended to have a higher concentration of skatole in the intermuscular fat than lambs fed ryegrass, the difference was not statistically significant. The values we observed for perirenal fat skatole concentration were actually high. Most of them (30 out of 34 lambs) were above the target value of 0.1 µg/g considered as high in lamb meat (Schreurs et al., 2007a), the data from the literature suggesting that the odour and flavour sensory threshold, which is used to define

minimal quantity detectable by nasal perception, is around 0.05 µg skatole/g fat in butter (Watkins et al., 2014) to 0.1 µg skatole/g fat in male pig meat (Bañón, Costa, Gil, & Garrido, 2003 and Lunde, Skuterud, Hersleth & Egelandsdal, 2010). This is probably because we chose experimental conditions and a site of fat depot which were favourable to obtaining high values for fat skatole content. Even in U lambs eating only cocksfoot, the mean value for fat skatole content was twice as much as that observed by Schreurs et al. (2007a) in lambs grazing perennial ryegrass. As the CP content of both forages was quite similar, these differences may be due to higher water soluble carbohydrates content in ryegrass than in cocksfoot, providing an available source of energy to improve the incorporation of amino acids into microbial protein and reduce the amount of tryptophan that is converted to skatole. The differences between both studies may also be due to the fact that skatole concentration was measured in perirenal fat in this study whereas in the tail-stub fat in Schreurs et al (2007a). Additionnally, differences between studies may also reflect differences in the methods used for fat skatole concentration analysis.

Our study found that perirenal fat skatole concentration was higher for L and M lambs than for U lambs. Surprisingly, perirenal fat skatole concentration in H lambs was lower than that of M lambs, and it was not statistically different from that of U lambs. This result may be partly linked to the high variability between individual animals in dietary choices (Prache et al., 2006). Also, a high variability between individual animals on the same feeding treatment has been observed in the skatole production in the rumen and in the effectiveness at clearing the skatole from the blood stream in the liver (Schreurs et al., 2007a; Zamaratskaia & Squires, 2008). Consequently, the concentration of this compound in the fat depots frequently ranges widely between individual animals, as

shown in previous studies conducted with both lambs and cattle (Schreurs, et al., 2007a and Young et al., 1999). All these sources of variation produce variability between replicates that may overwhelm treatment differences. It is worth noting however that although perirenal fat skatole concentration was not statistically different between H and U lambs, the intensity of 'animal' flavour in both the lean and the fat parts of the chops and the intensity of 'animal' odour in the lean part of the chops were all significantly higher in H lambs than in U lambs.

The variability between replicates in perirenal fat skatole concentration was particularly remarkable for M lambs, suggesting a high variability in diet choices, with some lambs possibly behaving like H lambs and others like L lambs. To explore this matter further, and take the between-animal variability in dietary choices into account, we used the $\delta^{15}N$ value of the LTL muscle as an indicator of the dietary proportion of alfalfa at the individual level. Studying the relationship between perirenal fat skatole concentration and $\delta^{15}N$ value of the LTL muscle enabled us both to overcome the issue linked to inter-individual variability in diet choices and to increase the statistical units. The relationship between perirenal fat skatole concentration and $\delta^{15}N$ value of the LTL muscle was significant and linear, although the points were somewhat scattered because of the remaining variability between individual animals fed a given diet in skatole formation in the rumen as well as in skatole clearance in the liver (Schreurs et al., 2008) and in nitrogen-use efficiency (Cheng, Sheahan, Gibbs, Rius, Kay, Meier, et al., 2013). The outcome of this added refinement supports the hypothesis that the perirenal fat skatole concentration increases linearly with the dietary proportion of alfalfa. However, this is the first report of such an application of the N isotope signature of the LTL muscle and the suitability of this method needs to be investigated further.

In this study, the panellists agreed to describe the specific flavour and odour of the chops associated with a high skatole concentration as 'animal' flavour and 'animal' odour. This perception is in line with previous studies (Young et al., 1997 and Rousset-Akrim, Young & Berdagué, 1997). The intensity of 'animal' odour in the lean part of the chop and of 'animal' flavour in both the lean and the fat parts of the chop were increased from the lowest level of alfalfa supplementation onwards and did not increase further with increasing levels of alfalfa supplementation. Similarly, although perirenal fat skatole concentration was linearly related to the $\delta^{15}N$ value of the LTL muscle, the intensities of 'animal' flavour in both the lean and the fat parts of the chops and of 'animal' odour in the lean part were not. This could be partly because we measured skatole concentration in perirenal fat, whereas the panellists evaluated the chops. The results of the present study show that although subcutaneous fat skatole concentration was correlated with perirenal fat skatole concentration, the skatole concentration was lower in subcutaneous than in perirenal fat, i.e. less favourable to the expression of differences between treatment groups. These results also suggest that there may be a threshold for fat skatole concentration above which the corresponding 'animal' odour and flavour plateau, as already observed in pork meat (Bonneau & Chevillon, 2012). We actually observed that the intensity of 'animal' flavour in both the lean and the fat parts of the chops rose from U lambs to L lambs and then levelled off for M and H lambs, i.e. the results of the present study suggest that the threshold above which the 'animal' flavour plateaus corresponds to a perirenal fat skatole concentration in the range 0.16-0.24 μg/g of liquid fat. It is noteworthy that the differences we observed in flavour intensity between U lambs and lambs consuming alfalfa is of similar amplitude than those observed between pasture-fed and grain-fed lambs, two production systems

known to lead to distinctive flavour characteristics (Priolo, Micol, Agabriel, Prache & Dransfield, 2002; Schreurs et al., 2008).

Conclusions

Perirenal fat skatole concentration was higher for lambs that consumed alfalfa than for those that consumed only cocksfoot. The intensity of 'animal' odour in the lean part of the chop and of 'animal' flavour in both the lean and fat parts of the chop were increased from the lowest level of alfalfa supplementation onwards and did not increase further with increasing levels of alfalfa supplementation. The outcome of this study therefore suggests that these sensory attributes may reach a plateau when perirenal fat skatole concentration is in the range 0.16-0.24 µg/g of liquid fat.

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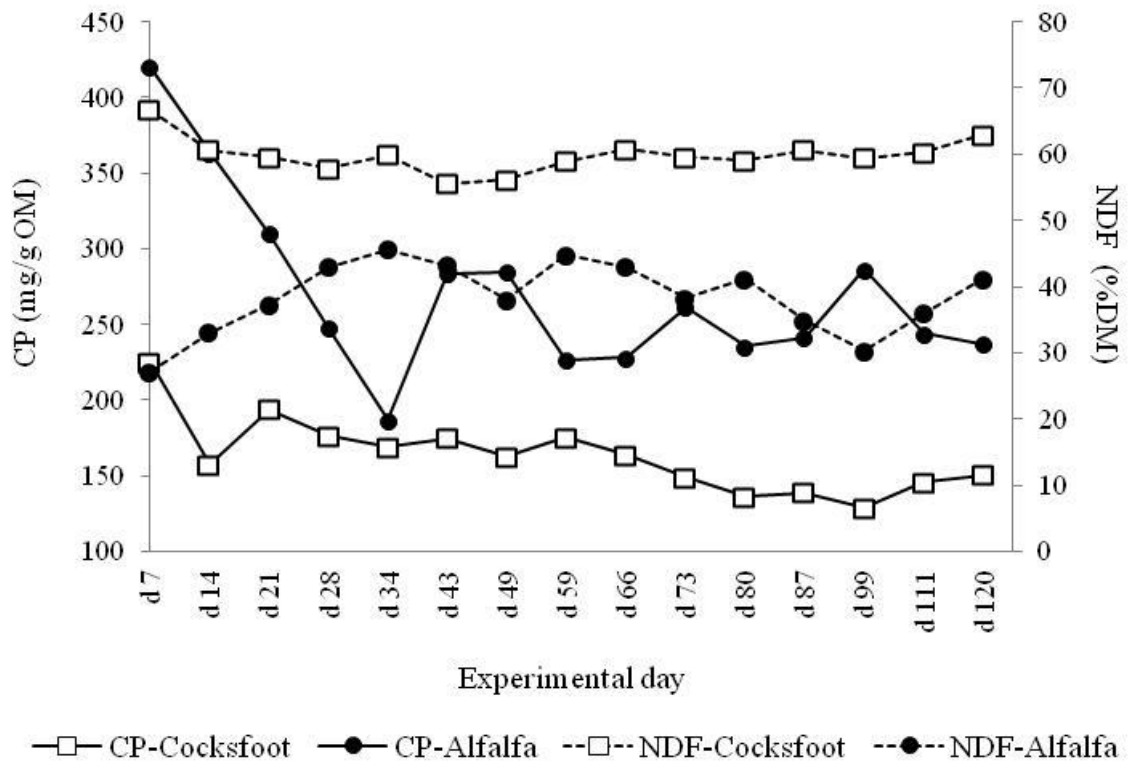
Figure 1: Variation in crude protein (CP) and neutral detergent fiber (NDF) content in the forages offered during the course of the experiment (d7 refer to day 7, d0 being on 29 May).
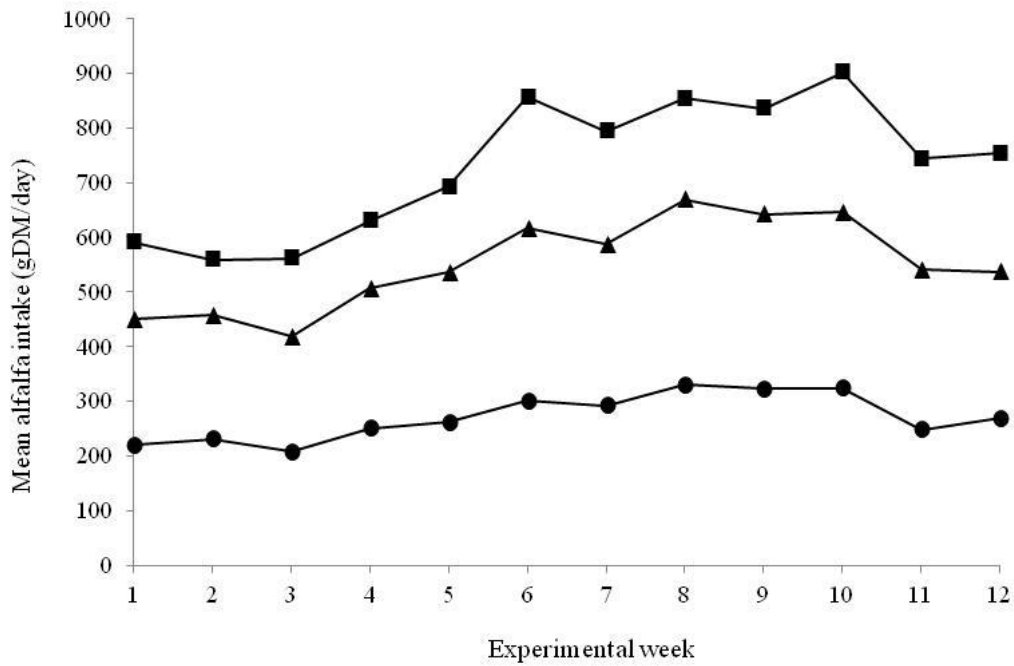
Figure 2: Variation in the mean daily alfalfa intake in lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa. Circles, triangles and squares refer to the low (L), medium (M) and high (H) levels of supplementation respectively. The supplementation level was calculated so that alfalfa should represent 25%, 50% and 75% of the voluntary dry matter (DM) intake in L, M and H lamb groups respectively.
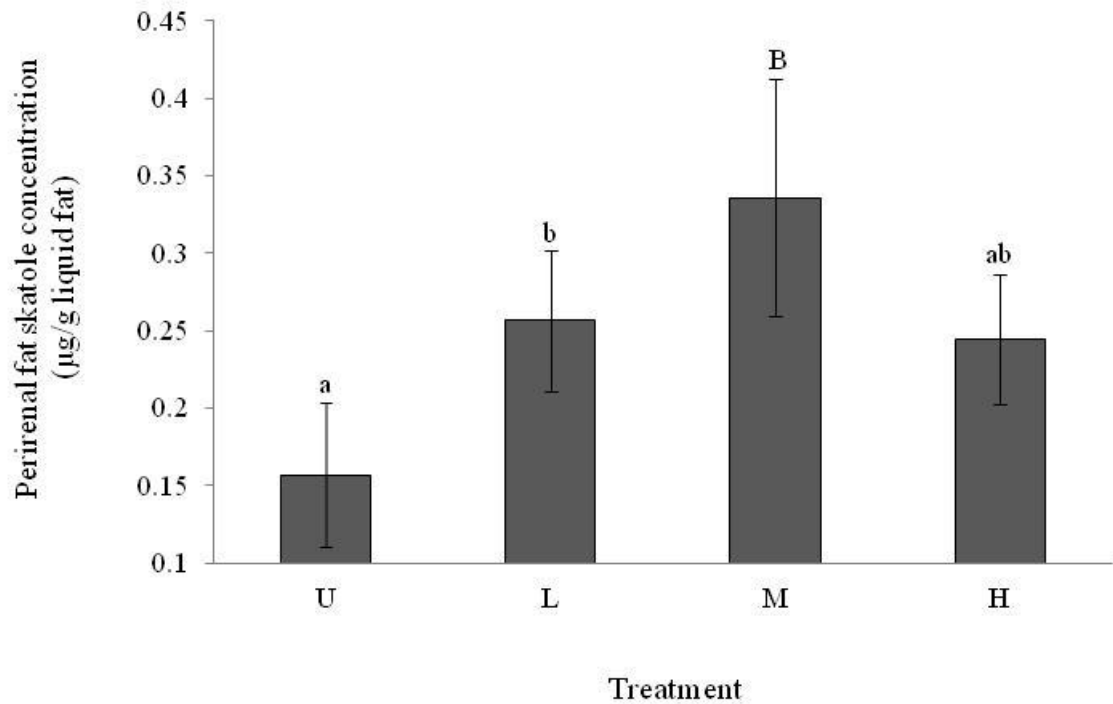
Figure 3: Perirenal fat skatole concentration in lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa (U, L, M and H refer to un-supplementation, low, medium and high level of supplementation respectively). Bars represent standard error of the mean (SD/√n, where n is the number of lambs in each group). Means with unlike superscripts differ (a, b: $P < 0.07$; A, b: $P < 0.05$).
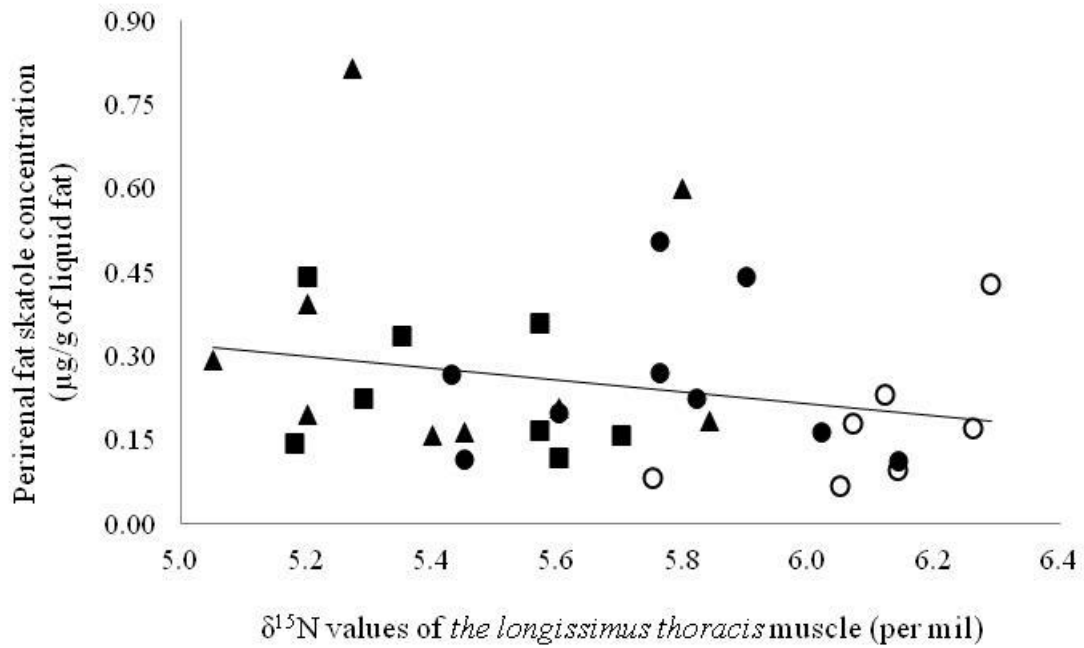
Figure 4. Relationship between perirenal fat skatole concentration and δ^{15}N value of the *longissimus thoracis et lumborum* muscle. White circles (○) refer to un-supplemented lambs, black circles (●) triangles (▲), and squares (■) refer to lambs receiving a low, medium or high level of alfalfa supplementation respectively.
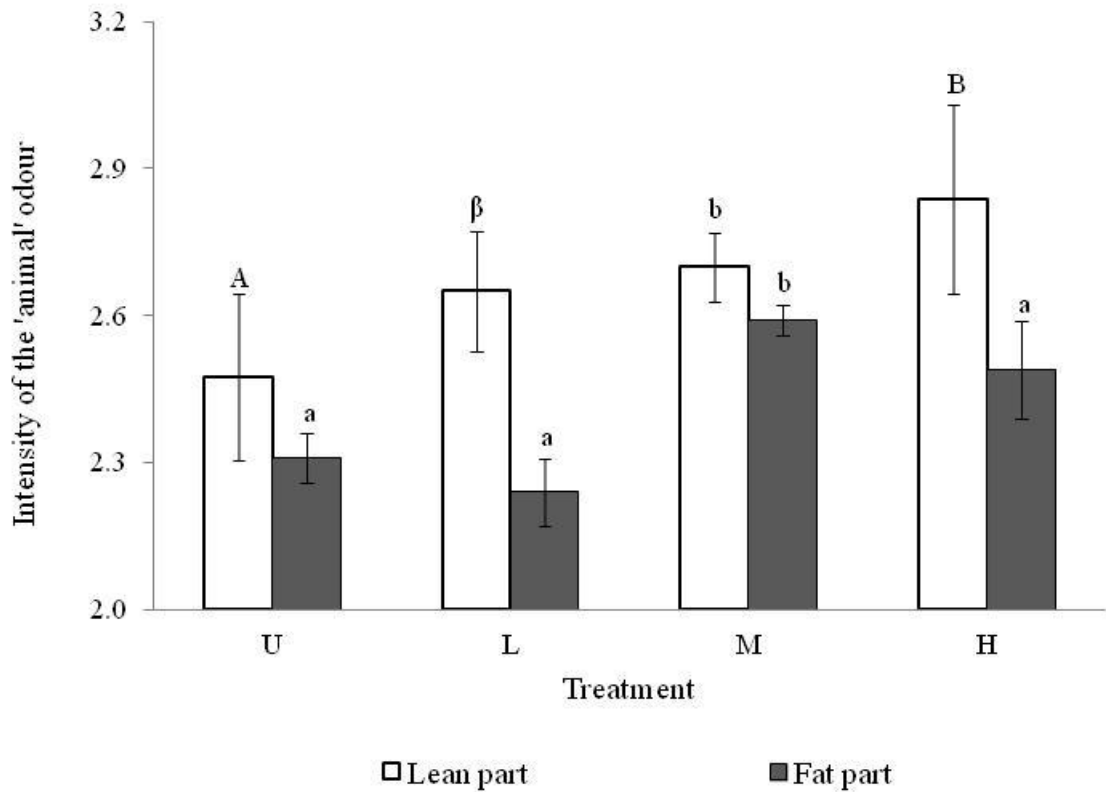
Figure 5. Mean intensity of the 'animal' odour (0-10 scale of increasing intensity) in the lean and fat parts of the chops in lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa. U, L, M and H refer to un-supplementation, low, medium and high level of supplementation, respectively. Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. For the lean part, means with unlike superscripts differ (A, β: $P < 0.06$; A, b: $P < 0.05$; A, B: $P < 0.01$). For the fat part, means with unlike superscripts differ (a, b: $P < 0.025$).
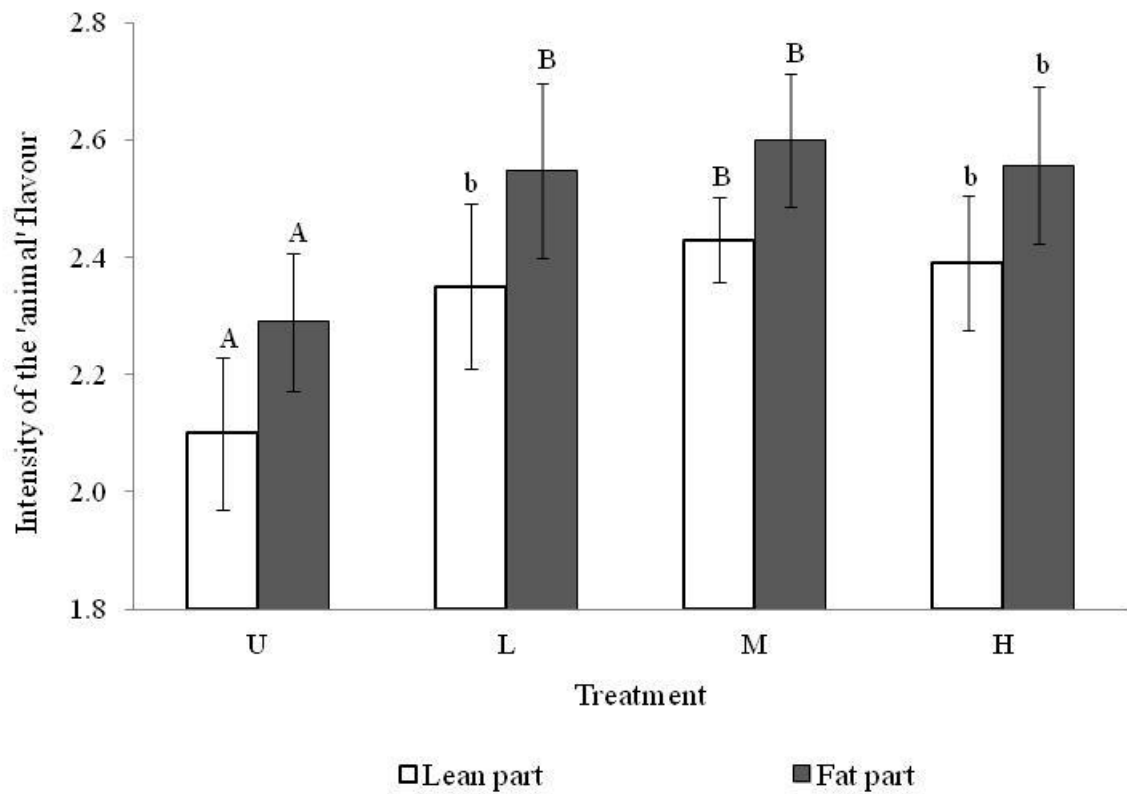
Figure 6. Mean intensity of the 'animal' flavour (0-10 scale of increasing intensity) in the lean and fat parts of the chops in lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa. U, L, M and H refer to un-supplementation, low, medium and high level of supplementation, respectively. Bars represents standard error of the mean (SD/$\sqrt{n}$), where n is the number of lambs in each group. For the lean part, means with unlike superscripts differ (A, b: $P < 0.05$; A, B: $P < 0.01$). For the fat part, means with unlike superscripts differ (A, b: $P < 0.05$; A, B: $P < 0.01$).

Table 1: Presentation order of lamb chops for sensory evaluation: example for the first panel session. U, L, M and H refer to treatment group (un-supplementation, low, medium and high level of alfalfa supplementation, respectively).

	Presentation order of lamb chops for sensory evaluation			
	1st	2nd	3rd	4th
Panellist1	H lamb	L lamb	M lamb	U lamb
Panellist 2	H lamb	L lamb	M lamb	U lamb
Panellist 3	H lamb	L lamb	M lamb	U lamb
Panellist 4	L lamb	U lamb	H lamb	M lamb
Panellist 5	L lamb	U lamb	H lamb	M lamb
Panellist 6	L lamb	U lamb	H lamb	M lamb
Panellist 7	M lamb	H lamb	U lamb	L lamb
Panellist 8	M lamb	H lamb	U lamb	L lamb
Panellist 9	M lamb	H lamb	U lamb	L lamb
Panellist 10	U lamb	M lamb	L lamb	H lamb
Panellist 11	U lamb	M lamb	L lamb	H lamb
Panellist 12	U lamb	M lamb	L lamb	H lamb

Table 2. Mean (standard deviation) daily forage intake and refusals. During pre-experimental period, lambs were individually fed indoors with freshly cut forages; during experimental period, each group of lambs grazed a cocksfoot pasture and was supplemented with different levels of freshly cut alfalfa.

Treatment[1]	U	L	M	H
Pre-experimental adaptation period				
Mean daily alfafa intake (g DM[2]/lamb)	-	184	382	563
		(4.0)	(3.5)	(5.3)
Mean daily cocksfoot intake (g DM[2]/lamb)	643	482	351	189
	(6.8)	(6.8)	(4.0)	(1.4)
Experimental period				
Mean daily alfalfa intake (g DM[2]/lamb)	-	269	545	716
Mean daily alfalfa refusals (g DM[2])	-	13	23	139
Refusals (proportion of the alfalfa offered, % DM[2])	-	3.7	3.7	15.7

[1] U, L, M and H refer to un-supplementation, low, medium and high level of alfalfa supplementation respectively

[2] DM: dry matter

Table 3. Animal performances and carcass characteristics in lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa.

Treatment [1]	U	L	M	H	RMSE [3]	*P value*
Number of lambs	8	9	9	8		
ADG [2] (g/day)	136b	147b	191a	224a	37.9	*P < 0.001*
Liveweight at slaughter (kg)	36.2b	36.5b	41.2a	43.7a	2.86	*P < 0.001*
Cold carcass weight (kg)	12.9b	13.3b	16.1a	17.6a	1.44	*P < 0.001*
Fat thickness (mm)	1.1b	1.1b	1.6a	2.1a	0.48	*P < 0.001*
Perirenal fat weight (g)	96b	124b	189a	196a	41.2	*P < 0.001*

Means with unlike superscripts differ ($P < 0.05$)

[1] U, L, M and H refer to un-supplementation, low, medium and high level of alfalfa supplementation respectively

[2] ADG: average daily gain during the experimental period

[3] RMSE: root mean square error

3. CAPÍTULO III

Dose-dependent response of meat and subcutaneous fat fatty acids composition to dietary alfalfa: cocksfoot proportion in grazing lambs [2]

[2] Artigo redigido de acordo com as normas da revista Meat Science.

Dose-dependent response of meat and subcutaneous fat fatty acids composition to dietary alfalfa:cocksfoot proportion in grazing lambs

Devincenzi T.[1, 2], Bauchart D[1], Nabinger C.[2], Prache S.[1*]

[1] Institut National de la Recherche Agronomique (INRA), UMR1213 Herbivores, F-63122 St-Genès-Champanelle, France.

[2] Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 7712, CEP 91501-970, Porto Alegre-RS, Brazil.

* Correspondence to Sophie Prache

e-mail: sophie.prache@ clermont inra.fr

phone : +33 4 73 62 40 63

Abstract

 We investigated the dose-dependent response of *Longissimus thoracis* (LT) muscle and subcutaneous fat fatty acids composition to increasing proportions of dietary alfalfa in lambs grazing a cocksfoot pasture. Four groups of nine male Romane lambs grazing a cocksfoot pasture were supplemented with various levels of alfalfa for at least 60 days before slaughter to obtain four dietary proportions of alfalfa. We found a linear increase in the proportion in C18:3n-3 and a linear decrease in C18:2n-6 / C18:3n-3 and PUFA n-6 / PUFA n-3 ratios with increasing th edietary proportion of alfalfa in both LT muscle and subcutaneous fat, which is beneficial for human health. However, palmitic acid (C16:0) and C16:0/C18:0 ratio increased linearly in LT muscle with the dietary

proportion of alfalfa. .

1. Introduction

Leguminous plants play an important role in organic and low-input production systems due to their ability to fix atmospheric nitrogen and therefore replace mineral fertilizers (MARASCHIN, 1997). The botanical composition of the pastures modulates the fatty acid (FA) composition of animal products (Farrugia, Martin, Baumont, Prache, Doreau, Hoste and Durand, 2008). Previous studies have shown that the presence of leguminous plants in the diet improves some nutritional parameters in lambs meat, such as C18:3 *n*-3 (Lourenço, Van Ranst, De Smet, Raes and Fievez, 2007; Prache, Gatellier, Thomas, Picard, Bauchart, 2011), and also improves the total polyunsaturated fatty acids (PUFA) of subcutaneous fat (Lourenço et al., 2007), which could imply on softer subcutaneous fat (Prache et al., 2011). However, the effect of the dietary level of leguminous plants on the FA composition of animal's tissues remains unknown. The objective of this study was therefore to investigate the dose-dependent response of *Longissimus thoracis* (LT) muscle and subcutaneous fat fatty acids composition to dietary alfalfa:cocksfoot proportion in grazing lambs.

2. Materials and Methods

The study was conducted at the Herbivore Research Unit at the INRA Clermont-Ferrand/Theix Research Centre, France. The animals were handled by specialized staff who ensured their welfare in accordance with European Union Directive No. 609/1986.

2.1. Experimental design, animals and diets

We compared four levels of fresh alfalfa (*Medicago sativa)* forage supplementation in lambs grazing a cocksfoot (*Dactylis glomerata*) pasture for at least 60 days before slaughter: no supplementation (U), low (L), medium (M) and high (H) level of supplementation. We used 36 non-castrated male Romane lambs from 9 rams and 28 dams. The supplementation level was calculated so that the alfalfa should represent 0%, 25%, 50% and 75% of the voluntary dry matter (DM) intake in U, L, M and H lambs respectively.

Two conterminal monocultures of alfalfa (1.0 ha) and cocksfoot (1.6 ha) were sown in October 2010. From 30 April 2012 onwards, both pastures were divided into four plots of similar area. In March 2012, both pastures received 67 kg P/ha and 100 kg K/ha. In April 2012, the cocksfoot pasture received 48 kg N/ha. Each week, one alfalfa plot was mown to ensure the provision of young forage throughout the experiment. The cocksfoot pasture was mown on 29 May to ensure a vegetative regrowth.

Lambs were born within 8 days (18 March-26 March 2012). Before weaning, the animals were managed uniformly and received no legumes in their diet (i.e. from the beginning of the gestation period for the dams). For approximately 50 days after birth, the lambs and their dams were housed in a sheepfold, and the lambs were offered a commercial concentrate. From 04 May to 10 May, the lambs were offered freshly cut cocksfoot grass distributed indoors. Lambs and their dams were then turned out to the cocksfoot pasture for 18 days until weaning on 29 May (day 0, d0), at 69 days on average.

On d1, the 36 lambs were assigned to nine blocks of similar animals according to birth weight and average daily gain (ADG) between birth and d0. They were then randomly

assigned from within the blocks to one of the four treatments. The treatments were thus applied to one lamb of each block. Mean lamb birth weight and ADG between birth and weaning were 4.2 (SD 0.71) kg and 301 (SD 49.6) g/d; lambs weighed 24.9 (SD 3.49) kg at weaning.

Pre-experimental adaptation period.

During this 35-d pre-experimental period, the lambs were individually fed *ad libitum* indoors. From d1 to d8, all lambs were fed freshly cut cocksfoot. From d9 to d35, U, L, M and H lambs were fed a diet containing 0%:100%, 25%:75%, 50%:50% and 75%:25% alfalfa forage: cocksfoot forage (on a DM basis). The levels of fresh alfalfa and fresh cocksfoot offered were calculated on the basis of their assigned proportion in the diet and the estimation of their DM content. The two forages were offered in separate tubs.

Experimental period.

From d36 until slaughter, each group of lambs was assigned to one of the four cocksfoot pasture plots and supplemented with freshly cut alfalfa using racks. Both cocksfoot pasture quality and availability were assumed not to limit lamb voluntary intake. The amount of fresh alfalfa offered to each group was based on the assigned proportion of alfalfa in the diet and the estimation of its DM content. Care was taken to use sufficiently large racks to avoid between-animal competition for alfalfa.

In both pre-experimental and experimental periods, the mean voluntary lamb intake level was estimated at 78.1 g DM/BW$^{0.75}$, where BW was the average body weight of the group (Hassoun & Bocquier, 2007). Alfalfa were cut at 6 cm above the ground

every morning at 8 a.m. and offered half in the morning at 9 a.m., and half in the afternoon at 4 p.m. after storage at 4 °C. Feed tubs and racks were emptied every morning before the distribution of the fresh forage, and the refusals were weighed, recorded and discarded. The estimation of the forage DM content was made daily (except on weekends) in duplicate using a microwave oven. Representative samples of offered and refused forages were collected daily for final DM measurements (made in duplicate). Water and salt blocks were always available. The salt blocks contained (g/kg, as-fed) 60 Ca, 20 P, 10 Mg, 280 Na, 17.5 Zn, 1.5 Fe, 5.5 Mn, 0.03 Co, 0.03 I, and 0.01 Se.

The cocksfoot pasture was grazed continuously during the experimental period, but the groups of lambs were changed weekly from one cocksfoot plot to another to avoid confounding effects of sward characteristics for cocksfoot forage intake level. All lambs received anthelmintic drenches monthly.

2.2. *Slaughter procedures*

The lambs were slaughtered at the INRA Clermont-Ferrand Centre experimental slaughterhouse, according to European Union welfare guidelines. Three, 2 and 4 blocks of lambs were slaughtered on d97, d100 and d121. Blocks were selected for slaughter on the basis of mean BW, with the priority given to the heaviest blocks. Lambs were thus slaughtered at mean age 177 (SD 12) days, after an experimental period ranging from 61 to 85 days according to block. The lambs had access to food and water until approximately 30 min before slaughter, and were transported by truck to the slaughterhouse located close to the experimental pastures (< 500 m). Immediately on arrival, the lambs were electrically stunned and slaughtered by throat cutting. The

carcasses were placed in a refrigerated room at 4 °C until 24 h *post mortem.*

2.3. Measurements

2.3.1. Animal body weight

Lambs were weighed once weekly before the alfalfa distribution in the morning.

2.3.2. Pasture availability

Fifty measurements of the cocksfoot sward surface height per plot were made weekly using a sward stick. Herbage mass was estimated weekly with a rising-plate meter (RPM, weight 430 g, 0.30 m × 0.30 m) using a double-sampling technique (Prache et al., 1989). For each measurement date, a regression of herbage mass on RPM height was established on 12 quadrats (0.30 m × 0.30 m, three randomized quadrats per plot). At each location, the RPM height was measured, and the herbage in the quadrat was cut 1.5 cm above ground level with a "mini mower", and oven-dried at 60 °C for 72 h. Fifty measurements of the RPM height were then made on each cocksfoot plot. Herbage mass was then estimated for each plot using the mean RPM height together with the regression of herbage mass on RPM height.

2.3.3. Carcass characteristics and meat and fat sampling

Carcass weight and subcutaneous fat thickness over the last thoracic rib were measured after 24 h shrinkage. A sample of approximately 10 g subcutaneous fat was taken 24 h *post mortem* from the posterior end of the loin, cut into small pieces and frozen in liquid nitrogen and stored at -80°C. A sample of the left *Longissimus thoracis* (LT) muscle was taken 24h *post mortem* from the last thoracic rib, cut into small pieces and frozen in liquid nitrogen and stored at -80°C. Subcutaneous fat tissue samples, and LT muscle

were both ground in liquid nitrogen in a model M20 mill (IKA-Werke, Staufen,Germany) to produce a fine homogenous powder, and then stored at −80 °C until fatty acid (FA) analysis.

2.3.4. Forage collection and analyses

During the experimental period, representative samples of the offered alfalfa were collected every 9 days on average. For the cocksfoot pasture, representative samples of the forage were taken every 9 days in each plot using the hand plucking technique (Cook, 1964). Briefly, approximately 250 g of fresh forage was collected, simulating the plant parts taken by the animal. This corresponded to 50% of the sward height (Prache & Peyraud, 2001). Bulked samples were then made, grouping the four plot samples. All the samples were dried for 72 h at 60 °C, milled in a 200 μm outlet mill, and then analyzed for crude protein (CP; AOAC, 1995) and neutral detergent fiber (NDF; Goering & Van Soest, 1970). Representative subsamples of each forage were frozen and stored at -20 °C for FA analysis. These subsamples were ground in liquid nitrogen in a domestic processor and then bulked by month and restored at -20°C until FA analysis. Total lipids were extracted from pooled herbage samples (around 160g dry matter (DM)) by grinding at 20000r.p.m. with chloroform/ methanol (2:1, vol/vol) followed by hexane/ethanol/HCl (25:10:10, vol/vol/vol), as reported in Bauchart et al. (1984), and determined by gravimetry. Herbage fatty acids of total lipids were extracted and transmethylated at room temperature for 2 x 20 min with sodium methylate (1M) followed by boron trifluoride in methanol (14%, vol/vol) according to Glass (1971). Herbage fatty acids analysis was conducted by gas–liquid chromatography using the Peri 2100-model chromatograph (PerichromSociety,Saulx-les-Chartreux,France) fitted

with a CP-Sil 88 glass capillary column (Varian, USA; length: 100m; internal diameter: 0.25mm) with H_2 as carrier gas, under conditions described in Scislowski et al . (2004).

2.3.5. Fatty acid composition of animal tissues lipids

 Meat and subcutaneous fat total lipids were extracted at room temperature by grinding 4 g of meat powder and 0.2 g of subcutaneous fat powder with chloroform-methanol 2:1 (vol/vol) according to the method of Folch, Lees, and Sloane Stanley (1957), then assayed gravimetrically. Fatty acids were transmethylated at room temperature with sodium methylate (1 M) and followed by boron trifluoride in methanol (14%, v/v) according to Glass (1971). The qualitative analysis of FA was achieved by gas–liquid chromatography (GLC) using the Perichrom 2000-model chromatograph (Perichrom Society, Saulx-les-Chartreux, France) fitted with a CP-Sil 88 glass capillary column (Varian, USA; length: 100 m, internal diameter: 0.25 mm) with H_2 as carrier gas. The FA were identified by comparing the retention times to the standad C4-C24 Fame (Supelco, Bellafonte, USA). The chromatographic conditions were as follows: the oven temperature was set at 70 °C for 30 s, then ramped from 70 to 175 °C at 20 °C/min, held at 175 °C for 25 min, ramped from 175 to 215 °C at 10 °C/min, and finally held at 215 °C for 41 min; injector and detector temperatures were 235 and 250 °C, respectively.

2.4. Data analysis

One U lamb was removed from fatty acid analisys because its degree of fatness was insufficient. Data were tested for normality by Shapiro-Wilk Test and underwent analysis of variance to test the effect of the feeding treatments using SAS software package and using Tukey test for pairwise comparisions. When the variance differed

between treatment groups, the data were analyzed using non-parametric statistics (Kruskall-Wallis test and Wilcoxon signed rank test for pairwise comparisons) Regression analyses were carried out using the GLM procedure of SAS to examine whether the LT muscle and subcutaneous FA had linear or curvilinear relation (quadratic effect) to the proportion of alfalfa in the diet.

3. Results

Pasture, intake and animal performance

All through the experimental period, one lamb in group H died from bloating and one lamb in group U died from causes unrelated to the experimental treatments.

Sward surface height over the whole cocksfoot pasture averaged 14.8 cm, ranging from 23.9 cm on 3 July to 10.0 cm on 23 August. Herbage mass over the whole cocksfoot pasture averaged 1816 kg DM/ha, ranging from 1540 kg DM/ha on 27 July to 2145 kg DM/ha on 29 September. Over the experiment, the dry matter (g/100g) average values were 22.9 (sd= ± 5.76), crude protein (CP) values averaged 163 (sd =± 24.54) mg/g of organic matter (OM), and mean neutral detergent fiber (NDF) values were 59.9 (sd= ±2.62) % DM.

For alfalfa, over the experiment, the dry matter average values were 20.5 (sd= ±7.43) g/100g, crude protein (CP) values averaged 270.6 (sd ± 58.82) mg/g of organic matter (OM), and mean neutral detergent fiber (NDF) values were 38.4 (sd= ±5.44) % DM. Full details of the nutritive value of the forages offered during the course of the experiment are given in Devincenzi et al (2014).

During experimental period, mean daily alfalfa intake was 269, 545 and 716 g DM for L, M and H lambs respectively, and refusals represented 3.7%, 3.7% and 15.7% of the

alfalfa offered for L, M and H lambs respectively. More detailed information about forage intake are given in Devincenzi et al., 2014. Average daily gain during the experiment was higher for M and H lambs than for L and U lambs (P < 0.001) (Table 1). Liveweight at slaughter and cold carcass weight were therefore higher for M and H lambs than for L and U lambs (P < 0.001). Consequently, subcutaneous fat thickness and perirenal fat weight were higher for H and M lambs than for L and U lambs (P < 0.001).

Fatty acid composition of the forages

Main FA composition of the forages is presented in Table 2. The proportion of C16:0 was higher in alfalfa than in cocksfoot (P < 0.05). Alfalfa had a higher proportion of ∑SFA than cocksfoot (P < 0.05). The proportion of linoleic acid (C18:2*n*-6 cis cis) was higher in alfalfa than in cocksfoot (P < 0.05). The proportion of linolenic acid (C18:3 *n*-3) and of ∑ *n*-3 PUFA were not significantly different between both forages (P > 0.05). Alfalfa presented a higher C18:2 *n*-6 /C18:3 *n*-3 ratio and a lower ∑PUFA/∑SFA ratio than cocksfoot (P < 0.05).

Fatty acid composition of the Longissimus thoracis (LT) muscle lipids

Main FA composition of the LT muscle is presented in Table 3. There was a tendency for an effect of the feeding treatment on total lipids content in LT muscle (P = 0.1046), total lipids content being in the order M and H lambs > L lambs > U lambs.

The proportion of C16:0 was affected by the feeding treatment (P = 0.0039), being higher for M and H lambs than for U lambs, the L lambs being intermediate. The proportion of C16:0 in the LT muscle increased linearly with the dietary proportion of alfalfa (P = 0.0091; Table 5).

The proportion of C18:0 was not significantly different between treatment groups. The proportion of C18:1 delta 9 cis was affected by the alfalfa proportions on lamb diets (P = 0.0049), being higher for M and H lambs than for U lambs, being L lambs intermediates. There was a tendency for the proportion of C18:1delta 9 cis in the LT muscle to increase linearly with the dietary proportion of alfalfa (P = 0.0512, Table 5). Consequently, the proportion of the sum of cis monounsaturated fatty acids (☐☐MUFA cis) was affected by the feeding treatment (P = 0.0071), being higher for H lambs than for U and L lambs and intermediate for M lambs. The proportion of the sum of cis monounsaturated fatty acids tended to increase linearly with the dietary proportion of alfalfa (P =0.0593, Table 5).

There was no effect of the feeding treatment on the proportion of C18:1 trans and on the proportion of the sum of trans monounsaturated fatty acids (☐☐MUFA trans).

There was no effect of the feeding treatments on C18:2n-6 cis cis in LT muscle (p=0.257), however it was observed a tendency to a quadratic decreasing on its proportion (P=0.0538, Table 5).

The proportion of C18:3 n-3 was affected by the feeding treatment (P = 0.0590), being higher for H lambs than for U lambs, the proportion for L and M lambs being intermediate and not significantly different from the other groups.

There was an effect of the feeding system on the proportion of C20:4n-6 (p<0.05), being higher for U lambs than for L, M and H lambs.

The $\sum$ PUFA n-6 / $\sum$ PUFA n-3 ratio was affected by the treatment (P = 0.0442), being higher for U lambs than for L, M and H lambs. Similarly, the C18:2n-6 / C18:3n-3 ratio was affected by the treatment (P = 0.0249), being higher for U lambs than for H lambs; the ratio for L and M was intermediate and no significantly different from the other

groups. The C18:2 *n*-6 / C18:3 *n*-3 ratio decreased linearly with the dietary proportion of alfalfa (P = 0.0222, Table 5). The C16:0 / C18:0 ratio was affected by the treatment (P = 0.0121), being lower for U lambs than for M and H lambs, L lambs being intermediate and not significantly different from the other groups. This ratio increased linearly with the dietary proportion of alfalfa (P=0.0160, Table 5).

Fatty acid composition in subcutaneous fat

Fatty acid composition of subcutaneous fat is presented in Table 4. Total lipid content in subcutaneous fat was affected by the feeding treatment (P = 0.05), being higher in H lambs than in U and L lambs. Total lipid content in subcutaneous fat was not significantly different between H lambs and M lambs and between M lambs and L lambs.

There was a tendency for an effect of the feeding treatment on the proportion of C16:0 in subcutaneous fat (P = 0.08), this proportion being higher for M and H lambs than in U lambs, and intermediate for L lambs. The proportion of C16:0 in subcutaneous fat increased linearly with the dietary proportion of alfalfa in the diet (P=0.0407, Table 6).

The proportion of C18:0 in subcutaneous fat was not affected by the feeding treatment (P > 0.05). The feeding treatment tended to affect the proportion of $\sum$SFA in subcutaneous fat (P = 0.0528), this proportion being lower for H lambs than for U lambs, L and M lambs being intermediates. The proportion of $\sum$ SFA showed a quadratic decrease with the dietary proportion of alfalfa (P=0.0501, Table 6).

There was no effect of the feeding treatment on the proportion of C18:1 trans and on the proportion of the sum of trans monounsaturated fatty acids ($\sum$MUFA trans). We also found no effect of the feeding system for C18:2 *n*-6 cis cis (P=0.758) on the subcutaneous fat.

There was an effect of the feeding treatment on the proportion of C20:4n-6 in subcutaneous fat (P = 0.0118), with a higher proportion in U lambs than in M and H animals, L lambs showing an intermediate value which was not significantly different from the proportion observed in the other groups. The proportion of C20:4n-6 in subcutaneous fat decreased linearly with the dietary proportion of alfalfa in the diet (P=0.0293, Table 6).

There was an effect of the feeding treatment on the proportion of C18:3n-3 in subcutaneous fat (P = 0.0300), with a higher proportion in H lambs than in U lambs, L and M lambs showing an intermediate proportion, which was not statistically different from the proportion observed in the other groups. The C18:3n-3 proportion in the subcutaneous fat increased linearly with the dietary proportion of alfalfa (P=0.0073, Table 6).

There was an effect of the feeding treatment on the C18:2n-6/C18:3n-3 ratio in subcutaneous fat (P < 0.05). This ratio was higher in U lambs than in H lambs, values for L and M lambs being intermediate and not statistically different from that of the other groups. The C18:2 n-6/C18:3 n-3 ratio showed a quadratic decrease with the dietary proportion of alfalfa (P = 0.0337, Table 6).

There was an effect of the feeding system on the proportion of C20:4n-6 (p<0.05). Values were higher in U lambs than in M and H lambs, being L lambs intermediates. The proportion of C20:4n-6 decreased linearly with the dietary proportion of alfalfa (P=0.0293, Table 6).

4. Discussion

We aimed through this study to investigate the dose-dependent response relating the

fatty acid profile in muscle and subcutaneous fat to the proportion of alfalfa in lamb diet. The experimental design was chosen both to ensure that the range of dietary alfalfa was sufficiently broad to obtain the response curves, and to limit the risks of bloating. The highest estimated dietary proportion of alfalfa during the experimental period (62.4%) was similar to the preference of ruminants on fields consisting of adjacent monocultures of grass and clover (Rutter, 2006). We initially planned to feed freshly cut forages to individually penned animals indoors (pre-experimental period), in order to achieve an optimal individual control of cocksfoot and alfalfa intake levels. However, this experimental scenario was thwarted by low levels of forage intake, which resulted in low ADG. We therefore changed the experimental design by turning out each group of lambs to one cocksfoot plot and supplementing twice daily with freshly cut alfalfa (experimental period). This change led to a marked improvement in ADG, but enabled the control of alfalfa intake only at group level.

Alfalfa presented higher CP values and lower NDF values, as expected. However, the proportion of C18:3 n-3 was not different between alfalfa and cocksfoot, at variance with the meta-analysis by Glasser, Doreau, Maxin and Baumont, 2013.

In the present study, the mean proportion of C16:0 was higher on alfalfa than in cocksfoot pasture. Our results are in line to those obtained by Glasser et al., 2013, who found about 45% more C16:0 for fresh alfalfa when compared to fresh Fescue grass.

The higher proportions of C18:2n-6 cis cis found for alfalfa were in line to the results reported by Glasser et al., 2013, for C18:2 n-6, which were about 48% higher on alfalfa when compared to fresh Fescue grass.

In the present study, the total lipid content in the LT muscle was situated in the range reported in previous studies for grazing lambs (Aurousseau et al 2004; Aurousseau,

Bauchart, Faure, Galot, Prache, Micol and Priolo, 2007; Lourenço, Van Ranst, De Smet, Raes and Fievez, 2007). We found very low values of total lipids content in the subcutaneous fat of U and L lambs when compared to M and H lambs. This may be due to a contamination of the subcutaneous fat by connective tissue, because of a lower degree of fatness at slaughter for U and L lambs than for M and H lambs, a difficulty which has also been reported by Lourenço et al., 2007.

The proportion of C16:0 increased linearly with the dietary proportion of alfalfa in both the LT muscle and the subcutaneous fat, and the 18% increase between U and H lambs' LT muscle is noticeable. This increase can be related to a higher proportion of C16:0 in alfalfa than in cocksfoot. However we can not excluded that our results could be confounded by the different degrees of fatness obteined in each treatment. Our results are in partilly in line with the one from Lorenço et al, 2007. The author found higher amounts of C16:0 on the intramuscular fat for lambs grazing a legume-rich pasture (>61% of *Leguminosae*) than for lambs grazing a biodiverse pasture, however, this difference was not significative when comparing to lambs from ryegrass rich pastures. . This result is important because high proportions of C16:0 in meat is undesirable to human's health because this FA is known by its atherogenic potential (Crupkin and Zambelli, 2008). We found no statistically significant effect of the forage or the feeding treatment on the proportion of C18:0 in the forages or in the animal tissues (LT muscle and subcutaneous fat). In LT muscle, as the proportion of C16:0 increased linearly with the dietary proportion of alfalfa, the C16:0/C18:0 ratio increased consequently, with a 27% increase between U and H lambs. The C16:0 has an atherogenic effect on humans and C18:0 has neutron effect, so lower values of C16:0/C18:0 ratio are desirable to the point of view of humans health.

The differences found for the sum of MUFA cis on LT muscle were mostly related by the amounts of C18:1 delta 9 cis.

The differences found between feeding treatments in the proportions sum of MUFA cis in LT muscle were mostly related to the differences in the proportion of C18:1 delta 9 cis. The increase in the proportion of C18:1 delta 9 cis in animal tissues when the dietary proportion of alfalfa increased is favorable from a human´s nutrition point of view. This effect can not be attributed to differences in the proportions of C18:1 delta 9 in the forages but to a higher proportion of C18:0 in alfalfa, many C18:1 isomers being formed from C18:0. However, as the oleic acid (C18:1 delta 9) is the major fatty acid in the triacylglycerol and polar lipid fraction, which are prone to increase with animal fatness, the dietary proportion of alfalfa and the animal's degree of fatness may be confounding factors in this study, as H and M lambs were fatter than U and L lambs.

We observed a tendency of a quadratic decrease of C18:2 n-6 cis cis concentration with the increase of the alfalfa proportions, but we did not observed an effect of the feeding systems, even alfalfa plants being 34% higher in C18:2 n-6 cis cis. In this sense, this behaviour could be probably associated to different levels of triacylglycerol and phospholipids. The phospolipid portion, which are richer in PUFA were probably more representative for animals from L0 and L25 than for L50 and L75, that presented higher degree of fatness.

 In our study, C18:3n-3 was present in similar proportions in alfalfa and cocksfoot lipids, but proportions of C18:3n-3 in both LT muscle and subcutaneous fat increased linearly with the dietary proportion of alfalfa. In this study, the proportion of C18:3n-3 increased by 28% in muscle LT and by 45% in subcutaneous fat between U and H lambs. These results are in line with those obtained by Lourenço et al (2007) when

comparing lambs grazing an intensive ryegrass pasture to lambs grazing a pasture containing 61% of forage legumes. However, the difference is of lower amplitude in our study than in that of Lourenço et al (2007), probably because the dietary proportion of legume in lambs grazing the legume-rich pasture was higher in Lourenço et al (2007) than in H lambs of the present study. Actually, animals do not eat at random and express preferences towards leguminous plants. Therefore, the proportion of legumes in the diet of the lambs grazing the pasture containing 61% legumes was probably higher than that of the H lambs (62.4%) As suggested by Lourenço et al (2007), it is likely that the increase in the proportion of C18:3 n-3 with the increased dietary proportion of alfalfa is due to a reduced ruminal microbial 'contact time' because of a lower diet NDF content and thus a higher outflow rate. The extent of the ruminal biohydrogenation is actually linked to the fiber content of the diet (Gerson, John and King, 1985). The linear increase of the proportion of C18:3n-3 in muscle LT and in subcutaneous fat is one of the main results of the present study because of the health benefits associated with the consumption of this FA. This result may be of importance for low-input and organic farming livestock systems, for which the presence of forage legumes is of major importance, because nitrogen-fixing plants improve pasture quality and reduce dependency on external outputs (Devincenzi et al, 2014). However, it should be noticed that an increase in the proportion of C18:3n-3 in the tissues of animals fed legume-rich diets could increase the risks of off-flavours linked to peroxidation of this FA (Priolo et al., 2001), beyond the greater occurrence of off-flavours in the meat linked to higher ruminal synthesis of skatole and indole in lambs grazing legume-rich diets (Devincenzi et al., 2014).

Arachidonic acid C20:4n-6 is an essential fatty acid, so it should be obtained from the

diet. We did not identify C20:4n-6 in either forage, however there was an effect of the feeding treatment on the proportion of this FA in both LT muscle and subcutaneous fat, the proportion of C20:4 n-6 decreasing linearly with the dietary proportion of alfalfa. . The presence of this FA probably originates from elongation and desaturation of shorter PUFA, such as C18:2 n-6.

It is largely known that a low C18:2 n-6 / C18:3 n-3 n-3 and PUFA n-6 / PUFA n-3 ratios are beneficial to the reduction of the risk of cardiovascular diseases (Givens et al 2006; Wood et al 2004; Simopoulos, 2002). In our work, C18:2 n-6 / C18:3 n-3 and PUFA n-6 / PUFA n-3 ratios in LT muscle decreased linearly with the dietary proportion of alfalfa, being 37% and 23% lower in H lambs than in U lambs. In our study, the PUFA n-6/PUFA n-3 ratio in the LT muscle lipids ranged from 1.79 to 2.33, which is much lower than the upper threshold limit of 5 reported by Nuernberg et al, 2004 and Webb et al., 2005. Similarly, C18:2 n-6 / C18:3 n-3 ratio in subcutaneous fat decreased linearly with the dietary proportion of alfalfa and was 38% lower in H lambs than in U.

5. Conclusions

An important result of this study is the linear increase of C18:3n-3 and the linear decrease of C18:2n-6/C18:3n-3 ratio in both muscle LT and subcutaneous fat with the dietary proportion of alfalfa, which is benefic to human health. This result is probably linked to the lower NDF content in alfalfa forage than in cocksfoot forage, which leads to a higher outflow rate and therefore a reduced microbial contact time within the rumen when the dietary proportion of alfalfa increases. However, issues regarding the risks of peroxidation and associated development of rancid odours and flavours of chops rich in

omega-3 FA need to be further addressed. Moreover, it should be noted that the pro-atherogenic C16:0 and the C16:0/C18:0 ratio increased with the dietary proportion of alfalfa.

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Table 1: Animal performances and carcass characteristics in lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa.

Treatment [1]	U	L	M	H	RMSE [3]	*P value*
Number of lambs	8	9	9	8		
ADG [2] (g/day)	136b	147b	191a	224a	37.9	*P < 0.001*
Liveweight at slaughter (kg)	36.2b	36.5b	41.2a	43.7a	2.86	*P < 0.001*
Cold carcass weight (kg)	12.9b	13.3b	16.1a	17.6a	1.44	*P < 0.001*
Fat thickness (mm)	1.1b	1.1b	1.6a	2.1a	0.48	*P < 0.001*
Perirenal fat weight (g)	96b	124b	189a	196a	41.2	*P < 0.001*

Means with unlike superscripts differ ($P < 0.05$)

[1] U, L, M and H refer to un-supplementation, low, medium and high level of alfalfa supplementation respectively

[2] ADG: average daily gain during the experimental period

[3] RMSE: root mean square error

Table 2: Fatty Acid proportion (% of total FAME[1]) of the forages during the experimental period.

	Cocksfoot	Alfalfa	S.E.M. [2]	Significance
C14 :0	0.74	0.61	0.060	n.s.
C16 :0	12.37	16.71	1.155	$P < 0.05$
C18 :0	1.95	2.86	0.372	n.s.
Σ SFA[3]	17.78	23.14	1.453	$P < 0.05$
C18 :1 delta 9 cis	1.89	1.95	0.379	n.s.
ΣMUFA cis [4]	4.36	4.21	0.467	n.s.
C18 :2 n-6 cis cis	12.40	16.61	1.117	$P < 0.05$
Σ PUFA n-6 [5]	16.77	17.75	1.121	n.s.
C18 :3 n-3	59.46	53.86	2.049	n.s.
Σ PUFA n-3 [6]	61.08	54.90	2.128	n.s.
Σ PUFA n-6 / Σ PUFA n-3	0.28	0.33	0.029	n.s.
C18 :2 n-6 / C18 :3 n-3	0.21	0.31	0.029	$P < 0.05$
∑PUFA/∑SFA	4.39	3.18	0.321	$P < 0.05$

[1] FAME = Fatty acids methyl esters
[2] Standard error of the mean.
[3] Σ SFA =Σ(C12:0; C14:0; C15:0; C16:0; C17:0; C18:0; C20:0; C22:0; C23:0; C24:0; Iso14 ; Iso16; Iso17)
[4] Σ MUFA cis(3) =Σ (C16 :1 ; C16 :1 delta 9 cis ; C18 :1 delta 7 cis ; C18 :1 delta 9 cis ; C18 :1 delta 11 cis ; C20 :1n-11 ; C22 :1n-9 ; C24 :1 n-9)
[5] Σ PUFA n-6=Σ(C18:2n-6 cis cis ; C18:3n-6 ; C22:4n-6 ; C22:5n-6)
[6] Σ PUFA n-3 = Σ(C18:3n-3 ;C20:5n-3; C22:5n-3)

Table 3: Mean ± standard deviation of total lipid content (g/100 g of fresh meat) and FA centesimal composition (% of total FAME[1]) of the *Longissimus thoracis* muscle lipids of lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa.

	U [2]	L [2]	M [2]	H [2]	*p-value*
Lipids	2.22 ± 1.093	2.18 ± 0.446	2.93 ± 0.789	2.82 ± 0.921	0.105
C14:0	3.35 ± 1.118	3.65 ± 1.32	3.46 ± 0.753	3.04 ± 0.752	0.655
C16:0	17.90 b ± 2.886	19.47 ab ± 1.255	20.87 a ± 1.321	21.16 a ± 0.831	0.004
C18:0	18.52 ± 1.810	19.35 ± 3.313	17.70 ± 1.544	17.46 ± 2.020	0.337
Σ SFA[3]	43.92 ± 4.881	46.73 ± 2.198	45.95 ± 1.618	45.31 ± 2.234	0.276
C18:1 delta 9 cis	27.21 c ± 2.522	27.61 bc ± 1.617	30.01 ab ± 1.462	30.35 a ± 2.042	0.005
Σ MUFA cis[4]	31.40 b ± 2.59	31.73 b ± 1.577	34.25 ab ± 1.753	34.54 a ± 2.236	0.007
Σ C18:1 trans[5]	4.23 ± 1.382	3.93 ± 1.155	4.26 ± 1.075	3.94 ± 0.607	0.869
Σ MUFA trans[6]	4.61 ± 1.418	4.33 ± 1.208	4.66 ± 1.079	4.30 ± 0.615	0.864
C18:2n-6 cis cis	6.54 ± 2.199	5.50 ± 1.796	5.04 ± 0.681	5.15 ± 1.2680	0.257
C20:4n-6	4.41 a ± 2.895	3.00 b ± 1.478	2.25 b ± 0.493	2.23 b ± 0.648	<0.050
Σ PUFA n-6 [7]	12.58 ± 5.25	9.92 ± 3.519	8.67 ± 1.067	8.86 ± 1.981	0.128
C18:3n-3	1.77 b ± 0.298	2.01 ab ± 0.293	1.98 ab ± 0.350	2.27 a ± 0.368	0.059
C20:5n-3	0.99 ± 0.517	0.97 ± 0.238	0.69 ± 0.237	0.78 ± 0.208	0.182
C22:5n-3	2.15 ± 1.115	1.92 ± 0.464	1.44 ± 0.393	1.51 ± 0.39	0.109
C22:6n-3	0.47 ± 0.212	0.49 ± 0.112	0.39 ± 0.080	0.44 ± 0.147	0.485
Σ PUFA n-3 [8]	5.38 ± 2.045	5.40 ± 0.840	4.51 ± 0.988	5.00 ± 1.031	0.422
CLA (9cis, 11trans)[9]	0.85 ± 0.294	0.93 ± 0.202	1.01 ± 0.261	1.04 ± 0.095	0.332
Σ PUFA[10]	20.07 ± 7.425	17.21 ± 4.008	15.13 ± 2.006	15.84 ± 2.840	0.159
Σ PUFA n-6 / Σ PUFA n-3	2.33 a ± 0.377	1.82 b ± 0.455	1.98 b ± 0.332	1.79 b ± 0.333	0.044
C18:2n-6 / C18:3n-3	3.64 a ± 0.874	2.84 ab ± 1.168	2.60 ab ± 0.480	2.29 b ± 0.456	0.025
Σ PUFA/ Σ SFA	0.48 ± 0.246	0.37 ± 0.105	0.33 ± 0.053	0.35 ± 0.074	0.170
C16:0/C18:0	0.97 b ± 0.163	1.04 ab ± 0.218	1.19 a ± 0.168	1.23 a ± 0.168	0.012

Means with unlike superscripts differ at the 5% probability level
[1] FAME= Fatty acids methyl esters
[2] Refer to the assigned dietary proportion of alfalfa (0%, 25%, 50% and 75% for U, L, M and H respectively).
[3] Σ SFA = Σ(C12:0; C14:0; C15:0; C16:0; C17:0; C18:0; C20:0; C22:0;Iso14; Iso15; Iso16, Iso17, Iso18).
[4] Σ MUFA cis = Σ(C14-1n-5 ; C15-1n-6 ; C16-1 delta 9 cis ; C17-1 delta 9 cis ; C18-1 delta 9 cis ; C18-1 delta 11 cis ;C18-1 delta 12 cis ; C18-1 delta 13 cis ; C18-1 delta 14 cis et 16 trans ; C22-1n-9 ; C24-1 delta 15)
[5] Σ C18:1 trans = Σ(C18-1 delta 6 trans ; C18-1 delta 9 trans; C18-1 delta 10-11 trans)
[6] Σ MUFA trans = Σ(C16-1 delta 9 trans ; C18-1 delta 6 trans ; C18-1 delta 9 trans ; C18-1 delta 10-11 trans)
[7] Σ PUFA n-6= Σ(C18-2n-6 trans trans ; C18-2n-6 cis trans ; C18-2n-6 trans cis ; C18-2n-6 cis cis ; C18-3n-6 ; C20-2n-6 ; C20-3n-6; C20-4n-6 ; C22-2n-6; C22-4n-6 ; C22-5n-6)
[8] Σ PUFA n-3 = Σ(C18-3n-3; C20-4n-3; C20-5n-3; C22-5n-3; C22-6n-3)
[9] CLA = Conjugated linolenic acid
[10] Σ PUFA = (Σ PUFA n-6 ; Σ PUFA n-3 ; Σ PUFA n-9 ; Σ CLA, Σ PUFA Conj)

Table 4: Mean ± standard deviation of total lipid content (g/100g of fat) and FA centesimal composition (% of total FAME[1]) of subcutaneous fat of lambs grazing a cocksfoot pasture and supplemented with different levels of fresh alfalfa.

	U [2]	L [2]	M [2]	H [2]	p-value
Lipids	22.20 c ± 12.228	30.72 bc ± 13.756	46.24 ab ± 11.290	61.18 a ± 15.343	0.050
C14 :0	5.90 ± 2.410	5.78 ± 3.692	4.59 ± 1.867	3.79 ± 1.259	0.360
C16 :0	20.63 b ± 1.095	21.28 ab ± 2.062	22.36 a ± 1.022	22.22 a ± 1.123	0.080
C18 :0	22.24 ± 4.86	20.72 ± 5.191	19.60 ± 2.723	18.85 ± 2.840	0.411
Σ SFA[3]	54.25 a ± 3.780	53.11 ab ± 4.186	51.38 ab ± 2.957	49.52 b ± 1.762	0.053
C18 :1 delta 9 cis	28.20 ± 4.164	30.36 ± 4.299	31.29 ± 1.532	32.42 ± 2.772	0.127
Σ MUFAcis[4]	31.884 ± 4.577	34.42 ± 4.798	35.44 ± 1.843	36.89 ± 3.229	0.102
Σ C18 :1 trans[5]	6.49 ± 1.977	4.96 ± 1.835	5.64 ± 1.389	5.85 ± 0.895	0.268
Σ MUFA trans[6]	6.97 ± 1.969	5.47 ± 1.827	6.07 ± 1.35	6.36 ± 0.926	0.261
C18 :2n -6 cis cis	1.98 ± 0.403	2.02 ± 0.429	1.97 ± 0.195	1.85 ± 0.280	0.758
C20 :4n -6	0.26 a ± 0.119	0.22 ab ± 0.111	0.14 b ± 0.036	0.14 b ± 0.034	0.012
Σ PUFA n-6 [7]	3.54 ± 0.555	3.47 ± 0.51	3.37 ± 0.365	3.53 ± 0.485	0.885
C18 :3n -3	1.02 b ± 0.303	1.21 ab ± 0.354	1.33 ab ± 0.228	1.48 a ± 0.255	0.030
C20 :5n -3	0.07 ± 0.088	0.10 ± 0.066	0.07 ± 0.065	0.07 ± 0.052	0.796
C22 :5n -3	0.48 ± 0.176	0.51 ± 0.125	0.40 ± 0.09	0.36 ± 0.055	0.063
C22 :6n -3	0.09 ± 0.117	0.07 ± 0.087	0.08 ± 0.060	0.07 ± 0.060	0.881
Σ PUFA n-3[8]	1.60 ± 0.598	1.90 ± 0.469	1.88 ± 0.3499	1.99 ± 0.332	0.615
CLA (9cis, 11trans)[9]	1.15 ± 0.428	1.10 ± 0.288	1.30 ± 0.526	1.38 ± 0.134	0.386
Σ PUFA[10]	6.89 ± 1.583	7 .0 ± 0.961	7.11 ± 1.32	7.34 ± 0.759	0.891
Σ PUFA n -6 / Σ PUFA n -3	2.24 ± 0.543	1.94 ± 0.656	1.82 ± 0.207	1.81 ± 0.311	0.277
C18 :2n -6 / C18 :3n -3	2.08 a ± 0.824	1.91 ab ± 1.021	1.54 ab ± 0.389	1.28 b ± 0.271	<0.050
ΣPUFA / ΣSFA	0.13 ± 0.036	0.13 ± 0.024	0.14 ± 0.034	0.15 ± 0.012	0.560
C16 :0 / C18 :0	0.96 ± 0.216	1.11 ± 0.38	1.16 ± 0.187	1.21 ± 0.242	0.365

Means with unlike superscripts differ at the 5% probability level

[1] FAME= Fatty acids methyl esters

[2] Refer to the assigned dietary proportion of alfalfa (0%, 25%, 50% and 75% for U, L, M and H respectively).

[3] Σ SFA = Σ(C12:0; C14:0; C15:0; C16:0; C17:0; C18:0; C20:0; C22:0;Iso14; Iso15; Iso16, Iso17, Iso18).

[4] Σ MUFA cis = Σ(C14-1n-5 ; C15-1n-6 ; C16-1 delta 9 cis ; C17-1 delta 9 cis ; C18-1 delta 9 cis ; C18-1 delta 11 cis ;C18-1 delta 12 cis ; C18-1 delta 13 cis ; C18-1 delta 14 cis et 16 trans ; C22-1n-9 ; C24-1 delta 15)

[5] Σ C18:1 trans = Σ(C18-1 delta 6 trans ; C18-1 delta 9 trans; C18-1 delta 10-11 trans)

[6] Σ MUFA trans= Σ(C16-1 delta 9 trans ; C18-1 delta 6 trans ; C18-1 delta 9 trans ; C18-1 delta 10-11 trans)

[7] Σ PUFA n-6= Σ(C18-2n-6 trans trans ; C18-2n-6 cis trans ; C18-2n-6 trans cis ; C18-2n-6 cis cis ; C18-3n-6 ; C20-2n-6 ; C20-3n-6; C20-4n-6 ; C22-2n-6; C22-4n-6 ; C22-5n-6)

[8] Σ PUFA n-3 = Σ(C18-3n-3; C20-4n-3; C20-5n-3; C22-5n-3; C22-6n-3)

[9] CLA = Conjugated linolenic acid

[10] Σ PUFA = (Σ PUFA n-6 ; Σ PUFA n-3 ; Σ PUFA n-9 ; Σ CLA, Σ PUFA Conj)

Table 5: Regression equations ($y= a \pm b$PA) for fatty acid composition of LT muscle lipids according to the dietary proportion of alfalfa (PA).

Dependent variable	a	b	P-value	R^2	RMSE
C16:0	17.99 (± 0.216)	0.05 (± 0.005)	0.009	0.98	0.201
C18:1 delta 9 cis	26.88 (± 0.548)	0.06 (± 0.013)	0.051	0.90	0.624
C18:2 n-6	6.55 (±0.063)	0.056 (0.005)	0.054	0.99	0.064
C18:3 n-3	1.78 (± 0.097)	0.006 (±0.0023)	0.102	0.81	0.110
C20:5 n-3	1.01 (± 0.085)	0.004 (± 0.0020)	0.160	0.70	0.097
Σ MUFA cis	31.05 (± 0.060)	0.06 (± 0.014)	0.059	0.88	0.684
C22:5 n-3	2.16 (± 0.113)	0.001 (± 0.003)	0.049	0.90	0.128
C18:2 n-6/C18:3n-3	3.55 (± 0.130)	0.021 (± 0.0031)	0.022	0.96	0.148
PUFA n-6/PUFA n-3	2.22 (± 0.165)	0.007 (± 0.004)	0.215	0.61	0.188
C16:0/C18:0	0.96 (± 0.023)	0.004 (± 0.0005)	0.016	0.97	0.027

Table 6: Regression equations ($y= a \pm b$PA $\pm$ cPA^2) for fatty acid composition of subcutaneous fat according to the dietary proportion of alfalfa (PA).

Dependent variable	a	b	c	P-value	R^2	RMSE
C16:0	20.64 (± 0.248)	0.029 (± 0.0060)	-	0.041	0.92	0.283
C18:1 delta 9 cis	28.35 (± 0.267)	0.065 (± 0.0064)	-	0.010	0.98	0.305
C18:3 n-3	1.03 (± 0.024)	0.007 (± 0.0005)	-	0.007	0.99	0.028
C20:4 n-6	0.263 (± 0.015)	-0.002 (± 0.0005)	-	0.029	0.94	0.018
Σ SFA	54.22 (± 0.177)	-0.015 (± 0.0139)	-0.001 (± 0.0002)	0.050	0.99	0.179
C18:2 n-6/C18:3n-3	2.09 (± 0.021)	-0.004 (± 0.0016)	-0.0001 (± 0.00002)	0.034	0.99	0.021

4. CAPÍTULO IV

Dose-dependent response of nitrogen stable isotope ratio to proportion of legumes in diet to authenticate lamb meat produced from legume-rich diets 2

Dose-dependent response of nitrogen stable isotope ratio to proportion of legumes in diet to authenticate lamb meat produced from legume-rich diets

Devincenzi T.[1,3,] Delfosse O.[2], Andueza D.[1], Nabinger C.[3], Prache S.[1*]

[1] Institut National de la Recherche Agronomique (INRA), UMR1213 Herbivores, F-63122 St-Genès-Champanelle, France.

[2] Institut National de la Recherche Agronomique (INRA), UMR0614 Fractionnement des Agro Ressources et Environnement, 2 esplanade Roland-Garros, BP 224, F-51686 Reims Cedex 2, France.

[3] Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 7712, CEP 91501-970, Porto Alegre-RS, Brazil.

* Correspondence to Sophie Prache

e-mail: sophie.prache@ clermont inra.fr

phone : +33 4 73 62 40 63

Abstract

This study investigated the dose-dependent response in lamb meat of stable nitrogen isotope ratio to the dietary proportion of legumes, and the ability of the nitrogen isotope signature of the meat to authenticate meat produced from legume-rich diets. Four groups of nine male Romane lambs grazing a cocksfoot pasture were supplemented with different levels of fresh alfalfa forage to obtain four dietary proportions of alfalfa (0%, 25%, 50% and 75%) for 98 days on average before slaughter (groups L0, L25, L50 and L75). We measured the stable nitrogen isotope ratio in the forages and in the *longissimus thoracis* muscle. The $\delta^{15}N$ value of the meat decreased

linearly with the dietary proportion of alfalfa. The distribution of the $\delta^{15}N$ values of the meat discriminated all the L0 lambs from the L75 lambs, and gave a correct classification score of 85.3% comparing lambs that ate alfalfa with those that did not.

Keywords: $\delta^{15}N$, nitrogen stable isotope ratio, lamb meat, authentication, legumes

1. Introduction

In recent year, there has been renewed interest in incorporating forage legumes within livestock systems in order to reduce reliance on bought-in concentrate feed, prevent the contamination of soil and water from excessive amounts of synthetic fertilizers and meet consumer demands for a healthy product produced in a natural way. Organic farming systems are particularly concerned due to the high cost of organic concentrate feed and because the organic farming list of specifications forbids the use of synthetic fertilizers. Moreover, there is a growing consumers' interest for the environment, animal welfare and the origin and method of food production and consequently for food products of low-input and organic production systems. The ability to authenticate food products from these agro-ecological systems has therefore become an important challenge for scientists, monitoring bodies, commercial organizations and farmers alike.

The nitrogen (N) isotope signature has recently been proposed as a valuable tool to authenticate meat produced from pasture-fed animals (Monahan, Moloney, Osorio, Röhrle, Schmidt & Brennam, 2012) and more specifically from organic beef (Schmidt, Quilter, Bahar, Moloney, Scrimgeour, Begley & Monahan, 2005; Bahar, Schmidt, Moloney, Scrimgeor, Begley & Monahan, 2008). Schmidt et al., 2005 observed a lower $^{15}N/^{14}N$ ratio in the meat of organically raised beef compared with conventionally raised

animals. They suggested that this difference might be related to differences in the composition of the diet, with more legumes in the diet of organically raised animals. The nitrogen stable isotope composition of animal products is directly related to the dietary $^{15}N/^{14}N$ ratio which is lower in legume-rich diets due to the capacity of leguminous plants to fix atmospheric nitrogen (De Niro & Epstein, 1981; Bahar et al, 2005). However, many of the studies using stable isotopes have limited their comparisons to the authentication of geographical locations, and no clear conclusions are drawn concerning the authentication of diet composition (Piasentier, Valusso, Camin, & Versini, 2003; Camin, Bontempo, Heinrich, Horacek, Kelly, Schlicht, Thomas, Monahan, Hoogewerff & Rossman, 2007; Vinci, Preti, Tieri, & Vieri, 2012; Yanagi, Hirooka, Oishi, Choumei, Hata, Arai, Kitagawa, Gotoh, Inada & Kumagai, 2012).

The aim of this study was to investigate the dose-dependent response in lamb meat of stable nitrogen isotope ratio to the proportion of legumes in the lambs' diet and the ability of the nitrogen isotope signature of the meat to authenticate meat produced from legume-rich diets.

2. Material and Methods

The experiment took place at the Herbivore Research Unit at the Institut National de la Recherche Agronomique (INRA) Clermont-Ferrand/Theix Research Centre, France. The animals were handled by specialized staff who ensured their welfare in accordance with European Union Directive No. 609/1986.

2.1 Experimental design, animals and diets

We compared four proportions of alfalfa (*Medicago sativa*) and cocksfoot grass

(*Dactylis glomerata* L.) in the animal's diet, using four groups of lambs. Each experimental treatment comprised nine non-castrated male Romane lambs that were fed, for 98 days on average before slaughter, a diet containing 0%:100% (L0), 25%:75% (L25), 50%:50% (L50) and 75%:25% (L75) fresh alfalfa forage : fresh cocksfoot forage. The lambs received no legumes in their diet before the experiment began.

Two conterminal monocultures of alfalfa (1.0 ha) and cocksfoot (1.6 ha) were sown in October 2010. From 30 April 2012 onwards, both pastures were divided into four plots of similar area. Each week, one plot was mown to ensure the provision of young forage throughout the experiment. In March 2012, both pastures received 67 kg phosphorus /ha and 100 kg potassium /ha. In April 2012, the cocksfoot pasture received 48 kg N/ha.

Lambs were born between 18 March and 26 March 2012. Before the experimental period, the animals were managed uniformly and received no legumes in their diet (i.e. from the beginning of the gestation period for the dams). Between birth and 10 May, the lambs and their dams were housed in a sheepfold, and the lambs were offered a commercial concentrate. From 04 May to 10 May, the lambs were offered freshly cut cocksfoot grass, distributed indoors. Lambs and their dams were then turned out to two unmown plots of the cocksfoot pasture from 11 May to 29 May. To remove the stemmy material over the whole cocksfoot pasture and ensure a vegetative regrowth, the cocksfoot pasture was topped on 29 May with a forage harvester, and the trimmings removed.

On 30 May, the 36 lambs were assigned to nine blocks according to birth weight and average daily gain (ADG) between birth and 29 May. They were then randomly assigned from within the blocks to one of the four treatments. Mean lamb birth weight

and ADG between birth and 29 May were 4.2 (standard deviation (SD) 0.71) kilogram (kg) and 301 (SD 49.6) gram per day (g/d); lambs weighed 24.9 (SD 3.49) kg on 29 May, i.e. at a mean age of 69 (SD 1.9) days. The lambs were weaned and treated against internal worms on 29 May, and then individually penned indoors for an adaptation period until 7 June. During this adaptation period, the lambs were fed freshly cut cocksfoot *ad libitum*.

Period 1. From 7 June until 3 July, the lambs were individually fed indoors. During this period, L0, L25, L50 and L75 lambs were fed a diet containing 0%:100%, 25%:75%, 50%:50% and 75%:25% alfalfa: cocksfoot freshly cut forages respectively (on a dry matter basis). The levels of alfalfa and cocksfoot offered were calculated every day (except on weekends) to ensure similar levels of Unité Fourragères Viande (UFV) for all the groups (INRA, 2007), and based on the estimation of the dry matter (DM) content of each forage offered. This last estimation was made in duplicate using a microwave oven. Both forages were cut daily at 8 *ante meridiem (*a.m.) and offered in separate tubs, half in the morning at 9 a.m. and half in the afternoon at 4 *post meridiem* (p.m.) (after storage at 4 °C between cutting and distribution). Feed tubs were emptied every morning before the distribution of the freshly cut forages, and refusals were weighed, recorded and discarded. Final dry matter measurement was made daily in duplicate for both forages by drying representative samples of the forages offered and refused in a forced-air oven for 72 hours (h) at 60 °C. We initially planned to use this feeding scenario throughout the fattening period between weaning and slaughter, in order to control the proportions of cocksfoot grass and alfalfa for each lamb. However, this plan was thwarted by low levels of forage intake, which resulted in low levels of ADG. We therefore adapted the experimental design during the second part of the study

(Period 2).

   Period 2. From 4 July 2012 until slaughter, each group of lambs was assigned to one of the four cocksfoot pasture plots and supplemented with freshly cut alfalfa using racks. The mean voluntary intake level of each lamb group was estimated at 78.125 g $DM/BW^{0.75}$, where BW was the average body weight of the group (Hassoun & Bocquier, 2007). The amount of fresh alfalfa offered to each group was based on the assigned proportion of alfalfa in the diet and the estimation of the dry matter content of fresh alfalfa. This estimation was made in duplicate using a microwave oven. Alfalfa was cut every morning at 8 a.m. and offered half in the morning at 9 a.m. and half in the afternoon at 4 p.m. (after storage at 4 °C between cutting and distribution). Care was taken to use sufficiently large racks to avoid competition for alfalfa. The racks were emptied every morning before the distribution of the freshly cut alfalfa and the refusals were weighed, recorded and discarded. Representative samples of offered and refused alfalfa were collected daily for final dry matter measurements made in duplicate by drying in a forced-air oven for 72 h at 60 °C. Water and salt blocks were always available. The salt blocks contained (g/kg, as-fed) 60 calcium, 20 phosphorus, 10 magnesium, 280 sodium, 17.5 zinc, 1.5 iron, 5.5 manganese, 0.03 cobalt, 0.03 iodine, and 0.01 selenium.

   The cocksfoot pasture was grazed continuously during period 2, but the experimental groups of lambs were changed weekly from one plot to another to avoid confounding effects of sward characteristics on the level of cocksfoot forage intake level.

2.2 Slaughter procedures

   Groups of lambs balanced for treatments were slaughtered at the INRA

Clermont-Ferrand Centre experimental slaughterhouse, according to European Union welfare guidelines, on 3 September 2012 (three lambs per group), 6 September (two lambs per group) and 27 September 2012 (four lambs per group). They were thus slaughtered at mean age 177 (SD 11.6) days after having been fed the experimental treatment for 98 days on average (range 86 to 112 days). The lambs had access to food and water until approximately 30 minutes before slaughter and were transported by truck to a slaughterhouse located less than 500 meters from the experimental pastures. Immediately on arrival, the lambs were electrically stunned and slaughtered by throat cutting.

2.3 Measurements

Animal body weight

Lambs were weighed once weekly before the alfalfa distribution in the morning.

Pasture availability

Fifty systematic measurements of the cocksfoot sward surface height per plot were made weekly using a sward stick.

N isotope ratio mass spectrometry

Nitrogen stable isotope ratio analysis of the forages was performed weekly on dried samples milled with a 200 micrometer (μm) outlet mill. During Period 2, for the cocksfoot pasture, snip samples representative of the plant material consumed by the lambs, i.e. about 50% of the extended tiller height (Prache & Peyraud, 2001), were taken weekly in each cocksfoot plot, dried for 72 h at 60 °C, and milled with a 200 μm outlet mill. Bulked samples were then made, grouping the four plot samples.

A sample of the left *longissimus thoracis* muscle was taken from the last thoracic rib 24 hours (h) *post mortem*, vacuum packed and frozen at −20 °C, freeze-

dried, and milled in a CYCLOTEC grinder with a 0.8 millimeter (mm) outlet grid of 200 μm.

Nitrogen stable isotope ratio analysis of meat and forage samples was carried out as follows. The sample powder was first homogenized, and an aliquot of about 4 milligram (mg) was then weighed in a tin capsule. The capsule was analysed using a EURO EA elemental analyser (Eurovector, Milan, Italy) connected to a delta PLUS advantage isotope ratio mass spectrometer (Thermo-Fischer, Bremen, Germany). The sample was fully oxidized. Nitrogen was converted into N_2 and carbon into CO_2. A Porapack-QS column was used to separate the two gases, which were then introduced into a mass spectrometer. After ionization, electrical field acceleration and magnetic field deviation steps, the ions were detected using Faraday cups, and the $^{15}N/^{14}N$ isotope ratio was calculated. Hereafter, $^{15}N/^{14}N$ isotope ratio is expressed using the $\delta^{15}N$ value, calculated as follows:

$$\delta^{15}N = ([(^{15}N/^{14}N_{sample}) - (^{15}N/^{14}N_{air})] / (^{15}N/^{14}N_{air})) \times 1000.$$

2.4 Data analysis

The data for the $\delta^{15}N$ value of the meat underwent a variance analysis using the General Linear Model (GLM) procedure of SAS software package (Inst. Inc., Cary, NC) to examine the effect of the feeding treatment. We used the Duncan test for pairwise comparisons. A regression analysis was carried out using the GLM procedure of SAS software package to examine whether the response of mean $\delta^{15}N$ of the meat had a linear or a curvilinear relation (quadratic effect) to the mean proportion of alfalfa in the diet.

Linear discriminant analysis was performed on $\delta^{15}N$ values of the *longissimus thoracis* muscle, followed by a cross-validation procedure to classify the meat samples

according to feeding treatments, using Minitab software v.13 (Minitab Inc., Paris). This analysis was performed to evaluate the reliability of the discrimination between pairwise groups, between lambs consuming no alfalfa (L0 lambs) and those consuming alfalfa (L25, L50 and L75), and between all four groups of lambs.

3. Results

3.1. Pasture, animal performance, and intake

During the study, one lamb in group L75 died from bloating and one lamb in group L0 died from causes unrelated to the experimental treatments.

Mean sward surface height over the whole cocksfoot pasture averaged 14.8 centimeter (cm), ranging from 23.9 cm on 3 July to 10.0 cm on 23 August.

Mean daily forages intake and refusals are reported in Table 1. During period 1, refusals represented 13.3 to 21.3% of the diet offered and were highly variable among animals. During period 2, refusals represented 5.3%, 4.3% and 16.2% of the alfalfa offered for L25, L50 and L 75 lambs respectively.

Mean dietary proportion of alfalfa was 27.7 %, 52.2 % and 74.8 % for L25, L50 and L75 lambs during period 1, and 25.8%, 48.7% and 62.4% for L25, L50 and L75 lambs during period 2 (Figure 1).

The ADG during the experiment was affected by the treatment ($P < 0.001$); it was lower for L0 and L25 lambs than for L50 and L75 lambs ($P < 0.001$). Mean ADG was 136, 147, 191 and 224 g/day for L0, L25, L50 and L75 lambs respectively.

3.2 δ^{15}N value of forages

Average δ^{15}N value was 3.3‰ (ranging from 2.9‰ to 4.2‰) for cocksfoot and 0.7‰ (ranging from −0.9‰ to 1.6‰) for alfalfa (Figure 2).

3.3 δ^{15}N value of longissimus thoracis muscle

The δ^{15}N value of the meat averaged 6.06‰ (5.8‰ to 6.3‰) for the L0 lambs, 5.77‰ (5.4‰ to 6.1‰) for the L25 lambs, 5.43‰ (5.1‰ to 5.8‰) for the L50 lambs and 5.44‰ (5.2‰ to 5.7 ‰) for the L75 lambs (Figure 3). Coefficient of variation of the δ^{15}N value of the meat was 3.41%, 4.21%, 5.11% and 3.70% for groups L0, L25, L50 and L75 respectively. The δ^{15}N value of the meat was significantly affected by the experimental treatment ($P < 0.001$). It was higher for the L0 lambs than for the L25 lambs ($P < 0.01$) and higher for the L0 lambs than for the L50 and L75 lambs ($P < 0.001$ for both comparisons). The δ^{15}N value of the meat was higher for the L25 lambs than for the L50 and L75 lambs ($P < 0.01$). It was not different between the L50 and L75 lambs.

The δ^{15}N value of the meat was linearly related to the proportion of alfalfa (PA, %) in the diet ($P = 0.024$) (Figure 4), with no significant quadratic effect ($P = 0.599$), the regression equation being:

δ^{15}N value of the meat = 6.04 ($\pm$0.071) − 0.0107 ($\pm$0.00169) PA,

Where $r^2 = 0.95$, RSD = 0.080, and $n = 4$.

3.4 Discriminant analysis

There was no overlap in the distribution of the δ^{15}N values of the *longissimus thoracis* muscle between the L0 and L75 lambs (Figure 3). Discriminant analysis correctly classified 100% of the L0 lambs and 100% of the L75 lambs. There was some overlap in the distribution of the δ^{15}N values of the *longissimus thoracis* muscle between the L0 and L50 lambs (two L0 lambs and two L50 lambs having a δ^{15}N value of the *longissimus thoracis* muscle of 5.8). The discriminant analysis correctly classified 100% of the L0 lambs and 77.8% of the L50 lambs (seven out of nine lambs), giving a

global correct classification score of 88.2%. The discriminant analysis between the L0 and L25 lambs correctly classified 75.0% of the L0 lambs (six out of eight lambs) and 77.8% of the L25 lambs (seven out of nine lambs), giving a global correct classification score of 76.5%. The discriminant analysis between lambs consuming no alfalfa (N, i.e. L0 lambs) and those who consumed alfalfa (A, i.e. L25, L50 and L75 lambs) correctly classified 75.0% of the N lambs (six out of eight) and 88.5% of the A lambs (23 out of 26), giving a global correct classification score of 85.3%. The linear discriminant analysis between the four experimental groups correctly classified 75% of the L0 lambs (six out of eight), 44.4% of the L25 lambs (four out of nine), 11.1% of the L50 lambs (one out of nine) and 0% of the L75 lambs, giving a global correct classification score of 32.4% of the samples.

4. Discussion

This work set out to study the dose-dependent response relating the proportion of legumes in the diet to the $\delta^{15}N$ value of lamb meat and to investigate the discriminatory ability of the $\delta^{15}N$ value of the meat for the authentication of meat produced from legume-diets.

The experimental design was chosen both to ensure that the range of alfalfa proportions was sufficiently broad to obtain the response curve, and to limit the risk of bloating. The highest proportion of alfalfa also mimicked the preferences of ruminants on fields consisting of adjacent monocultures of grass and clover (Rutter, 2006).

We initially planned to feed freshly cut forages to individually penned animals indoors (Period 1), in order to achieve an optimal individual control of cocksfoot and alfalfa intake levels. However, this experimental scenario was thwarted by low levels of

forage intake, which resulted in low levels of ADG. Accordingly, we changed the experimental design during the second part of the study (Period 2) by (i) turning out each group of lambs to one cocksfoot plot and (ii) supplementing each group twice daily with freshly cut alfalfa. This change led to a marked improvement in ADG, but enabled the control of alfalfa intake only at group level.

During Period 1, the proportion of alfalfa in the diet was close to the assigned value for all the groups. During Period 2, the estimated proportion of alfalfa in the diet was close to the assigned value for L25 and L50 groups, but it was lower for L75 group (62.4% *vs.* 75.0%). However, the level of alfalfa refusals demonstrated that this forage was offered *ad libitum* to L75 lambs. The area and the management of the cocksfoot pasture were planned to ensure *ad libitum* intake (calculated as equal to 78.125 g $DM/BW^{0.75}$), and consequently a satisfactory estimation of the proportion of alfalfa in the diet. The stocking rate was low (22.5 lambs/hectare) and the cocksfoot regrowth was in a vegetative stage throughout the experiment. However, we experienced drought conditions from mid-August onwards, which resulted in a lower mean sward surface height on the cocksfoot pasture than initially planned. The mean ADG for L25 lambs (147 g/d) indicated that voluntary intake may not always have been reached for this group. It follows that the proportion of alfalfa in the diet could have been somewhat over-estimated for L25 lambs. However, values for L50 and L75 lambs ADG (191 and 224 g/d) indicated that voluntary intake was reached for both groups, and so the proportion of alfalfa in the diet was satisfactorily estimated. Concerning the L0 lambs, although their voluntary intake may not always have been reached, this was irrelevant to the estimation of the diet composition, as they ate only cocksfoot.

Cocksfoot and alfalfa $\delta^{15}N$ values lay in the range reported in previous studies

(Rossmann, Kornexl, Versini, Pichmayer & Lamprecht, 1998; Schwertl, Auerswald, Schaüfele & Schnyder, 2005). The ^{15}N level of the nitrogen compounds in plants is lower for leguminous plants, because they utilize the nitrogen in the air, which is rich in ^{14}N, as a nitrogen source (Virginia & Delwiche, 1982). The temporal changes in δ^{15}N observed during the course of the experiment were greater for alfalfa than for cocksfoot grass. The lower δ^{15}N values occasionally observed for alfalfa could be explained by the fact that we sporadically used an older field that was conterminal to the experimental field, as a result of a shortage of forage on the initially planned alfalfa pasture due to the onset of drought conditions. Owing to a more developed root system, older plants may have a greater ability to fix air nitrogen, increasing ^{14}N in plant nitrogen compounds, and so decreasing the δ^{15}N value (Högberg, 1997).

The δ^{15}N values of the *longissimus thoracis* muscle were somewhat more variable between individual animals for the L25 and L50 lambs than for the L75 lambs, probably reflecting the inter-individual variability in animal preferences (Prache, Roguet & Petit, 1998; Prache et al., 2006) and above all the higher competition for alfalfa. The inter-individual variability observed in this study is in line with that observed in beef meat (Bahar et al., 2005) and cattle hair (Schwertl et al., 2005).

There was a significant effect of the feeding treatment on the δ^{15}N value of the *longissimus thoracis* muscle, and one of the main results of the present study is that there was a negative linear relationship between the mean proportion of alfalfa in the diet and the mean δ^{15}N value of the lamb muscle. The declining value of muscle δ^{15}N clearly reflected the replacement of the cocksfoot pasture with alfalfa. In this regard, we note that as the between-animal variability in diet preferences may be high (Prache et al., 2006), the δ^{15}N value of the *longissimus thoracis* muscle may give relevant

information to estimate the proportion of alfalfa in the diet for individual animals fed within groups.

A second main result of this study is that the δ^{15}N value of the *longissimus thoracis* muscle is a useful tool for discriminating lambs that consume legumes from those that do not. Although the discriminant analysis did not satisfactorily separate all the treatment groups, the distribution of the δ^{15}N values of the muscle for lambs that ate legumes (L25, L50 and L75 lambs) from those that did not (L0 lambs) gave a correct classification score of 85.3%. Moreover, the distribution of the δ^{15}N values of the muscle for L0 and L75 lambs enabled us to discriminate the two groups fully. Hence N isotope composition in the meat can give relevant information to discriminate meat from lambs fed legume-rich diets, such as lambs produced in low-input and organic production systems. Actually, leguminous plants are more widespread in these production systems, which avoid or limit the use of synthetic fertilizers (Prache et al., 2011).

However, as δ^{15}N values of the *longissimus thoracis* muscle depend on δ^{15}N values in the diet, they may be modulated by the level and nature of the fertilization used on grasslands. In this regard, we note that organic fertilizers (for example, farmyard manure) increase the ^{15}N in both soil and plants (Bateman, Kelly & Woolfe, 2007) and consequently in the meat of grazing animals (Yanagi et al., 2012).

Furthermore, compared with the present feeding scenario, in which the proportion of alfalfa in the diet remained steady throughout the fattening period, the proportion of legumes in a grazing animal's diet may vary greatly according to management practices of grasslands. There may be a high variability between pastures that are successively grazed in the contribution of legume species to the sward biomass

(Bahar et al., 2008). There may also be a high variability between successive days during the grazing-down of a pasture, because animals may switch from the preferred legume species to the less preferred graminae species (Prache et al., 2006). These issues raise further questions such as the time of appearance and persistence of δ^{15}N values of the *longissimus thoracis* muscle relative to changes in diet.

Finally, it is likely that the N isotope signature of the meat cannot reveal intensive indoor fattening with concentrates containing legumes (such as soya bean, dehydrated alfalfa, or chickpea for example) (Bahar et al., 2005; Prache et al., 2009; Biondi, D'Urso, Vasta, Luciano, Scerra, Priolo, Ziller, Bontempo, Caparra & Camin, 2013). To yield a robust tool for the authentication of the meat produced from lambs pasture-fed legume-rich pastures, the N isotope signature of the meat may therefore need to be combined with other methods, particularly those used for pasture-feeding authentication (Prache, 2007; Dian, Andueza, Jestin, Prado & Prache, 2008).

5. Conclusion

This study demonstrates that the δ^{15}N value of the *longissimus thoracis* muscle decreases linearly with the proportion of legume in the diet and can give relevant information to authenticate meat from lambs fed legume-rich diets.

These results may be of interest for the authentication of meat produced in low-input and organic production systems, in which leguminous plants are more widespread. In this regard, further work should be directed at combining δ^{15}N value of the meat with other methods to increase the reliability of the discrimination and avoid the risk of bias.

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Table 1. Mean (standard deviation) daily forage intake and refusals. During period 1, lambs were individually fed indoors with freshly cut forages; during period 2, each group of lambs grazed a cocksfoot pasture and was supplemented with the assigned level of freshly cut alfalfa.

Feeding treatment[1]	L0	L25	L50	L75
Period 1				
Daily alfafa intake (kg DM[2]/lamb)	-	0,184	0,382	0,563
		(0,0403)	(0,0357)	(0,0535)
Daily cocksfoot intake (kg DM[2]/lamb)	0,643	0,482	0,351	0,189
	(0,0682)	(0,0680)	(0,0408)	(0,0136)
Refusals (proportion of the diet offered, % DM[2])	21.3	20.1	13.3	13.5
	(11.37)	(12.21)	(10.37)	(10.59)
Period 2				
Daily alfalfa intake (kg DM[2]/lamb)	-	0,269	0,545	0,716
Mean daily alfalfa refusals (kg DM[2])	-	0,013	0,023	0,139
Refusals (proportion of the alfalfa offered, % DM[2])	-	3,7	3,7	15,7

[1] Refer to the assigned dietary proportion of alfalfa (0%, 25%, 50% and 75% for L0, L25, L50 and L75 respectively).

[2] dry matter.
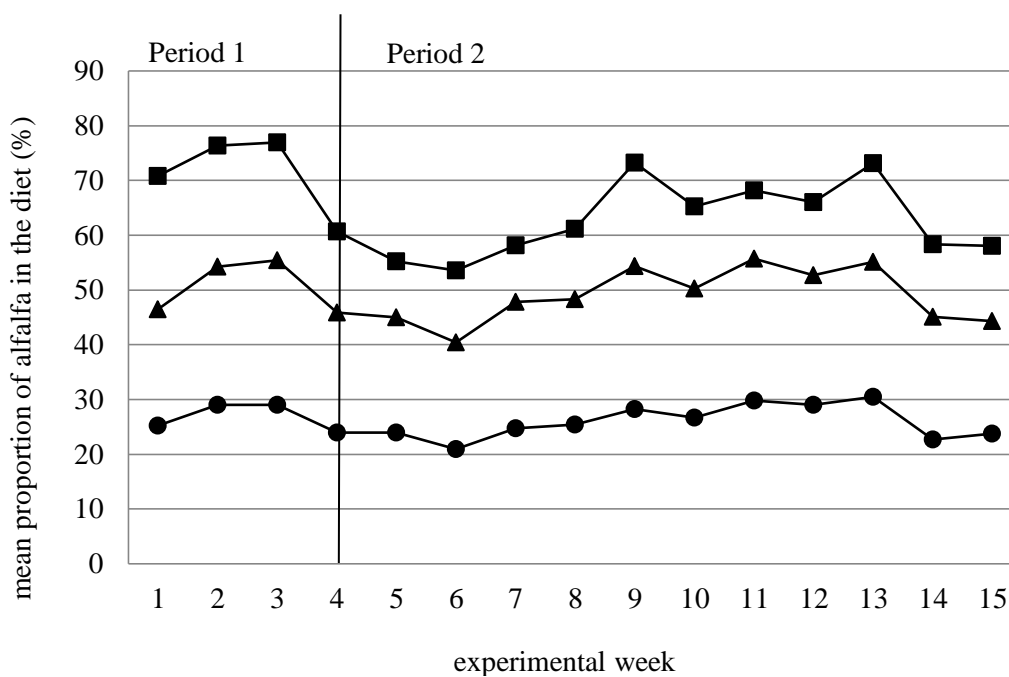
Figure 1 Mean proportion of alfalfa in the diet of lambs according to the experimental week. Circles (●), triangles (▲) and squares (■) refer respectively to groups of lambs assigned to consume 25%, 50% and 75% of alfalfa in their diet. During Period 1, the lambs were individually fed indoors with freshly cut cocksfoot and alfalfa. During Period 2, they were pasture-fed on a cocksfoot sward and supplemented with freshly cut alfalfa.
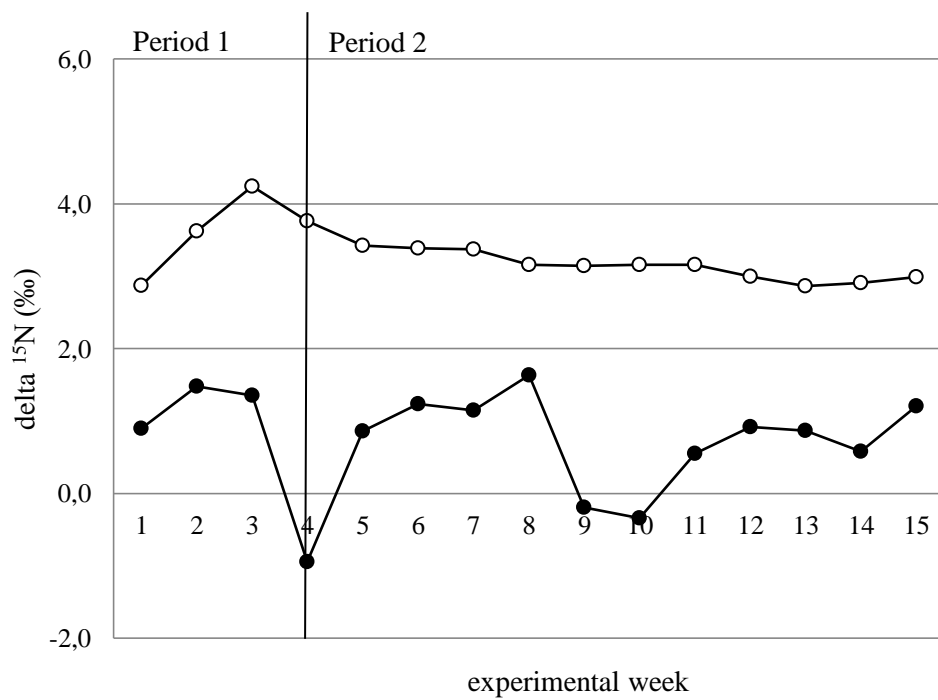
Figure 2 Changes in $\delta^{15}N$ values of the forages according to the experimental week.

White (○) and black (●) circles refer to the cocksfoot pasture and to alfalfa respectively.
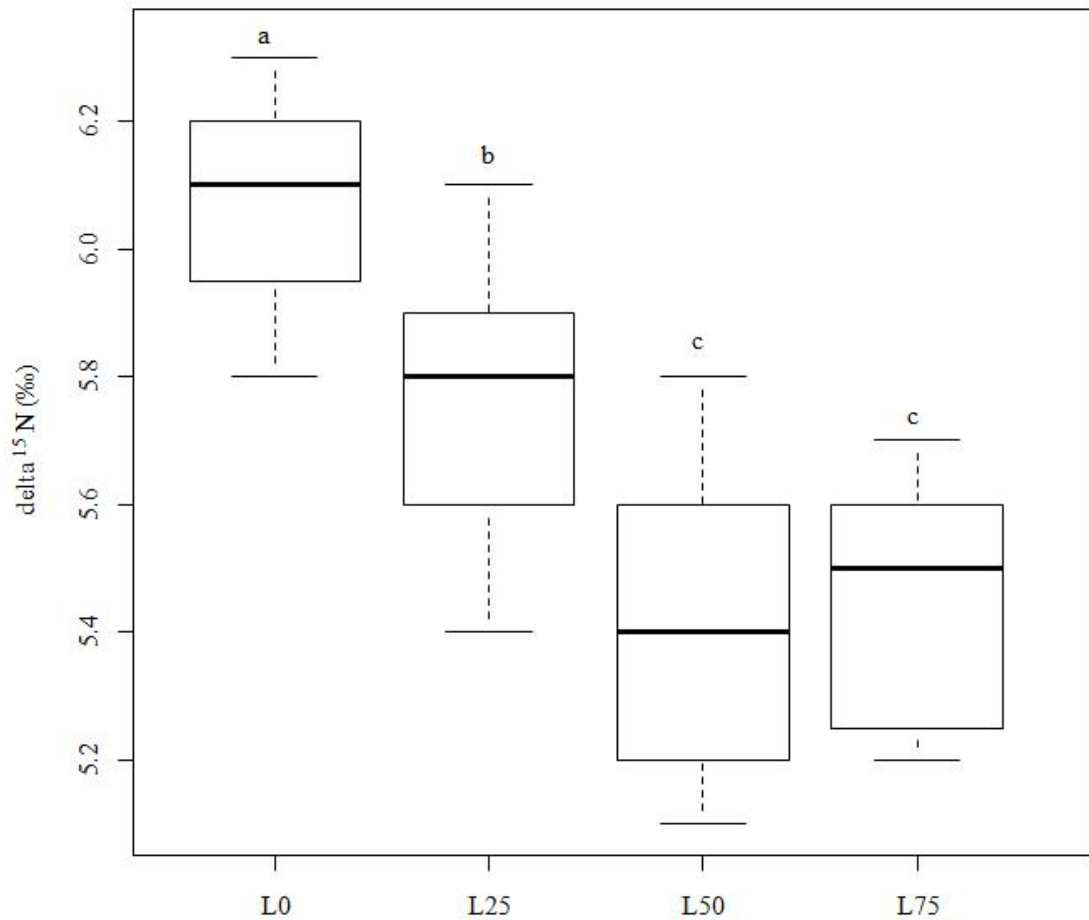
Figure 3 Box plot representation of the $\delta^{15}N$ value of the *longissimus thoracis* muscle according to the level of fresh alfalfa supplementation in lambs grazing a cocksfoot pasture. The level of alfalfa supplementation was adjusted to achieve a proportion of alfalfa in the diet of 0% (L0), 25% (L25), 50% (L50) and 75% (L75).

The box contains the middle 50% of the data, the upper edge of the box indicates the 75th percentile of the data set, and the lower edge indicates the 25th percentile. The bold line in the box indicates the median value of the data. The ends of the vertical lines indicate the minimum and maximum data values. Box plots not bearing a common letter are significantly different ($P < 0.01$).
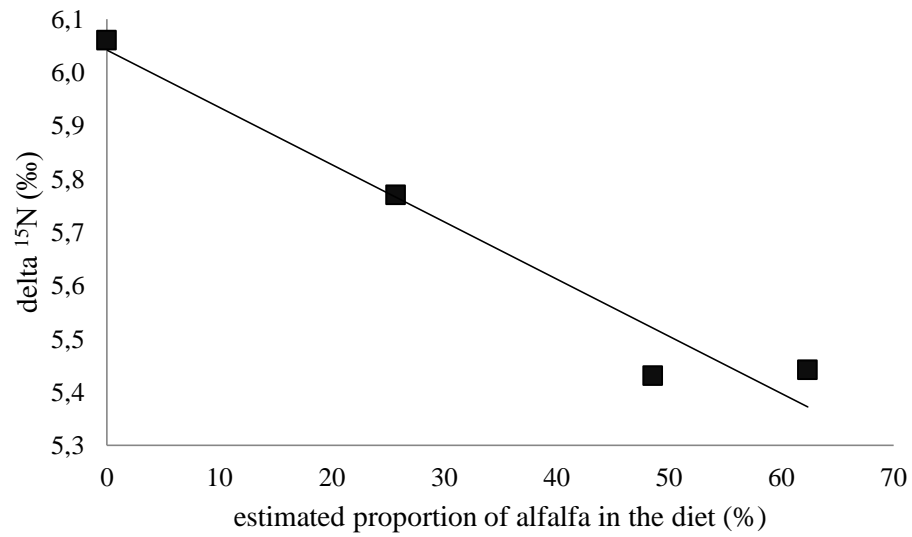
Figure 4 Relationship between mean proportion of alfalfa in the diet and δ^{15}N in lambs'

longissimus *thoracis* muscle.

5. CAPÍTULO V

Fatty acid profile of grass-based beef production on natural grassland of Southern Brazil

Fatty acid profile of grass-based beef production on natural grassland of Southern Brazil

Thais Devincenzi T.[1], Carlos Nabinger C. N.[1] et al...

[1] Universidade Federal do Rio Grande do Sul

(UFRGS), Av. Bento Gonçalves 7712, CEP 91501-970, Porto Alegre-RS, Brazil.

[2] Embrapa Pecuária Sul

* Correspondence to Thais Devincenzi

e-mail: thais_devincenzi@yahoo.com.br

phone : +55 51 96 98 09 01

Abstract

Producing beef on natural grasslands may represent an alternative to local farmers to add value on meat products of Pampa Biome, agreeing to the consumers claim for products that incorporate environmental conservancy. But, besides being " nature friendly", the meat product from natural grasslands must also supply the demands related to the nutritional composition. We evaluated the fatty acid profile on beef of steers finished on natural grassland, natural(NG), grassland fertilized and oversown with winter species (ING) and annual summer pasture (SP). We used 43 Aberdeen Angus steers 15-18 months of age which were randomly distributed between treatments: NG (n=16); ING (n=18); SP (n=9). Animals were slaghter at same fat tchickess. Fatty acid profile was perfeormed by gas chromatography. Meat of steers finished grazing Natural grassland and Improved natural grasslands were poorest in C14:0 and richer on n-3 polyunsaturated fat acids than meats from steers grazing Sorghum pasture.

Finishing steers on production systems based on improved natural grasslands promote meat with a more beneficial fatty acid profile to human health.

Introduction

In Southern Brazil, livestock beef production still relies mainly on rangelands of Pampa Biome (Sebrae/Senar/Farsul, 2005, Freitas et al., 2014). This biome is characterized by its herbage vegetation with a vast floristic biodiversity (Boldrini, 2002) and consists in a natural pastoral ecosystems (Nabinger et al., 2009). Besides the important ecological role, as conservancy of fauna diversity, water quality, sequestration of carbon, etc this rangelands are also associated to Gaucho´s culture. All this characteristics makes Pampa biome conservation mandatory (Bencke, 2009), for both environmental and cultural point of view. This high diversity of forages species also implies in more complex management of grasslands, which leads farmers to chose monoespecifc pastures or even to change livesctock to croplands (Nabinger & Sant'Anna, 2007). This choice, had result in estimated losses of about 440.000 ha of natural pastures by year in the last 10 years (Hasenack et al., 2007). In contrast, many information have been produced about southern brazilian natural grasslands manegement (Moojen & Maraschin, 2002, Carvalho et al., 2009, Nabinger et al., 2009, Ferreira, 2011), showing that this resource can be very productive when well maneged. Some practices like stocking rate adjusting and fertilizers and winter species over sown can improve significantly its productivity (Ferreira et al., 2011). So, producing beef on natural grasslands may represent an alternative to local farmers to add value on meat products of Pampa Biome, agreeing to the consumers claim for products that incorporate environmental conservancy. (Font-i-Furnols & Guerrero, 2014).

Besides being " nature friendly", the meat product from natural grasslands must also supply the demands related to sensorial quality and nutritional composition. Devincenzi et al., 2012, working with a trained panelists, found odour and flavour differences on beef from steers finished on natural grasslands, improved natural grasslands and sorghum pasture, suggesting that the improving in forage biovidersity could lead to different flavour composition.

Related to the nutritional composition of meat it is weel known that the increasing of diets polyunsaturated fatty acids from omega 3 family (PUFA n-3), leads to an increase on PUFA n-3 on meat products (Ribeiro et al., 2011), which are beneficial to humans health (Simopoulos, 1991). Hence, the fatty acid profile can be positively modified when animals are fed with pasture (French et al., 2000; Ribeiro et al., 2011), because this feedstuffs are rich on PUFA n-3. Nevertheless it is also know that there exists differences on fatty acid composition between grasslands (O´Kelly & Reich, 1976; Glasser et al., 2013), so, even for grass feeding animals, differences on meat fatty acid profile could be found.

To study the meat quality on different levels of biodiversity on production system based on natural graslands pampa biome, we evaluated the fatty acid profile on beef of steers finished on natural grassland, natural grassland fertilized and oversown with winter species (improved natural grassland) and annual summer pasture (Sorghum).

2. Materials and Methods

The study was conducted in a private beef farm located at Dom Pedrito's country rural area (31°21'39.26"S 54°34'56.20" O) between Decemeber of 2009, and November of 2010. The climate, according to Köppen's classification system, was a Cfa 2 (sub-

tropical climate) and the soil was a Mollisol with vertisol's properties (Chernossolo Háplico Órtico Vértico; Streck et al., 2003). The experimental area was composed by five paddocks with approximately 15 ha each.

2.1. Experimental design, animals, grazing management and measurements

The fatty acid profile on steers *Longissimus* muscle (LM) raised and finished on three different pastures which represents increasing levels of inputs. The treatments were three pasture based systems for finishing beef cattle: NG=Natural Grassland, based solely on naural grassland; ING= Improved Natural grassland, natural grassland over sown with ryegrass, birdsfoot and trefoil + fertilization, and SP= Annual summer-pasture (Sorghum).

On NG system a mechanical-mowing was done in May and September, 2010, aiming the control of undesirable species and the sward structure maintenance. On ING system, the soil acidity was corrected with three tons of lime per hectare in November 2009. In March 2010 a fertilization using 200 kg/ha of diammonium phosphate (DAP: 18-45-00) was done. In May 2010, a mechanical mowing and urea application (100 kg/ha) was conducted. At that time were oversown 36 kg of *Lolium multiflorum* Lam. pure live seed/ha, 5.4 kg of *Lotus corniculatus* cv. São Gabriel pure live seed/ha), both spreaded.

On SP system an hybrid forage sorghum (*Sorghum bicolor* x *Sorghum sudanense)* cv AGR 2501 was sown in 01/01/2010. The sowing density was 22 seeds/m with 0.45 m distance between rows in a no-tillage system. Soil acidity correction and fertilization was preceded according to a prescription based on soil analysis.

systems were composed by two plots each, in which the animals from those treatments were distributed equally and kept until the end of the experimental period. We kept two

paddocks in NG and ING due to the highest heterogeneity of those treatments when they are compared to mono-specific pastures (e.g. sorghum pasture), therefore, monitoring their productive conditions (herbage mass, sward height, floristic composition) would be important for their characterization.

On pre-experimental period animals were kept on natural grassland without any input. On NG and ING animals started grazing on 12/01/2009, with 353 ± 20.8 kg of liveweigh and 15 months of age. On SP, animals started grazing on 02/03/2010 with 354 ± 27.4 kg of liveweight and about 18 mounts.

For NG and ING systems animals were kept in continuous grazing with variable stocking rate aiming a forage allowance of 13 kg of dry matter per 100 kg of body weight. The forage allowance was controlled by adjusting the stocking rate using regulators (Mott & Lucas, 1952). Stocking rate was adjusted monthly, at the same time of forage mass evaluation and forage accumulation rate measurements. On NG and ING systems, the average forage mass and height were respectively 1802 kg DM/ha and 8.9 cm. Pasture data and management are detailed presented in (Devincenzi et al., 2012). On SP system, animals were kept in a strip stocking. The animals started grazing when sorghum sward reached 90 cm height on average and the stocking rate was adjusted to demote the height until 30 cm in a one-week period.

Animals were monthly weighed with a 12 hours period of solid and liquid fasting. At weighing procedures, to accompany fat deposition on the animals, it was taken ultrasonic measurements of fat thickness (FT) between the 12th and 13th rib. Production and acquisition of ultrasonic images were obtained by a main unit – eco camera Aloka SSD 500 V (Eletro Medicina Berger, Ltda) – equipped with a linear transducer UST 5049 with 3.5MHz frequency and 17.2 cm length. To allow comparisons between

treatments, animals were slaughtered in the same degree of physiological maturity.

Three millimeters of fat thickness (FT) was adopted as slaughter criteria.

2.2. Floristic evaluation

At the end of the fattening phase, which is the period of highest body fat deposition on the animals, it was proceeded the floristic evaluation in the natural grasslands-based production systems (NG and ING) using Botanal method (Tothill, 1978). This evaluation indicated the species which had most contribution in the forage mass (Table 1). Although *Lotus corniculattus* was spread on ING treatment, its contribution on herbage mass was not representative.

2.3. Feed collection and analysis

Forage samples were collected one day before each slaughter (06/09/2010 for SP treatment, 10/21/2010 for ING treatment, and 11/29/2010 for NG treatment). Two trained assessors collected forage samples following, observing and simulating the intake behavior of two animals during two meals (morning and afternoon). Each assessor followed one animal. Approximately 250 g of fresh forage was manually collected observing the species and plant parts grazed by the each animal, according to Moraes et al., 2005.

2.4. Slaughter procedures

Slaughtering procedures were carried out at a commercial slaughterhouse, in the city of Bagé, Rio Grande do Sul state (SIF 232) on 06/10/2010 for SP group (n=9), 10/22/2010 for ING group (n=18), and 11/30/2010 and NG group (n=16). Slaughter followed the slaughterhouse routine and carcasses were evaluated according to the Brazilian

carcasses grading system Ordinance 612/1989 (Brasil, 1989).

2.5 Meat sampling

A portion of the *Longissimus* muscle (LM) from the right side of each carcass was taken between the 12th and 13th rib after 24h chilling. The portions were packed in permeable plastic, then in brown paper, indentified and then frozen in a domestic freezer at -18 ºC until the analysis. A 1-cm-thick sample were taken off from the frozen portion. Samples were arranged on a tray covered with permeable plastic thawed in a refrigerator at 1.5-5 ºC for about 20 hours. After that, bones and subcutaneous fat were removed and the meat minced in a multiprocessor, freeze dried and vacuum packed.

2.6 Lipid and fatty acid analysis

For lipid analysis, the freeze dried samples were ground in liquid nitrogen in a micromower model M20mill (IKA-Werke, Stoaufen, Germany) to produce a fine and homogenous powder. Total lipids were extracted to the method described by Bligh & Dyer (1959). Briefly, 1.5 g of freeze dryed sample was extracted with a solution of chloroform/methanol/destilated water in a 5/10/4 v/v/v proportion. After the evaporation of the solvent under vacuum, total lipid were determinate by gravimetry.

Fatty acids were esterifies using Hartman & Lago (1973) methodology. The fatty acids methyl esters were analyzed using a Shimadzu GC 2010 plus gas chromatograph with a capillary column (RT-2560, 100m length, 0.25mm internal diameter and 0.2μm film thickness) with H_2 as gas vector. The chromatographic conditions were as follows: the oven temperature was set at 100C °C for 4min, then ramped from at 3°C/min to 240°C follow by 12 minutes at 240°. Vector gas pressure was kept at 247.9 kPa. Fatty acid

methyl esters were identified by comparing the retention times with a standard (47885-U Supelco® 37 Component FAME Mix, vaccenic and conjugated linoleic acid -CLA standards). Retention times and areas were recorded and integrated by the software GC SOLUTION, VERSÃO 2.3. Results were expressed as a percentage of the total peak areas of the identified fatty acid methyl esters.

The Atherogenic Index (AI) was calculated from the data of the fatty acid composition. The AI was obtained by following equation:

$$AI= (C12:0+4*C14:0+ C16:0)/(\sum MUFA+\sum n\text{-}6 +\sum n\text{-}3),$$

2.7 Statistical analysis

Data were tested for its normality by Shapiro-Wilk Test and underwent to analysis of variance to test the effect of the treatments using proc mixed of SAS package considering the production system as fixed effect and individual animals as random effect. Differences between treatments were tested by Tukey at 5% of significance.

3. Results and discussion

3.1 Fatty acid profile of the offered feeds

Sorghum forage (SP) presented high contents of saturated fatty acids (SFA), which must be due to the important contribution of palmitic acid, C16:0, on its fatty acid profile (Table 2). This is in line with the statement from O´Kelly & Reich (1976), which showed that tropical grasses present C16:0 as mainly fatty acid. The contribution of C16:0 in SP was 65.5% higher than in ING and 40.5 % higher than in NG. Pastures from ING treatment stood out for its high levels of C18:3 *n*-3, the alpha-linolenic acid. The proportion of C18:3 *n*-3 (9c, 12c, 15c) in ING was 80.6% higher than in SP, and

84.94% higher than in NG pastures. Pastures of ING production system were oversown with *Lolium multiflorum* ryegrass, a winter grass, which is rich in C18:3 *n*-3 (Bauchart, et al., 1984, Glasser et al., 2013) and as consequence, the sum of *n*-3 family fatty acids was very expressive on ING. The pasture from NG production system presented high proportions of C18:3 (9t 12c 15c), an isomer of linolenic acid. The Natural Grasslands in the geographic region of Campanha of Rio Grande do Sul province are characterized by its richness on winter grasses, as *Piptochaetium stipoides*, *Piptochaetium ruprechtianum, Stipa setigera*, *Stipa papposa* and *Briza minor* (Boldrini, 1997). There are a very small number of studies describing fatty acid profile of natural grassland at Rio Grande do Sul Brazil. Freitas (2010), studying on the same geographical region, found very similar values of C18:3 *n*-3 between a fertilized natural grassland (39.6% of total indentified fatty acids) and improved natural grassland (41.1% of total indentified fatty acids), while for NG, C18:3*n*-3 contents represented 35.8% of total identified fatty acids, however, the author did not identified other isomers of linolenic acid C18:3 *n*-3.

3.2. Animal performance values and characteristics at slaughter

Growth performances data, mean body weight at slaughter and carcass characteristics are presented on Table 3. No differences were found between production systems for fat thickness at slaughter (P=0.4014), indicating that the animals were slaughtered at the same physiological maturity. On the other hand, animals from SP were slaughter at lower weight than the animals from NG and ING (p=0.0023), as well they had higher average daily gain (p<0.001). Natural Grasslands and Improved Natural Grassland are mostly composed by subtropical species which, besides presenting elevated nutritional quality (Ferreira et al., 2009), are less productive than Sorghum, a tropical C4 grass

characterized by being a very productive pasture. The higher productivity of sorghum pasture and also its regular bromatological characteristics allowed to obtain higher gains in a short period (Restle et al., 1996; Muehlmann et al., 1997; Devincenzi et al., 2012). However, as an annual pasture, the utilization period of the pasture was limited by its cycle, so it leads us to slaughter younger animals (-5 months) and to obtain lighter carcasses for steers finished on SP system.

3.3. Intramuscular fatty acid composition

Total lipids and the intramuscular fatty acid composition of the *Longisssimus* muscle (LM) of steers are presented on Table 4.

Mean value of total lipid content was 2.11% (s.e.m=0.22) and there was no effect of treatment (p=0.0837) which confirm, once again, that animals were slaughtered at the same fattening point on all production systems and differences between fatty acid compositions must be from the production system. In our study the mean fat content (2.11%) can be considered low when compared to values found by Freitas et al., 2014, who finished Hereford steers in ryegrass pastures and obtained mean values of 3.7% of fat content. On the other hand, our results can be considered in line with the study of Rossatto et al., 2010, who found values of 2.99% for Angus steers grazing a Bachiaria pasture in southeast Brazil and those from Muchenje et al., 2009, who found values of 1.18% of fat content for Angus steers grazing Natural Pastures on South Africa in a low input beef production.

In ruminants, the relationship between consumed FA and their recovery on animal tissue is more complex than in monogastric animals due to the biohydrogenation (Jouany et al., 2007), however, diet is the main factor on modulate FA profile on animal

products (Scollan et al., 2006).

The central goal of this study was to describe the FA profile of meats from different production systems, which is clearly coupled to diet. However, we do not expect to make directly relationships between diet FA composition and meat FA composition manly because in our experimental scenario several sources of variation took place, as the individual selective behavior and individual metabolism of FA in each animal, which were not monitored. However knowing the FA profile of this vegetation can give us tools to improve the discussion and to make new hypotheses on future works. There was an effect of the production system for both miristic (C14:0) and palmitic acid (C16:0) on LM. For C14:0 concentrations, the values were found to be higher for SP than for NG and ING systems (p=0.0067). The proportion of C16:0 was higher on LM of SP steers than for ING ones (p=0.037), being the values for NG group, intermediates. Miristic acid (C14:0) and palmitic acid (C16:0) are recognized by its atherogenic potential (Keys, Anderson & Grande, 1965). These fatty acids have a saturated chain and they are not susceptible to eventual modifications in the rumen compartment, so our results could be partially explained by the feed composition, once the SP and NG pastures were richer in C16:0 (31.68 ± 0.615 for SP and 22.55 ± 1.891 for NG) than ING pastures (19.14 ± 0.839).The proportions of C18:0 on LM were not affected by the production system (P= 0.4910). Means values were 18.54% (± 2.236) of total identifed FA, being in line with previous values reported for pasture finished animals (Freitas et al., 2014, Muchenje et al., 2009). Besides being a saturated fatty acid, C18:0 is reported as presenting neutral effects for human health, or even being beneficial, because it is converted on C18:1on animal tissue (Bauchart et al., 2010; Huang et al., 2011)

There was an effect of the of production system on LM total saturated fatty acids (SFA)

(P= 0.0231). The proportions of SFA were higher on SP when compared to ING, being NG meats intermediates. The C16:0 had the most important contribution on SFA amounts (>53%), followed by C18:0, which did not suffer effect of the treatments.

There was an effect of the production systems for the total monounsaturated fatty acids (MUFA) content on LM (p=0.036) being higher for ING compared to NG and SP treatments. The oleic acid (C18:1n-9c) was the major component of the total lipid content on LM (39,41% ±2.746) of total identified FA). The ING group tended to present higher amounts of oleic acid (C18:1n-9c) compared to NG and SP (p=0.057), probably explaining the higher MUFA contents for ING group.

The total polyunsaturated fatty acids (PUFA) proportions on LM were affected by the production systems (p=0.002). It was observed higher proportions of PUFA on the NG and ING groups on SP. The most part of PUFA was composed by linoleic acid (C18:2n-6c) (40.2 to 46.9%), which was found in higher proportion on LM of NG steers. The dietary composition on C18:2 n-6c was rather similar between foodstuffs on this study, and moreover, this FA is prone to undergo to several transformations on rumen. So, a possible clue for the higher proportions of C18:2n-6c found on LM of NG group, could be the elevated botanical diversity on NG pastures, which according to Lourenço et al., 2007, could induce changes on rumen bacterial population (Lourenço et al, 2007), and lead to different isomerization rates of C18:2n-6c.

The production systems implied on different proportions of linolenic acid on meat. Linolenic acid (C18:3 n-3 9c12c15c) was higher on LM of NG and ING groups, than on SP treatment (P<0.001). The same effect was found for the long chain polyunsaturated fatty acids (C20:5 n-3, EPA, p=0.008; C22:2 n-6, p=0.006 and C22:5 n-3, DPA, p=0.005) and consequently for total PUFA (p=0.020) and total n-3 fatty acids

(p=0.002). In animal tissue, the linolenic acid is converted by dessaturation and elongation process to long chain polyunsaturated fatty acids, mainly EPA and DHA. High amounts of linolenic acid, EPA and DHA are very desirable on human´s diets. They are essential fatty acids, so they must be obtained from diet. Their beneficial effects are mainly related to the permeability of brain cell membranes (Martin et al., 2006) and protective effects against vascular diseases.

The most efficient way to improve linolenic acid and all the others long chain fatty acids in meat products is by improving the amounts of linolenic acid on diet (Raes et al., 2004; Ribeiro et al., 2011). In our study, the amounts of alpha-linolenic acid C18:3 n-3 c9, c12, c15 on forrage were numerically higher for forage of ING (56.87% of total indentified FA, sd= ±1.181) than for NG (30.75% of total identified FA, sd= ±0.092) and SP (31.49 ± % of total FA, sd=2.497). This was an expected result, once ING were oversown with Ryegrass (*Lolium multiflorum*. Lam), a winter specie, and it represented about 1/3 of the total available forage mass at the last month of the steers on this treatment. Winter species are prone to have higher content of C18: 3 n-3 (c9, c12,c15) (Glasser et al., 2013). Besides that, the nitrogen input performed on pasture of this feeding system (100kg of Urea/ha) could also improve the levels of C18:3n-3 on forages on ING system (Glasser et al., 2013). Our study found similar values for the pastures described by Freitas et al., 2014, which worked on the same geografical region of the present study. Lourenço et al. (2007) also found higher values of linolenic acid for pastures with high composition of ryegrass than for high diverse pastures.

On the other hand, pastures from NG and SP systems were similar on C18:3n3 (9c12c15c) proportions. Surprisingly the LM did not behavior as the same way, being the meat composition from NG similar to those from ING being richer on C18:3n3

(9c12c15c). The pastures of NG systems presented high amounts of C18:3 9t, 12c, 15c, another isomer of linolenic acid. Maybe the composition of NG pastures, which are relatively riches in native C3 species, could be associated to the proportions of C18:3n-3 (9c12c15c) found on meats from this NG treatment. However, in this sense it remains the question if C18:3 (9t, 12c, 15c) from the diet could be converted to C18:3n3 (9c12c15c) on animal tissue.

The sum of the proportions of n-3 and n-6 fat acids on LM were affected by the production system (P>0.05). For the fatty acids from n-6 family, higher levels were found on NG group than on ING and SP (p<0.001), this result can be explained by the higher participation of C18:2n-6 found on LM of NG system. Nevertheless, concerning the fatty acids from n-3 family, higher amounts of this FA were verified on NG and ING than on SP group (p=0.002). The proportion of n-3 FA on meats from production systems based on natural grassland was 42.2% higher than on SP.

The n-6/n-3 ratio were lower on LM for ING group (p<0.001). Mean values were 1.11:1, for ING and 1.4:1, for NG and on SP. It is important to underline that for the all productions systems evaluated on our study, the LM presented n-6/n-3 ratios that were below from the 5:1 more recently prescribed by the German, Austrian and Swiss Society of Nutrition (DACH, 2000).

The CLA proportions were not affected by the production system (p= 0.067). The CLA (mainly C18:2 cis9, trans11) and other isomers, also known by rumenic acid is found in small amounts in ruminants products, and its beneficial properties as anticarcinogenic, antidiabetic and as promoting fat weight loss are largely related in literature (Pariza et al., 2009). Usually, the feeding systems have a high impact on CLA content, especially when comparing concentrate to pasture feeds (De La Torre et al.,2006). The CLA is an

intermediate of the ruminal biohydrogenation of C18:2n-6, apparently, C18:2n-6 contents on the forages were very similar between the studied feeding systems, so it could be reflected on the similar CLA contents found between the meats. The atherogenic index (AI) was higher on LM of SP than for ING (p=0.0021), and NG meats, presented intermediate values. The AI indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated - monounsaturated fatty acids (MUFA) and polyunsaturated from n-6 and n-3 families, the first being considered pro-atherogenic (contributing the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, there- by preventing the appearance of micro and macro coronary diseases) Ulbritch & Southgate, 1991. Olive oil, for example, presents an AI of 0.14, while, coconut oil presents an AI of 13.63 (Ulbritch & Southgate, 1991). In our study, the values were in line to those found by Russo et al., 1995 for organic beef and to those found by Nfor et al., 2014 for Zebu cattle from central african region. It is important to highlight that the values for AI found for beef in our study are even lower to those found by Garaffo et al., 2011 for tuna species.

4. Conclusions

The most important findings of this study can be summarized as follows: Different production systems based in diverse pasture compositions are able to promote beef with distinguish fatty acid profiles. Meat of steers finished grazing Natural grassland and Improved natural grasslands were poorest in C14:0 and richer on n-3 polyunsaturated fat acids than meats from steers grazing Sorghum pasture. Finishing steers on

production systems based on improved natural grasslands promote meat with a more beneficial fatty acid profile to human health.

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Table 1: Five mainly botanical species and its contribution on total herbage mass (%) obtained by Botanal evaluation.

Natural Grassland	Improved Natural Grassland
Coelorachis selloana (20.2%)	*Lolium multiflorum* (25.6%)
Paspalum notatum (14.1%)	*Paspalum notatum* (22.4%)
Paspalum dilatatum (9.05%)	*Piptochaetium stipoides* (13.9%)
Piptochaetium stipoides (8.6%)	*Axonopus affinis* (5.2%)
Botriochloa laguroides (7.1%)	*Paspalum ditatatum* (4.2%).

Table 2: Centesimal composition of fatty acid (% of total identified FAME[1]) on forage of three different feeding systems.

	Natural Grassland [a]	Improved Natural Grassland [b]	Sorghum pasture[c]
C14:0	2.61 ± 0.279	0.76 ± 0.017	1.92 ± 0.228
C16:0	22,55 ± 1.891	19.14 ± 0.839	31.68 ± 0.615
C18:0	3.77 ± 0.044	2.33 ± 0.152	5.02 ± 0.298
C18:1delta 9c	4.64 ± 0.388	1.56 ± 0.167	2.60 ± 0.340
C18:2*n*-6c	13.23 ± 1.018	13.54 ± 1,894	12.29 ± 0.293
C18:3 9t12c15c	20.03 ± 3.911	3.83 ± 0.920	10.13 ± 0.732
C18:3 *n*-3 (9c12c15c)	30.75 ± 0.092	56.87 ± 1.181	31.49 ± 2.497
C20:4 *n*-6	0.95 ± 0.157	0.86 ± 0.347	1.25 ± 0.340
C24:0	1.47 ± 0.312	1.10 ± 0.317	3.68 ± 0.883
SFA[1]	30.40 ± 2.438	23.33 ± 0.387	42.30 ± 0.793
MUFA[2]	4.64 ± 0.388	1.56 ± 0.167	2.60 ± 0.340
PUFA[3]	51.73 ± 3.845	61.57 ± 2.45	42.87 ± 1.426
n-6 [4]	14.18 ± 1.176	14.40 ± 1.55	13.48 ± 0.632
n-3 [5]	30.75 ± 0.092	56.87 ± 1.181	31.49 ± 2.497
n-6/*n*-3	0.46 ± 0.040	0.25 ± 0.032	0.43 ± 0.054

[1] FAME= Fatty acids methyl esters
[a] Natural Grassland of Pampa Biome.
[b] Improved Natural Grassland with inputs of DAP + Urea and oversown with *Lolium multiflorum* Lam and *Lotus corniculatus.*
[c] Sorghum pasture (*Sorghum bicolor* × *Sorghum sudanense*)
[1] SFA = Σ(C12:0; C14:0; C16:0; C18:0; C24:0).
[2] MUFA cis =(C18:1 *n*-9 cis)
[3] PUFA trans = Σ(C18:2*n*-6 cis;C18:3 9cis 12 trans, 15 trans; C18:3 *n*-3(9 cis 12 cis 15 cis); C18:2cis 9 trans11; C20:4 *n*-6).
[4] *n*-6= Σ (C18:2*n*-6 cis; C20:4 *n*-6)
[5] *n*-3= Σ (C18:3 *n*-3 9 cis 12 cis 15 cis)

Table 3: Weight at slaughter (kg BW$^{(1)}$), average daily gain (ADG - kgBW$^{(1)}$/day), Age at slaughter (days) and fat thickness (mm) of steers finished on three different feed systems.

	Natural Grassland [a] (n=16)	Improved Natural Grassland [b] (n=18)	Sorghum pasture[c] (n=8)	p-value	SEM
Weight at slaughter (kg BW)	514,8 a	496,9 a	458,1 b	0,0023	6,15
ADG (kgBW/day)	0,466 b	0,491 b	0,833 a	<0,00011	0,02
Age at slaughter (days)	1012 a	955 a	860 b	0.0009	17.32
Fat thickness (mm)	1,9	2,6	2,1	0,4014	0,22

Means with unlike superscripts differ at the 5% probability level
[1] BW=Body weight
[a]Angus steers grazing for 364 days on Natural Grassland of Pampa Biome.
[b]Angus steers grazing for 325 days on Improved Natural Grassland with inputs of DAP + Urea and oversown with *Lolium multiflorum* Lam and *Lotus corniculatus.*
[c]Angus steers grazing for 128 days on a Sorghum pasture (*Sorghum bicolor* × *Sorghum sudanense*)

Table 4: Total fat content (%) and fatty acid centesimal composition (% of indentified total FAME[(1)]) ± standard derivation on *Longissimus* muscle of steers finished in three different feeding systems.

	Natural Grassland (n=16)	Improved Natural Grassland (n=18)	Sorghum pasture (n=8)	p-value
Total lipid content	2.03 ± 0.179	1.82 ± 0.142	2.53 ± 0.321	0.0837
C14:0	1.48 b ± 0.201	1.40 b ± 0.225	1.75 a ± 0.101	0.0067
C14:1	0.22 ± 0.069	0.22 ± 0.053	0.28 ± 0.108	0.1899
C16:0	25.49 ab ± 1.188	24.62 b ± 0.9752	26.35 a ± 1.777	0.0037
C16:1	2.30 ± 0.438	2.52 ± 0.427	2.46 ± 0.423	0.370
C18:0	18.72 ± 2.429	18.10 ± 2.058	19.20 ± 2.329	0.4910
C18:1*n*-9t	0.09 ± 0.014	0.08 ± 0.006	0.12 ± 0.021	0.4922
C18:1t11	2.88 ± 0.501	2.67 ± 0.384	2.73 ± 0.282	0.3637
C18:1*n*-9c	38.37 ± 2.721	40.58 ± 2.725	38.73 ± 1.962	0.0565
C18:1c11	1.34 ± 0.195	1.43 ± 0.184	1.26 ± 0.114	0.0871
C18:2*n*-6c	2.95 a ± 0.559	2.31 b ± 0.402	2.05 b ± 0.446	0.0003
C18:3 *n*-9c12t15c	0.09 ± 0.012	0.11 ± 0.006	0.09 ± 0.0167	0.3898
C18:3*n*-3 (9c12c15c)	0.93 a ± 0.152	0.99 a ± 0.202	0.70 b ± 0.120	0.0014
C18:2 c9t11 + 18:1 t9c11	0.58 ± 0.051	0.57 ± 0.061	0.53 ± 0.055	0.0872
C20:4*n*-6	0.14 ab ± 0.026	0.15 a ± 0.034	0.10 b ± 0.042	0.0079
C22:2*n*-6	0.11 a ± 0.025	0.12 a ± 0.035	0.08 b ± 0.022	0.0057
C20:5*n*-3	0.48 a ± 0.112	0.49 a ± 0.012	0.33 b ± 0.091	0.0075
C22:5*n*-3	0.80 a ± 0.175	0.80 a ± 0.177	0.55 b ± 0.137	0.0051
C22:6*n*-3	0.06 ± 0.016	0.07 ± 0.025	0.05 ± 0.014	0.0577
SFA	47.38 ab ± 3.117	45.65 b ± 2.604	48.97 a ± 1.881	0.0231
MUFA	46.25 b ± 2.689	48.59 a ± 2.702	46.34 b ± 2.044	0.0393
PUFA	6.29 a ± 1.057	5.74 a ± 0.904	4.57 b ± 0.849	0.0019
n-6/*n*-3	1.40 a ± 0.079	1.10 b ± 0.132	1.36 a ± 0.072	<.0001
n-6	3.21 a ± 0.595	2.59 b ± 0.448	2.23 b ± 0.485	0.0004
n-3	2.29 a ± 0.432	2.37 a ± 0.481	1.64 b ± 0.351	0.0020
CLA	0.58 ± 0.051	0.57 ± 0.061	0.53 ± 0.055	0.0872
AI	0.64 ab ± 0.064	0.58 b ± 0.052	0.66 ± 0.050	0.0021

Means with unlike superscripts differ at the 5% probability level
[(a)]Angus steers grazing for 364 days on Natural Grassland of Pampa Biome.
[(b)]Angus steers grazing for 325 days on Improved Natural Grassland with inputs of DAP + Urea and oversown with *Lolium multiflorum* Lam and *Lotus corniculatus.*
[(c)]Angus steers grazing for 128 days on a Sorghum pasture (*Sorghum bicolor* × *Sorghum sudanense*)
[(1)]FAME= Fatty acids methyl esters
[(2)]SFA = Σ(C12:0; C14:0; C15:0; C16:0; C17:0; C18:0;C20:0; C22:0).
[(3)] MUFA cis = Σ(C14:1 ; C16:1T ; C16:1 ; C18:1 *n*-9 trans ; C18:1 trans 11 ;C18:1 C18:1 *n*-9 cis; C20:1 *n*-9; C22:1T)
[(4)] PUFA trans = Σ(C18:2*n*-6 cis;C18:3 9cis 12 trans, 15 trans; C18:3 *n*-3(9 cis 12 cis 15 cis); C18:2cis 9 trans11; C20:3*n*-3; C20:4 *n*-6; C22:2 *n*-6 ; C20:5 *n*-3; C22:4; C22:5*n*-6; C22:5 *n*-3; C 22:6 *n*-3).
[(5)] *n*-6= Σ (C18:2*n*-6 cis; C20:4 *n*-6; C22:2 *n*-6 ; C22:5*n*-6)
[(6)] *n*-3= Σ (C18:3 *n*-3; C20:3*n*-3; C20:5 *n*-3; C22:5 *n*-3; C 22:6 *n*-3)
[(7)]CLA = Conjugated linolenic acid (18:2 cis 9 trans 11)
[(8)] AI=(C12:0+4*C14:0+ C16:0)/(ΣMUFA+Σn-6 +Σn-3
)

6. CAPÍTULO VI

6.1 CONSIDERAÇÕES FINAIS

A qualidade de um produto cárneo, depende tanto da qualidade intrínseca e objetiva do produto (cor, sabor, textura, composição química, etc...) como também das características extrínsecas e preferências subjetivas expressas pelos consumidores. Com relação as características extrínsecas, os consumidores de carne aportam uma crescente importância para a preservação dos recursos naturais, redução de insumos, respeito ao ambiente e bem-estar animal. A pesquisa tem mostrado que sistemas de produção pecuários extensivos podem ser eficientes do ponto de vista de utilização de energia e capazes de mitigar a emissão de gases de efeito estufa, sendo uma alternativa o atual modelo de produção. Contudo, a qualidade intrínseca do produto, não deve ser comprometida, sob pena de desagradar ao consumidor, visto que alguns atributos de qualidade da carne ainda são fundamentais.

Alguns estudos demonstram que elementos de comunicação tais como selos de certificação e marcas relativos ao modo de produção ou de sua origem podem modificar a apreciação sensorial dos produtos pelos consumidores. Por outro lado, esses sinais de qualidade podem confundir o consumidor. Um exemplo são os produtos oriundos da agricultura orgânica (AB) na França. As normas técnicas para a produção desses produtos garante o modo de produção com respeito ao meio ambiente e aos animais, e sem a utilização de insumos químicos, mas não faz nenhuma menção de garantia de qualidade intrínseca do produto. Contudo, 91% dos consumidores que escolhem produtos com o selo AB o fazem visando garantir aspectos relativos à saúde e/ou pela qualidade ou sabor dos produtos (Agence Bio 2014), estando portanto, sujeitos a ter desapontamentos com relação a esses aspectos.

A União Européia vem abordando a problemática da qualidade dos produtos em sistemas de produção com baixo aporte de insumos, um exemplo é o projeto de pesquisa Low Input Breeds. No Brasil, tecnologias para a implantação de sistemas de produção com baixo impacto ambiental tem sido estudados e colocados em prática pelo programa ABC (Agricultura de baixo carbono) através dos SIPAS (Sistemas Integrados de Produção Agropecuária), porém ainda com pouca ênfase na qualidade do produto.

O desenvolvimento de uma parte desse estudo foi realizado na França, país com o terceiro rebanho ovino da união europeia. O consumo de carne de cordeiro na França é de aproximadamente, 3,6 kg/por habitante por ano, um valor que pode ser considerado baixo. Além disso, os franceses são habituados a consumir animais terminados em confinamento, portanto a carne de animais terminados em pastagens pode ter baixa aceitação sensorial. A

realização de um experimento de dose resposta com forragens frescas mimetizando níveis de leguminosas em uma pastagem de gramíneas foi difícil de se colocar em prática. Primeiramente os animais foram alojados em gaiolas individuais de modo a se determinar o consumo exato de gramínea e leguminosa. No entanto, problemas relativos ao comportamento ingestivo começaram a ocorrer, pois negligenciu-se uma das partes mais importantes do processo de consumo de pasto, a seleção da dieta. Além disso, fornecer forragem fresca cortada diariamente para 36 animais era extremamente laborioso para a equipe executora. Dessa forma, a melhor solução encontrada foi deixar os animais pastejando nas parcelas de gramínea e suplementar com alfafa fresca. Assim estimou-se o consumo voluntário de cada grupo de animais e ofertou-se as proporções de alfafa correspondentes aos tratamentos.

A sequência de trabalhos desenvolvidos na presente tese mostra que em sistemas com baixo aporte de insumos, utilizando difererentes níveis de alfafa na dieta de cordeiros, a concentração de escatol na gordura perirenal foi mais elevada para os animais que consumiram alfafa do que para os que estavam em um regime alimentar apenas com gramíneas.Também houve efeito dos níveis de alfafa na concentração de escatol na gordura perirenal, a qual aumentou até a proporção de 50% (nível médio) e surpreendentemente diminuiu no nível mais elevado de alfafa. Esse resultado pode estar ligado a variabilidade individual, que foi particularmente mais possibilitada no lote 50 (M) onde a quantidades de alfafa disponíveis permitiram maior expressão das preferências alimentares de cada animal. Há ainda que se considerar a variabilidade individual da produção ruminal de escatol, do metabolismo do escatol no fígado etc. Interessante notar que os valores da concentração de escatol obtidos, mesmo no tratamento apenas com gramíneas, já são acima do limiar sensorial descrito por alguns autores. Os resultados da análise sensorial são consistentes com os resultados de concentração de escatol na gordura perirenal, sugerindo que a partir de uma concentração 0,26 µg de escatol/g na gordura líquida ocorre estabilização da intensidade de percepção dos odores e flavours relacionados a este composto.

O aumento de oferta de alfafa aos cordeiros, possibilitou o incremento de ácido linolênico na carne dos mesmos, o que é positivo do ponto de vista da saúde humana, no entanto, também foi verificado o incremento do ácido palmítico, que possui efeito aterogênico e trombogênico em humanos, sugerindo que o exesso de leguminosas na dieta de animais em pastejo pode representar perdas no que se refere a saudabilidade do alimento.

Com relação a possibilidade de autentificar a carne oriunda sistemas de produção ricos em leguminosas, a metodologia da relação de isótopos estáveis de N (valor δ^{15}N) consistiu numa ferramenta eficaz. No entanto, deve ser combinada com outras metodologias, pois a alteração dos padrões de deposição dos isótopos deve ocorrer de forma similar se os animais forem alimentados com grãos de leguminosas, como farelo de soja, por exemplo.

Para o segundo contexto trabalhado, é importante destacar que o estado do Rio Grande do Sul é possuidor de um ambiente pastoril natural raro no mundo, para o qual a pecuária é a alternativa mais sustentável de utilização, além de possuir importância histórica e cultural. Ao longo de mais de 30 anos o Departamento de Plantas Forrageiras e Agrometreorologia vem desenvolvendo

pesquisas de manejo das pastagens naturais no estado do Rio Grande do Sul, demonstrando que esse recurso pode ser muito produtivo. O capítulo V pretendeu somar-se a esse conjunto de informações relativas aos sistemas de produção com base em pastagem natural, porém sob a ótica da qualidade do produto. Detaca-se que foram obtidos altos teores de ácidos graxos da família ômega-3, com destaque para o ácido eicosapentanóico (C20:5 *n*-3), que é considerado um ácido graxo funcional na carne dos animais terminados em pastagem natural e pastagem natural melhorada. Os dados da presente tese apontam que a produção de carnes com perfil lipídico bastante favorável pode ser obtida em pastagens naturais do bioma Pampa. Espera-se que esses resultados tenham utilidade prática no sentido de subsidiar iniciativas de valorização do produto com fins de conservação e utilização sustentável desse recurso forrageiro de alta importância ecológica .

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7. APÊNDICES

Apêndice 1. Idade ao abate, tempo de permanência no tratamento, consumo de alfafa, dactylys proporção de alfafa na dieta na fase 1 do experimento referente aos Capítulos II, III e IV. (Tratamento 0, 25, 50 e 75 correspondem a 0%, 25%, 50% and 75% de alfafa na dieta.

Animal (n°)	Tratamento	Idade ao abate (dias)	Tempo no tratamento (dias)	Consumo Alfafa fase 1 04/06 a 03/07 (kgMS/dia)	Consumo Dactilis fase 1 04/06 a 03/07 (kgMS/dia)	Proporção Alfafa fase-1
20415	0	169	88	0,000	0,699	0,000
20428	0	193	110	0,000	0,614	0,000
20462	0	169	89	0,000	0,672	0,000
20473	0	166	88	0,000	0,723	0,000
20484	0	190	110	0,000	0,660	0,000
20492	0	166	88	0,000	0,624	0,000
20499	0	189	112	0,000	0,650	0,000
20542	0	187	110	0,000	0,500	0,000
20437	25	170	91	0,199	0,454	0,304
20438	25	191	110	0,208	0,496	0,295
20445	25	167	88	0,206	0,530	0,280
20461	25	190	110	0,208	0,404	0,340
20485	25	190	110	0,085	0,493	**0,147**
20505	25	165	86	0,206	0,591	0,258
20537	25	164	88	0,183	0,500	0,268
20554	25	164	91	0,160	0,511	0,238
20555	25	185	110	0,203	0,362	0,360
20419	50	169	88	0,370	0,379	0,494
20443	50	167	88	0,394	0,406	0,492
20453	50	170	89	0,313	0,266	0,540
20467	50	190	110	0,410	0,377	0,521
20469	50	190	110	0,431	0,343	0,557
20471	50	166	88	0,402	0,372	0,520
20478	50	190	112	0,377	0,321	0,540
20527	50	188	110	0,346	0,338	0,506
20536	50	167	89	0,395	0,352	0,529
20417	75	193	110	0,503	0,176	0,741
20436	75	170	89	0,618	0,184	0,770
20454	75	167	88	0,505	0,163	0,756
20470	75	166	86	0,608	0,196	0,756
20487	75	190	110	0,584	0,202	0,743
20494	75	190	112	0,495	0,203	0,709
20496	75	169	89	0,570	0,192	0,748
20549	75	163	88	0,617	0,192	0,763

Apêndice 2. Entrada de dados para PA (peso ao abate), PCF(peso de carcaça fria) δ15N, E concentração em escatol referentes aos Capítulos II, III e IV. (Tratamento 0, 25, 50 e 75 correspondem a 0%, 25%, 50% and 75% de alfafa na dieta.

N° ANIMAL	Tratamento	PA	PCF	δ15N	ESCATOL (µg/g)
20415	0	37,4	12,48	5,8	0,00
20428	0	31,6	12	6,1	0,23
20462	0	33,1	11,3	6,3	0,43
20473	0	39,1	14,62	6,1	0,10
20484	0	39,4	13,82	6,1	0,18
20492	0	37,2	13,66	6,3	0,17
20499	0	37,1	12,72	5,8	0,08
20542	0	34,6	12,3	6,1	0,07
20437	25	37,8	13,76	5,8	0,51
20438	25	35,6	12,62	5,9	0,44
20445	25	38,1	13,48	5,8	0,23
20461	25	34,8	12,68	5,8	0,27
20485	25	34,7	11,38	6,1	0,11
20505	25	37,6	13,22	6,0	0,17
20537	25	36,6	14,24	5,5	0,12
20554	25	36,4	14,22	5,6	0,20
20555	25	37,3	14	5,4	0,27
20419	50	46,9	18,86	5,6	0,21
20443	50	44,2	18,38	5,4	0,16
20453	50	40	14,48	5,8	0,60
20467	50	38,1	15,3	5,3	0,82
20469	50	40,8	14,58	5,1	0,29
20471	50	44,4	17,64	5,8	0,19
20478	50	40,9	15,28	5,5	0,17
20527	50	34	13,16	5,2	0,20
20536	50	41,2	16,94	5,2	0,40
20417	75	39	15,16	5,2	0,15
20436	75	41,7	17,86	5,6	0,17
20454	75	43,3	17,66	5,4	0,34
20470	75	45	18,52	5,6	0,12
20487	75	43	16,6	5,2	0,44
20494	75	47,1	18,9	5,6	0,36
20496	75	42,1	16,54	5,3	0,22
20549	75	48,2	19,92	5,7	0,16

Apêndice 3. Entrada de dados para análise sensorial referentes ao Capítulo II. (Tratamento 0, 25, 50 e 75 correspondem a 0%, 25%, 50% and 75% de alfafa na dieta.

N° ANIMAL	Tratamento	Intensidade odor animal-parte magra	Intensidade flavour animal - parte magra	Intensidade odor animal-parte gorda	Intensidade flavour animal-parte gorda
20415	0	2,46	1,84	2,38	2,18
20428	0	1,88	1,79	1,77	1,94
20462	0	3,41	2,38	2,77	2,58
20473	0	2,10	1,89	2,50	2,31
20484	0	2,91	2,71	2,67	3,01
20492	0	2,33	1,81	2,04	2,03
20499	0	2,29	2,16	2,43	2,14
20542	0	2,40	2,26	1,89	2,14
20437	25	2,31	2,12	2,31	2,19
20438	25	3,41	3,17	2,95	3,11
20445	25	2,44	2,19	2,74	2,75
20461	25	2,55	2,66	2,14	2,62
20485	25	2,79	2,16	2,09	2,19
20505	25	2,95	2,81	2,06	2,56
20537	25	2,91	2,28	2,22	3,03
20554	25	2,10	1,96	2,03	2,17
20555	25	2,40	1,79	1,58	2,31
20419	50	3,04	2,12	2,95	2,91
20443	50	2,56	2,26	2,73	3,15
20453	50	2,36	2,19	2,35	2,16
20467	50	2,52	2,34	2,59	2,36
20469	50	2,80	2,73	2,68	2,57
20471	50	2,50	2,04	2,21	1,98
20478	50	2,69	2,97	2,48	2,72
20527	50	2,81	2,82	2,61	2,63
20536	50	3,01	2,45	2,72	2,93
20417	75	1,93	1,98	1,99	2,25
20436	75	1,78	1,86	1,92	2,00
20454	75	2,87	1,91	2,47	1,85
20470	75	2,91	2,65	2,83	2,93
20487	75	3,43	2,53	2,84	3,00
20494	75	3,17	2,74	2,53	2,77
20496	75	2,99	2,57	2,46	2,55
20549	75	3,63	2,90	2,93	3,10

Apêndice 4. Valores de análise bromatológica das forragens referentes aos Capítulos II, III, IV. (NDF: Neutral fiber detergent)

Amostra	Data de amostragem	Teor de Matéria Seca (%)	Proteína Bruta (mg/g)	NDF (%)
Dactilis	05/06/2012	19,60	224,83	66,71
Dactilis	12/06/2012	13,82	157,56	60,58
Dactilis	19/06/2012	16,89	193,90	59,53
Dactilis	26/06/2012	15,45	176,52	57,88
Dactilis	02/07/2012	14,96	169,11	59,91
Dactilis	11/07/2012	15,63	174,71	55,53
Dactilis	17/07/2012	14,49	162,39	56,18
Dactilis	27/07/2012	15,44	175,22	58,93
Dactilis	03/08/2012	14,36	163,68	60,75
Dactilis	10/08/2012	13,07	148,74	59,57
Dactilis	17/08/2012	12,10	136,34	59,04
Dactilis	24/08/2012	12,31	138,89	60,68
Dactilis	05/09/2012	11,59	128,72	59,42
Dactilis	17/09/2012	13,13	145,80	60,21
Dactilis	28/09/2012	13,65	150,63	62,92
Alfafa	05/06/2012	36,26	420,33	27,17
Alfafa	12/06/2012	30,90	363,37	33,05
Alfafa	19/06/2012	26,86	310,27	37,23
Alfafa	26/06/2012	21,80	247,78	43,01
Alfafa	02/07/2012	16,69	186,83	45,66
Alfafa	11/07/2012	25,34	283,54	43,18
Alfafa	17/07/2012	25,50	284,90	37,95
Alfafa	27/07/2012	20,28	226,60	44,84
Alfafa	03/08/2012	20,45	228,29	43,00
Alfafa	10/08/2012	23,57	261,80	38,20
Alfafa	17/08/2012	20,97	235,87	41,13
Alfafa	24/08/2012	21,37	241,18	34,85
Alfafa	05/09/2012	25,41	286,37	30,30
Alfafa	17/09/2012	21,67	244,16	36,00
Alfafa	28/09/2012	21,29	237,28	41,10

Apêndice 5. Nomenclatura dos ácidos graxos

Ácido Graxo	
Ácido Mirístico	C14:0
Ácido Miristelaídico	C14:1T
Ácido Mirístoleico	C14:1
Ácido Pentadecanóico	C15:0
Ácido Trans-10-Pentadecenóico	C15:1T
Ácido Cis-10-Pentadecenóico	C15:1
Ácido Palmítico	C16:0
Ácido Palmitelaídico	C16:1T
Ácido Palmitoleico	C16:1
Ácido Heptadecanóico	C17:0
Ácido Trans-10-Heptadecenóico	C17:1T
Ácido Cis-10-Heptadecenóico	C17:1
Ácido Esteárico	C18:0
Ácido Elaídico	C18:1n9t
Ácido Petroselainóico	C18:1T
Ácido Trans-11-Vacenênico	C18:1 t11
Ácido Petroswlinóico	C18:1
Ácido Oleíco	C18:1n9c
Ácido Cis-11-Vacênico	C18:1 c11
Ácido Nonadecanóico	C19:0
Ácido Linolelaídico	C18:2n6t
Ácido Trans-7-Nonadecenóico	C19:1 t7
Ácido Trans-10-Nonadecenóico	C19:1 t10
Ácido Linoleíco	C18:2n6c
Ácido Araquídico	C20:0
Ácido Trans-9-Trans-12-Trans-15 Linolênico	C18:3 9t12t15t
Ácido Trans-9-Trans-12-Cis-15 e Trans-9-Cis-12-Trans-15 Linolênico	C18:3 9t12t15c e 9t12c15t
Ácido Cis9-Trans-12-Trans-15 Linolênico (γ-Linolênico)	C18:3 9c12t15t
Ácido Cis-9-Cis-12-Trans-15 Linolênico	C18:3 9c12c15t
Ácido Cis-11-Eicosanóico	C20:1n9
Ácido Cis-9-Trans-12-Cis-15 Linolênico	C18:3 9c12t15c
Ácido Trans-9-Cis-12-Cis-15 Linolênico	C18:3 9t12c15c
Ácido Linolênico - (Cis-9-Cis-12-Cis-15)(α-Linolênico)	C18:3n3 (9c12c15c)
Ácido Heneicosanóico	C21:0
Ácido Cis-9-Trans-11-Octadecadienóico (CLA)	C18:2 c9t11 + 18:1 t9c11
Ácido Cis-10-Cis-12-Octadecadienóico (CLA)	C18:2 c10c12
Ácido Trans-9-Trans-11-Octadecadienóico (CLA)	C18:2 n6t9t11 + outros
Ácido Cis-11,14-Eicosadienóico	C20:2n9
Ácido Behênico	C22:0
Ácido Cis-8,11,14-Eicosatrienóico	C20:3n6
Ácido Brassídico	C22:1T
Ácido Eurúcico	C22:1n9
Ácido Cis-11,14,17-Eicosatrienóico	C20:3n3

Apêndice 6. Entrada de dados de perfil de ácidos graxos utilizadas no capítulo III; AGST=ácidos graxos saturados totais, AGMIc=ácidos graxos, C181d9c =Ácido oleico, monoinsaturados cis.

Trat	Rep	Animal	C12:0	C14:0	C16:0	C18:0	AGST	C181d9c	AGMIc
1	1	415	0,46	3,90	18,33	17,33	43,47	25,01	29,02
1	2	428	0,72	5,28	20,86	18,11	48,96	28,56	32,66
1	3	462	0,25	2,81	18,50	20,95	46,41	30,48	34,39
1	4	473	0,36	3,69	18,27	16,48	42,28	29,50	34,25
1	5	484	0,37	3,47	20,69	19,63	48,36	27,85	32,01
1	6	499	0,24	2,49	16,19	20,42	43,45	23,90	27,89
1	7	542	0,22	1,83	12,44	16,74	34,51	25,16	29,59
2	1	437	0,41	3,63	18,35	18,61	44,67	28,89	33,05
2	2	438	0,15	2,11	19,66	23,31	49,00	29,13	32,74
2	3	445	0,64	4,99	18,86	14,71	42,48	24,27	28,80
2	4	461	0,25	2,87	18,51	23,53	49,29	27,53	31,32
2	5	485	0,32	2,55	17,79	21,15	45,92	26,37	30,23
2	6	505	0,67	6,27	20,17	14,98	46,13	27,50	32,26
2	7	537	0,37	4,16	21,97	16,74	46,98	29,51	34,02
2	8	554	0,39	3,62	19,93	20,13	48,12	27,06	31,05
2	9	555	0,26	2,72	19,97	21,03	48,01	28,20	32,10
3	1	419	0,28	3,88	22,19	15,82	45,39	33,11	37,88
3	2	443	0,29	3,39	20,56	17,45	45,13	30,27	34,40
3	3	453	0,32	3,67	19,92	18,29	45,75	30,73	34,92
3	4	467	0,32	3,61	22,33	18,89	48,92	29,35	33,35
3	5	469	0,33	2,89	21,19	18,75	47,06	29,33	33,63
3	6	471	0,56	5,02	22,33	14,82	46,29	31,02	35,83
3	7	478	0,25	2,40	18,45	19,32	44,09	28,49	32,30
3	8	527	0,26	2,82	19,88	17,11	43,76	28,54	32,62
3	9	536	0,36	3,43	20,98	18,86	47,17	29,28	33,37
4	1	417	0,10	2,06	21,52	20,42	47,58	31,42	35,20
4	2	436	0,46	4,41	21,90	13,80	43,95	30,82	35,80
4	3	454	0,28	3,06	19,91	17,47	44,42	26,60	30,77
4	4	470	0,26	2,75	21,16	17,48	44,75	30,18	34,32
4	5	487	0,25	3,26	21,53	16,45	45,06	31,24	35,55
4	6	494	0,35	3,66	22,25	19,56	49,79	29,18	32,74
4	7	496	0,17	2,28	20,05	17,94	43,34	29,65	33,70
4	8	549	0,19	2,85	20,96	16,60	43,63	33,68	38,25

Apêndice 6. Continuação..

Trat	Rep	Animal	sC181t	sAGMlt	AGPIn6t	C1826cc	C204n6	n6	C183n3
1	1	415	2,69	3,04	1,09	8,80	5,53	16,73	1,93
1	2	428	4,33	4,78	0,94	4,78	2,35	8,49	1,55
1	3	462	5,70	6,04	0,97	3,70	1,92	7,03	1,31
1	4	473	3,26	3,62	0,79	7,30	4,28	13,05	1,68
1	5	484	5,83	6,33	1,08	4,70	1,79	7,98	1,72
1	6	499	5,17	5,50	0,52	7,04	5,00	13,32	2,21
1	7	542	2,64	2,94	0,35	9,46	10,01	21,47	1,98
2	1	437	3,32	3,68	0,54	6,33	3,57	11,07	1,78
2	2	438	3,93	4,29	0,86	3,75	1,80	6,80	2,10
2	3	445	1,92	2,22	1,25	9,49	6,24	17,96	1,91
2	4	461	5,31	5,70	1,05	3,74	1,65	6,76	2,23
2	5	485	5,29	5,79	0,92	5,32	2,97	9,71	2,58
2	6	505	2,60	2,96	0,81	6,55	4,12	12,12	1,53
2	7	537	4,05	4,45	0,87	5,15	1,84	8,22	1,90
2	8	554	4,25	4,67	0,97	4,76	2,70	8,88	2,00
2	9	555	4,72	5,22	0,87	4,38	2,16	7,79	2,06
3	1	419	3,53	3,94	1,12	4,41	1,96	7,96	1,42
3	2	443	3,77	4,09	0,82	6,24	2,84	10,44	1,88
3	3	453	3,47	3,90	0,78	5,25	2,61	9,15	1,96
3	4	467	4,72	5,08	1,02	4,07	1,57	6,94	2,01
3	5	469	5,60	6,03	1,26	4,31	1,57	7,46	1,75
3	6	471	2,64	3,05	0,50	5,33	2,50	8,81	1,73
3	7	478	5,97	6,34	1,01	5,54	2,17	9,12	2,55
3	8	527	4,71	5,21	1,09	5,01	2,87	9,41	2,43
3	9	536	3,95	4,30	1,03	5,19	2,16	8,78	2,09
4	1	417	4,33	4,66	1,11	3,26	1,16	5,67	2,47
4	2	436	3,78	4,20	1,19	5,50	2,41	9,54	2,09
4	3	454	4,41	4,82	0,96	7,59	2,76	11,84	2,77
4	4	470	3,68	3,99	1,28	5,41	2,37	9,46	2,52
4	5	487	4,83	5,17	0,84	4,44	2,17	7,79	1,84
4	6	494	4,20	4,57	1,03	4,34	1,54	7,20	1,97
4	7	496	3,03	3,36	1,12	5,73	3,22	10,77	2,62
4	8	549	3,29	3,64	1,17	4,94	2,19	8,64	1,86

Apêndice 6. Continuação..

Trat	Rep	Animal	C205n3	C225n3	C226n3	n3	9c11t	AGPI	n6n3
1	1	415	1,12	2,42	0,45	5,98	0,50	24,48	2,80
1	2	428	0,54	1,23	0,32	3,64	0,75	13,61	2,33
1	3	462	0,63	1,32	0,32	3,57	1,35	13,15	1,97
1	4	473	0,86	1,92	0,40	4,87	0,74	19,85	2,68
1	5	484	0,43	1,09	0,26	3,50	1,10	13,29	2,28
1	6	499	1,78	2,87	0,75	7,61	0,93	23,16	1,75
1	7	542	1,54	4,19	0,78	8,49	0,61	32,96	2,53
2	1	437	1,14	2,40	0,49	5,81	0,76	18,60	1,90
2	2	438	1,00	1,78	0,46	5,34	0,91	13,98	1,27
2	3	445	1,49	2,83	0,73	7,04	0,53	26,50	2,55
2	4	461	0,78	1,43	0,43	4,87	1,11	13,69	1,39
2	5	485	0,99	2,14	0,59	6,29	1,10	18,05	1,55
2	6	505	0,98	1,81	0,50	4,82	0,76	18,66	2,52
2	7	537	0,68	1,39	0,39	4,36	1,09	14,55	1,89
2	8	554	0,85	1,84	0,45	5,14	1,05	16,16	1,73
2	9	555	0,83	1,68	0,36	4,92	1,02	14,67	1,58
3	1	419	0,43	1,00	0,30	3,15	0,90	12,79	2,53
3	2	443	0,66	1,36	0,50	4,40	0,71	16,37	2,37
3	3	453	0,69	1,48	0,39	4,53	0,74	15,42	2,02
3	4	467	0,49	1,03	0,31	3,83	1,05	12,64	1,81
3	5	469	0,54	1,12	0,27	3,68	1,26	13,28	2,03
3	6	471	0,61	1,46	0,43	4,23	0,81	14,82	2,08
3	7	478	0,89	1,76	0,40	5,61	1,36	17,28	1,63
3	8	527	1,21	2,25	0,48	6,37	1,36	18,41	1,48
3	9	536	0,73	1,50	0,42	4,75	0,87	15,16	1,85
4	1	417	0,80	1,38	0,36	5,01	1,06	12,57	1,13
4	2	436	0,58	1,17	0,44	4,28	1,12	16,05	2,23
4	3	454	0,96	2,06	0,37	6,16	1,08	19,99	1,92
4	4	470	0,97	1,70	0,50	5,75	0,98	16,95	1,64
4	5	487	0,67	1,32	0,39	4,22	1,21	14,22	1,85
4	6	494	0,51	1,12	0,26	3,86	0,94	12,90	1,86
4	7	496	1,09	2,08	0,76	6,55	1,04	19,60	1,64
4	8	549	0,66	1,23	0,41	4,16	0,93	14,47	2,07

Apêndice 6. Continuação. LNALA= C182n6/C183n3; Lt= Lipídios Totais; AG= Ácidos Graxos.

Trat	Rep	Animal	LNALA	AGPI/AGS	C16C18	LT	AG
1	1	415	4,55	0,56	1,06	1,65	1,24
1	2	428	3,09	0,28	1,15	3,47	2,15
1	3	462	2,83	0,28	0,88	2,05	1,77
1	4	473	4,34	0,47	1,11	1,72	1,42
1	5	484	2,73	0,27	1,05	4,01	2,54
1	6	499	3,19	0,53	0,79	1,60	0,99
1	7	542	4,77	0,96	0,74	1,03	0,54
2	1	437	3,55	0,42	0,99	1,61	1,35
2	2	438	1,78	0,29	0,84	2,51	1,52
2	3	445	4,97	0,62	1,28	1,47	1,18
2	4	461	1,68	0,28	0,79	2,66	2,41
2	5	485	2,07	0,39	0,84	1,94	1,58
2	6	505	4,28	0,40	1,35	2,19	1,45
2	7	537	2,71	0,31	1,31	2,72	1,85
2	8	554	2,38	0,34	0,99	2,44	1,56
2	9	555	2,13	0,31	0,95	2,09	1,90
3	1	419	3,11	0,28	1,40	3,76	2,79
3	2	443	3,32	0,36	1,18	2,82	1,87
3	3	453	2,67	0,34	1,09	2,05	1,95
3	4	467	2,03	0,26	1,18	4,25	2,65
3	5	469	2,46	0,28	1,13	3,68	2,25
3	6	471	3,07	0,32	1,51	2,41	2,03
3	7	478	2,17	0,39	0,95	2,04	1,66
3	8	527	2,06	0,42	1,16	2,80	1,82
3	9	536	2,48	0,32	1,11	2,58	1,55
4	1	417	1,32	0,26	1,05	3,67	2,48
4	2	436	2,63	0,37	1,59	2,15	2,18
4	3	454	2,75	0,45	1,14	2,00	1,64
4	4	470	2,15	0,38	1,21	2,59	2,24
4	5	487	2,42	0,32	1,31	4,31	2,78
4	6	494	2,20	0,26	1,14	3,48	2,74
4	7	496	2,19	0,45	1,12	1,65	1,63
4	8	549	2,66	0,33	1,26	2,67	2,14

Apêndice 7. Entrada de dados de perfil de ácidos graxos das forragens.
LNALA= C182n6/C183n3, FA= fatty acids.

	July, 2012	Ago, 2012	Sept, 2012	July, 2012	Ago, 2012	Sept, 2012
	Dactyle	Dactyle	Dactyle	Alfafa	Alfafa	Alfafa
C14	0,72	0,82	0,67	0,39	0,70	0,74
C16	12,14	13,39	11,58	17,52	18,44	14,18
C18	2,82	1,59	1,46	2,27	3,95	2,36
∑AGS	17,81	18,90	16,63	23,00	26,18	20,23
C181d9c	2,97	1,54	1,17	1,43	3,24	1,19
∑AGMIc	5,95	3,60	3,54	5,37	4,21	3,04
C182n6cc	10,77	13,69	12,75	17,69	17,61	14,54
∑AGPIn6c	15,27	14,57	20,48	20,43	17,92	14,90
C183n3	59,44	61,27	57,65	50,14	50,64	60,81
∑AGPIn3	60,97	62,94	59,35	51,19	51,68	61,83
n6/n3	0,25	0,23	0,35	0,40	0,35	0,24
LA/LNA	0,18	0,22	0,22	0,35	0,35	0,24
AGPI/AGS	4,28	4,10	4,80	3,11	2,66	3,79
FA	0,92	0,79	0,8	0,59	0,68	0,91

Apêndice 8. Entrada de dados de perfil de ácidos graxos do capítulo V. LT= Lipídios totais.

Animal	Trat	Pot	REP	LT	C14:0	C14:1	C16:0	C16:1	C18:0
230561	1	1	1	1,26	1,54	0,18	26,27	2,10	19,94
230584	1	2	2	2,40	1,52	0,27	27,19	2,57	17,56
230652	1	2	3	2,33	1,52	0,13	25,66	1,69	23,48
230656	1	1	4	1,79	1,41	0,25	24,87	2,01	18,43
230662	1	1	5	1,33	1,42	0,16	25,08	2,30	17,23
230674	1	2	6	3,06	1,63	0,22	26,11	2,31	20,17
230680	1	2	7	1,66	1,71	0,18	26,89	1,92	20,91
230681	1	1	8	2,96	1,26	0,18	25,09	1,83	20,35
230682	1	1	9	1,63	1,16	0,18	23,50	1,96	19,69
230688	1	1	10	2,05	1,25	0,20	23,85	2,46	17,88
230693	1	2	11	1,60	1,44	0,22	25,51	2,38	17,59
230707	1	1	12	2,70	1,47	0,26	24,41	2,68	17,99
230710	1	2	13	3,13	1,59	0,19	25,95	2,18	20,52
230712	1	2	14	1,03	1,26	0,35	24,46	2,68	13,17
230719	1	2	15	1,47	1,98	0,38	27,51	3,44	15,93
230511	2	1	1	1,91	1,41	0,23	25,42	2,67	17,67
230552	2	1	2	2,32	1,61	0,20	25,27	2,31	21,38
230648	2	2	3	1,30	1,36	0,17	26,17	2,52	17,86
230650	2	2	4	0,93	1,40	0,22	24,73	2,66	17,10
230653	2	2	5	0,83	1,20	0,22	23,86	2,44	17,34
230665	2	1	6	1,60	1,09	0,29	22,63	2,80	14,91
230668	2	2	7	1,73	1,43	0,22	24,56	2,46	17,06
230669	2	2	8	3,26	1,53	0,20	24,56	2,03	20,26
230671	2	1	9	2,09	1,47	0,34	23,69	2,66	18,64
230673	2	1	10	2,09	1,33	0,20	23,68	1,97	19,92
230675	2	2	11	1,61	1,23	0,13	24,21	2,05	20,81
230687	2	1	12	1,60	1,97	0,22	24,97	3,23	19,92
230700	2	1	13	1,20	1,21	0,19	23,75	2,31	16,96
230702	2	2	14	2,53	1,26	0,25	24,38	2,39	16,35
230706	2	2	15	1,84	1,17	0,11	24,30	1,70	21,06
230711	2	1	16	2,44	1,50	0,22	24,84	3,19	14,65
230715	2	1	17	2,07	1,27	0,25	25,31	2,78	16,06
230726	2	2	18	1,41	1,80	0,26	26,78	3,14	17,92
230553	3	1	1	2,12	2,35	0,50	28,42	3,18	17,29
230575	3	1	2	2,32	1,65	0,28	26,79	2,31	17,37
230651	3	1	3	1,90	1,68	0,21	26,95	2,40	18,27
230664	3	1	4	2,05	1,69	0,16	24,94	1,70	24,53
230672	3	1	5	4,10	1,95	0,34	27,14	2,50	18,60
230676	3	1	6	2,07	1,61	0,24	25,60	2,20	19,67
230705	3	1	7	3,84	1,56	0,28	25,85	2,68	18,24
230717	3	1	8	1,80	1,50	0,18	25,15	2,26	19,65

Apêndice 8. continuação

Animal	Trat	Pot	REP	C18:1 t11	C18:1n9c	C18:1c11	C18:2n6c	C18:3n3	C18:2
230561	1	1	1	2,92	37,70	1,20	2,39	0,82	0,53
230584	1	2	2	2,38	38,60	1,27	2,79	0,85	0,50
230652	1	2	3	3,78	33,97	1,09	2,39	0,80	0,55
230656	1	1	4	2,67	39,41	1,34	3,27	0,99	0,56
230662	1	1	5	2,35	38,79	1,49	4,14	1,24	0,58
230674	1	2	6	3,42	36,96	1,23	2,44	0,78	0,60
230680	1	2	7	3,37	34,78	1,11	2,76	0,96	0,56
230681	1	1	8	2,89	36,28	1,20	3,59	1,12	0,53
230682	1	1	9	3,16	39,96	1,38	2,73	0,83	0,59
230688	1	1	10	2,52	43,04	1,28	2,14	0,69	0,59
230693	1	2	11	3,07	38,50	1,42	3,30	0,98	0,67
230707	1	1	12	2,70	39,11	1,37	3,35	1,12	0,61
230710	1	2	13	3,50	36,70	1,20	2,47	0,81	0,61
230712	1	2	14	2,04	44,28	1,78	3,46	1,01	0,67
230719	1	2	15	2,40	37,54	1,68	3,09	0,96	0,51
230511	2	1	1	2,70	40,32	1,48	2,29	0,90	0,56
230552	2	1	2	3,45	35,23	1,20	2,54	1,29	0,60
230648	2	2	3	2,76	39,25	1,48	2,52	0,96	0,50
230650	2	2	4	2,28	40,44	1,55	2,63	1,10	0,52
230653	2	2	5	2,39	40,48	1,47	2,96	1,30	0,58
230665	2	1	6	2,11	46,07	1,71	2,38	0,84	0,60
230668	2	2	7	2,49	43,30	1,41	1,72	0,74	0,61
230669	2	2	8	2,94	40,20	1,36	1,64	0,89	0,55
230671	2	1	9	2,86	42,00	1,28	1,69	0,74	0,64
230673	2	1	10	3,16	38,24	1,38	2,71	1,17	0,59
230675	2	2	11	3,16	38,45	1,31	2,32	0,95	0,57
230687	2	1	12	2,62	37,47	1,19	2,31	1,09	0,54
230700	2	1	13	2,94	41,07	1,57	2,92	1,35	0,69
230702	2	2	14	2,79	42,53	1,53	2,32	1,01	0,66
230706	2	2	15	2,72	39,66	1,33	2,50	0,74	0,45
230711	2	1	16	2,17	44,85	1,91	1,76	0,70	0,61
230715	2	1	17	2,27	43,06	1,34	2,06	0,94	0,55
230726	2	2	18	2,20	37,92	1,22	2,38	1,08	0,48
230553	3	1	1	2,69	37,39	1,18	1,86	0,60	0,55
230575	3	1	2	2,71	41,05	1,42	1,67	0,55	0,53
230651	3	1	3	2,44	39,61	1,37	2,11	0,75	0,46
230664	3	1	4	3,12	35,12	1,05	2,09	0,70	0,48
230672	3	1	5	2,65	38,98	1,28	1,67	0,63	0,53
230676	3	1	6	2,31	39,79	1,29	2,22	0,74	0,45
230705	3	1	7	2,99	40,47	1,26	1,78	0,65	0,60
230717	3	1	8	2,99	37,41	1,23	3,03	0,94	0,58

Apêndice 8. continuação

Animal	Trat	Pot	REP	C20:4n6	C22:2n6	C20:5n3	C22:5n3	C22:6n3	SFA
230561	1	1	1	0,12	0,08	0,39	0,73	0,06	49,53
230584	1	2	2	0,14	0,10	0,48	0,80	0,06	47,87
230652	1	2	3	0,10	0,08	0,37	0,58	0,06	52,67
230656	1	1	4	0,15	0,10	0,54	0,88	0,09	46,36
230662	1	1	5	0,17	0,12	0,62	1,12	0,07	45,30
230674	1	2	6	0,11	0,09	0,30	0,56	0,04	49,70
230680	1	2	7	0,12	0,15	0,46	0,78	0,08	51,44
230681	1	1	8	0,17	0,13	0,68	1,01	0,08	48,54
230682	1	1	9	0,16	0,10	0,44	0,73	0,07	46,12
230688	1	1	10	0,12	0,10	0,42	0,61	0,06	44,47
230693	1	2	11	0,17	0,12	0,57	0,78	0,06	46,15
230707	1	1	12	0,16	0,12	0,61	0,90	0,09	45,57
230710	1	2	13	0,10	0,10	0,31	0,60	0,05	49,89
230712	1	2	14	0,17	0,16	0,52	1,07	0,08	40,08
230719	1	2	15	0,14	0,15	0,50	0,81	0,04	46,95
230511	2	1	1	0,15	0,12	0,53	0,73	0,07	45,99
230552	2	1	2	0,13	0,03	0,49	0,71	0,06	50,09
230648	2	2	3	0,22	0,10	0,40	0,80	0,05	47,00
230650	2	2	4	0,18	0,16	0,66	1,06	0,07	44,78
230653	2	2	5	0,22	0,18	0,74	1,21	0,14	43,81
230665	2	1	6	0,17	0,12	0,55	0,86	0,06	39,91
230668	2	2	7	0,10	0,06	0,36	0,69	0,06	44,55
230669	2	2	8	0,11	0,11	0,32	0,51	0,03	47,96
230671	2	1	9	0,12	0,11	0,36	0,58	0,06	45,33
230673	2	1	10	0,20	0,16	0,54	0,94	0,06	46,72
230675	2	2	11	0,14	0,13	0,51	0,86	0,08	47,95
230687	2	1	12	0,13	0,13	0,55	0,82	0,09	48,40
230700	2	1	13	0,16	0,15	0,58	0,88	0,06	43,36
230702	2	2	14	0,16	0,12	0,48	0,77	0,07	43,56
230706	2	2	15	0,14	0,11	0,32	0,68	0,04	48,19
230711	2	1	16	0,14	0,12	0,36	0,62	0,05	42,13
230715	2	1	17	0,14	0,12	0,43	0,69	0,07	44,05
230726	2	2	18	0,15	0,14	0,68	1,01	0,11	47,86
230553	3	1	1	0,13	0,07	0,32	0,55	0,05	49,64
230575	3	1	2	0,08	0,05	0,25	0,42	0,03	47,51
230651	3	1	3	0,02	0,11	0,36	0,58	0,05	48,35
230664	3	1	4	0,13	0,07	0,30	0,51	0,05	53,17
230672	3	1	5	0,10	0,06	0,27	0,51	0,05	49,31
230676	3	1	6	0,12	0,07	0,38	0,60	0,06	48,42
230705	3	1	7	0,09	0,07	0,24	0,44	0,04	47,23
230717	3	1	8	0,16	0,11	0,52	0,86	0,08	48,12

Apêndice 8. Continuação

Animal	Trat	Pot	REP	MUFA	PUFA	*n*-6	*n*-3	*n*-6/*n*-3	AI
230561	1	1	1	45,13	5,26	2,60	2,02	1,28	0,65
230584	1	2	2	46,08	5,97	3,03	2,21	1,37	0,65
230652	1	2	3	42,11	5,15	2,58	1,84	1,40	0,68
230656	1	1	4	46,71	6,84	3,53	2,52	1,40	0,58
230662	1	1	5	46,25	8,37	4,43	3,08	1,44	0,57
230674	1	2	6	45,09	5,13	2,65	1,70	1,56	0,66
230680	1	2	7	42,44	6,03	3,03	2,27	1,33	0,71
230681	1	1	8	43,79	7,58	3,89	2,91	1,33	0,60
230682	1	1	9	47,88	5,91	3,00	2,11	1,42	0,53
230688	1	1	10	50,46	4,99	2,36	1,80	1,31	0,53
230693	1	2	11	46,87	6,89	3,60	2,42	1,49	0,59
230707	1	1	12	47,22	7,12	3,64	2,74	1,33	0,57
230710	1	2	13	44,76	5,26	2,68	1,79	1,50	0,66
230712	1	2	14	52,30	7,53	3,80	2,72	1,40	0,50
230719	1	2	15	46,69	6,31	3,38	2,30	1,47	0,68
230511	2	1	1	48,35	5,57	2,56	2,24	1,14	0,59
230552	2	1	2	43,78	6,05	2,71	2,58	1,05	0,65
230648	2	2	3	47,21	5,72	2,85	2,20	1,29	0,61
230650	2	2	4	48,52	6,61	2,98	2,90	1,03	0,56
230653	2	2	5	48,49	7,62	3,39	3,41	0,99	0,52
230665	2	1	6	54,13	5,87	2,68	2,34	1,15	0,46
230668	2	2	7	50,81	4,55	1,89	1,86	1,02	0,56
230669	2	2	8	47,61	4,37	1,87	1,78	1,05	0,60
230671	2	1	9	50,07	4,50	1,93	1,76	1,10	0,55
230673	2	1	10	46,53	6,65	3,07	2,74	1,12	0,55
230675	2	2	11	46,22	5,76	2,59	2,40	1,08	0,57
230687	2	1	12	45,66	5,87	2,57	2,58	1,00	0,65
230700	2	1	13	49,49	7,07	3,24	2,92	1,11	0,51
230702	2	2	14	50,55	5,80	2,61	2,36	1,10	0,53
230706	2	2	15	46,50	5,23	2,75	1,80	1,53	0,57
230711	2	1	16	53,15	4,64	2,02	1,76	1,15	0,54
230715	2	1	17	50,63	5,23	2,32	2,18	1,06	0,55
230726	2	2	18	45,78	6,28	2,69	2,90	0,93	0,66
230553	3	1	1	45,93	4,33	2,07	1,54	1,35	0,76
230575	3	1	2	48,57	3,81	1,81	1,25	1,44	0,65
230651	3	1	3	46,92	4,65	2,24	1,75	1,28	0,66
230664	3	1	4	42,19	4,54	2,30	1,58	1,45	0,69
230672	3	1	5	46,59	4,00	1,83	1,48	1,24	0,70
230676	3	1	6	46,64	4,85	2,42	1,80	1,35	0,63
230705	3	1	7	48,64	4,01	1,95	1,40	1,39	0,62
230717	3	1	8	45,29	6,49	3,31	2,40	1,38	0,61

8. VITA

Thais Devincenzi é filha de Rui Osório Devincenzi e Lélia Maria Devincenzi. Nasceu em 9 de janeiro de 1984 no município de Porto Alegre , Rio Grande do Sul, onde cursou o ensino fundamental nos colégios Instituto Porto Alegre, Leonardo da Vinci e Colégio de Aplicação da UFRGS, concluído em 1998. O ensino médio foi finalizado no ano de 2001 no Colégio de Aplicação da UFRGS. Em 2003, ingressou no Curso de Agronomia da Universidade Federal do Rio Grande do Sul, onde desenvolveu atividades como bolsista de iniciação científica com bolsa do CNPq no Departamento de Plantas Forrageiras e Agrometeorologia. Graduou-se Engenheira Agrônoma em agosto de 2008. Em 2009 ingressou no curso de Mestrado junto ao Programa de Pós-graduação em Zootecnia da Universidade Federal do Rio Grande do Sul, na área de concentração Plantas Forrageiras, com bolsa do CNPq e submetendo sua dissertação a exame em 25 de março de 2011. Em abril de 2011 ingressou no curso de Doutorado junto ao Programa de Pós-graduação em Zootecnia da Universidade Federal do Rio Grande do Sul, na área de concentração Plantas Forrageiras, também com bolsa do CNPq. Durante o curso de doutorado realizou estagio sanduiche por um periodo de um ano no Institute National de la Recherche Agronomique (INRA), na cidade de Theix, França sob orientação da pesquisadora Sophie Prache. Em 13 de abril de 2015 submete sua tese a exame.