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**PRODUÇÃO DE CORDEIROS EM PASTAGENS TROPICAIS E SEUS  
REFLEXOS NOS ATRIBUTOS QUALITATIVOS DA CARNE**

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## TESE

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" Não sei por quais  
caminhos Deus me  
conduz, mas conheço  
bem meu guia "

Martinho Lutero

♪ Ando despacito

Porque ya tuve prisa

Y llevo esta sonrisa

Porque ya lloré de más

Hoy me siento más fuerte,

Más feliz quién sabe

Y tengo la certeza

**De que muy poco sé, o nada sé ♪**

Composição: Almir Sater / Renato Teixeira

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## PRODUÇÃO DE CORDEIROS EM PASTAGENS TROPICAIS E SEUS REFLEXOS NOS ATRIBUTOS QUALITATIVOS DA CARNE<sup>1</sup>

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Orientadora: Dr. Cesar H. E. C. Poli

**Resumo:** A qualidade da carne produzida pelos ruminantes é um reflexo do alimento consumido pelos mesmos. Primeiramente realizamos uma abordagem metanalítica e verificamos que o tipo de sistema alimentar e o nível de tocoferol na dieta dos cordeiros enfluenciaram na qualidade da carne de cordeiro produzida. Assim, o objetivo foi avaliar a produção de forragem e composição química da dieta dos cordeiros alimentados com pastagens tropicais e seus reflexos sobre a qualidade da carne. Foram utilizados 54 cordeiros, machos castrados, com idade e peso inicial de 3-4 meses e  $20,4 \pm 3,97$  kg, distribuídos aleatoriamente em três sistemas de terminação baseados em pastagens tropicais: 1) Capim aruana (*Panicum maximum* cv. IZ-5); 2) Feijão guandu (*Cajanus cajan*) e 3) Consórcio (*Panicum máximo* cv. IZ-5 e *Cajanus cajan*, sendo cada espécie semeada em metade da superfície do piquete. Para testar o efeito tanino, metade dos cordeiros de cada tratamento recebeu polietilenoglicol (PEG) duas vezes ao dia. A amostragem da pastagem foi realizada a cada 21 dias, totalizando 4 coletas. Foi reaizada simulação de pastejo para as amostragens para composição química da pastagem. Avaliou-se a produção de forragem, composição química, perfil de ácidos graxos e antioxidantes (tocoferol e taninos). Os cordeiros foram abatidos após 92 dias de experimento, com peso médio de  $25,7 \pm 4,36$  kg. O perfil de ácidos graxos, concentração de tocoferóis, a coloração e a oxidação lipídica do músculo longissimus dos cordeiros foram avaliados. As pastagens apresentaram variação de massa de forragem, produção de colmos, produção de lâminas foliares e altura ao longo do tempo da avaliação. A inserção de leguminosas na dieta de cordeiros não altera a concentração de tocoferol, mas, ao longo dos períodos de avaliação, os níveis de tanino condensado e tanino total aumentam na dieta contendo leguminosas. A maioria dos ácidos graxos estavam relacionados com o conteúdo de fibra em detergente neutro da dieta. A participação da leguminosa na dieta dos cordeiros aumenta o teor de omegas 3 e 6 disponíveis na dieta de cordeiros. A inclusão de PEG nas dietas dos cordeiros aumentou o valor do ângulo da tonalidade e diminuiu a intensidade de vermelho da gordura subcutânea. O sistema de pastagem exclusivamente de feijão guandu resultou em menor concentração de ácidos graxos saturados no músculo do que o sistema exclusivo de capim aruana. A carne de cordeiros terminados exclusivamente em Capim Aruana apresentou menor relação n-6: n-3 e menor oxidação lipídica após 6 dias de armazenamento do que animais mantidos em feijão guandu ou em pastagem consorciada.

**Palavras-chave:** *Cajanus cajan*, *Panicum maximum*, tanino, TBARS, teor de vermelho, tocoferol

<sup>1</sup>Tese de Doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil 113p.), março de 2018.

## PRODUCTION OF LAMBS IN TROPICAL PASTURES AND ITS REFLECTIONS ON THE QUALITATIVE ATTRIBUTES OF MEAT<sup>1</sup>

Author: Viviane da Silva Hampel

Adviser: Dr. Cesar H. E. C. Poli

**Abstract:** The quality of the meat produced by ruminants is a reflection of the food consumed by those offered to them. First, we performed a meta-analytic approach and found that the type of food system and the level of tocopherol in the diet of lambs influenced the quality of the lamb meat produced. We used 54 male lambs with age and initial weight of 3-4 months and  $20.4 \pm 3.97$  kg randomly distributed in three finishing systems based on tropical pastures: 1) Aruana grass (*Panicum maximum* cv. IZ-5); 2) Pigeon pea (*Cajanus cajan*) and 3) Consortium (*Panicum maximum* cv. IZ-5 and *Cajanus cajan*, each species being sown on half of the surface of the picket. To test the tannin effect, half of the lambs from each treatment received polyethylene glycol (PEG) twice daily. Pasture sampling was performed every 21 days, totaling 4 collections. Grazing simulation was performed for the chemical composition of the pasture. The forage production, chemical composition, profile of fatty acids and antioxidants (tocopherol and tannins) were evaluated. The lambs were slaughtered after 92 days of experiment, with weight of  $25.7 \pm 4.36$  kg. The fatty acid profile, concentration of tocopherols, coloration and lipid oxidation of lamb longissimus muscle were evaluated. The present variation pasture forage mass, stalk production, production of leaf blades and height throughout the evaluation time. The insertion of legumes in the diet of lambs does not alter the concentration of tocopherol, but during the evaluation periods, the levels of condensed tannin and total tannin increase in the diet containing legumes. Most of the fatty acids studied were related to the neutral detergent fiber content of the diet. The participation of the legume in the diet of lambs increases the content of omegas 3 and 6 available in the diet of lambs. The inclusion of PEG in lamb diets increased the value of the hue angle and decreased the red intensity of the subcutaneous fat. The grazing system exclusively of pigeon pea resulted in a lower concentration of saturated fatty acids in the muscle than the exclusive system of Aruana grass. Meat from lambs exclusively in Aruana grass presented lower n-6: n-3 ratio and lower lipid oxidation after 6 days of storage than those maintained on Pigeon pea or Mixed pasture.

**Key-words:** *Cajanus cajan*, *Panicum maximum*, redness, tanin, TBARS, tocopherol

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<sup>1</sup>Doctoral Thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil (113p.), March, 2018

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## LISTA DE ABREVIATURAS E SÍMBOLOS

### **Capítulo II**

FA	Total fatty acid content
° C	Degrees
BIC	Bayesian information criterion
Cielab	Dimensional colour measurement system
CLA	Conjugated linoleic acid content
g 100 g <sup>-1</sup>	Grams per hundred grams
H <sup>+</sup>	Hydrogen
L *	Brightness
mgkg <sup>-1</sup> DM	Milligrams per kilogram of dry matter
MUFAs	Monounsaturated fatty acid content
n-3	Fatty acid content of omega 3
n-6	Fatty acid content of omega 6
n-6:n-3	Ratio of n-6: n-3
PUFAs	Polyunsaturated fatty acid content
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SFAs	Saturated fatty acid content
TBARS	Lipid peroxidation characteristic
VC	Variance component

### **Capítulo III e Capítulo IV**

AG	Aruana grass ( <i>Panicum maximum</i> cv. IZ-5)
PP	Pigeon pea ( <i>Cajanus cajan</i> )
A	Área of each paddock (ha)
a*	Redness
ADF	Acid detergent fiber
ADL	Acid detergent lignin
ATI	Alpha-tocopherol intake
b*	Yellowness
BW	Body weight
BWr	Weight of put-and-take lambs
BWS	Weighed
BWt	Average body weight of the test lambs
C*	Chroma
CC	Carcass conformation
CCW	Cold carcass weight
CP	Crude protein
Cr <sub>2</sub> O <sub>3</sub>	Chromium oxide
CY	Carcass yield
DAR	Daily accumulation rate

DM	Dry matter
EE	Ether extract
ES	Ethereal stratum
FM	Forage mass
FP	Fecal production
H°	Hue angle
HCW	Hot carcass weight
ISDMD	Dry matter in situ digestibility
L*	Lightness
LP	Leaf production
LTL	<i>Longissimus thoracis et lumborum</i>
Mixed	Half of the area with <i>Panicum</i> and half with <i>Cajanus cajan</i>
MUFA	Monounsaturated fatty acids
N	Number of days lamb remained on paddock
NDF	Neutral detergent fiber
OMI	Organic matter intake
PUFA	Polyunsaturated Fatty acids
SFA	Saturated fatty acids
SFT	Subcutaneous fat thickness
SP	Stem production
SR	Stocking rate
TBARS	Thiobarbituric Acid-Reactive Substances method
TC	Condensed tannin
TH	Hydrolyzable tannin
TT	Total tannin
WBSF	Shear force
WRC	Water retention capacity

## **CAPÍTULO I**

## 1. INTRODUÇÃO

Os sistemas de produção a pasto, são um diferencial da produção ovina gaúcha. Essa forma de produzir pode alterar a quantidade e a qualidade da carne produzida, através do consumo de compostos bioquímicos (taninos e tocoferóis) das forragens pelos animais. Além do elevado potencial de produção das forrageiras, as características bioquímicas que elas possuem podem gerar um relevante impacto na produção de carne, com reflexos na saúde humana. Dos compostos bioquímicos presentes nas forrageiras, e com grande potencial para melhoria da qualidade da carne, devido as suas características antioxidantes, destacam-se os compostos secundários, os taninos condensados (POLI et al., 1998; LIU et al., 2012) e os tocoferóis (TURNER et al., 2002; RIPOLL et al., 2013). A dieta do animal tem uma forte relação com a qualidade do produto final, no entanto, muito pouco se sabe sobre o possível efeito das pastagens tropicais em relação aos compostos bioquímicos, além dos sistemas de alimentação no desempenho dos animais e na qualidade da carne. Sabe-se apenas que as pastagens tropicais podem constituir importantes fontes de polifenóis e tocoferóis (JACKSON et al., 1996), mas muito pouco explorados.

Os tocoferóis presentes nas plantas têm grande importância devido a sua ação antioxidante sobre as macromoléculas celulares como DNA, proteínas e ácidos graxos contra a ação de radicais livres (SADO et al., 2013). Uma de suas principais funções é remover radicais livres e desempenhar um papel importante na prevenção do estresse oxidativo em tecidos biológicos, o qual pode ser agravado quando há desequilíbrio vitamínico na formulação de dietas (ABDEL-HAMEID et al., 2012). A vitamina E é um dos antioxidantes mais utilizados em dietas de animais pelo fato de retardar a oxidação lipídica e as perdas por exsudação da carcaça, além de fornecer estabilidade da cor da carne (LÓPEZ - BOTE et al., 2001).

Além de incrementar o desempenho animal, os taninos condensados podem gerar um produto final (carne ou leite) de qualidade, devido a sua ação antioxidante. Os taninos têm ação antioxidante, anti-inflamatória e anticancerígena (LENTINI et al., 2010; ZHANG et al., 2010), podendo ser um meio de melhorar a qualidade da carne com potencial para atingir a saúde humana. A ação antioxidante do tanino condensado, assim como dos tocoferóis têm um efeito importante também na manutenção da cor e na rancificação da carne, promovendo melhor conservação e maior tempo de prateleira do produto (LÓPEZ -BOTE et al., 2001; SOARES, 2002; LIU et al., 2012).

Apesar de haver importantes trabalhos no mundo com a utilização de taninos condensados e tocoferóis (POLI et al., 1998; WAGHORN, 2008; LIU et al., 2012; LOUVANDINI et al., 2011; RIPOLL et al., 2013), ainda é pouco conhecido o efeito desses compostos. Oriundo das gramíneas e leguminosas estivais cultivadas em nosso país, sobre os aspectos relacionados a produtividade e a qualidade da carne de ruminantes. A introdução de forrageiras tropicais como capim aruana (*Panicum maximum*) nos sistemas de criação, pode ser uma alternativa para manter uma adequada oferta de forragem, pois esta apresenta uma alta qualidade nutricional, com boa tolerância ao pastejo e produção adequada de forragem (GERDES et al., 2005; PAULINO et al., 2015). Para otimizar a produção animal, pode-se introduzir o uso do concentrado ou

leguminosas como o feijão guardu (*Cajanus cajan*), cultivadas exclusivas ou oferecidas como banco de proteína. Essa leguminosa apresenta alto teor de proteína bruta e taninos condensados (Vitti et al., 2005; Louvandini et al., 2011).

A utilização de pastagens tropicais, e a incorporação do uso de compostos secundários na criação animal pode melhorar a qualidade da carne e incrementar a produção no sistema. Portanto, é de grande importância a avaliação dos sistemas de produção e qualidade da carne de ruminantes em função da qualidade do alimento ingerido pelos animais.

## 2. REVISÃO BIBLIOGRÁFICA

### 2.1. Produção de ovinos em pastagens tropicais

Até o ano de 2050, o setor agrícola terá o desafio de aumentar produção em mais de 60% para suprir a demanda alimentar do mundo (FAO, 2012). Neste cenário, a carne será uma fonte estratégica de proteína para a dieta humana, crescendo substancialmente. A demanda projetada mostra um positivo aumento nas carnes de aves e suínos, seguido de bovino e carne de ovinos. Espera-se um crescimento na produção e consumo de carne de ovinos (22% em volume; 4% no preço em termos reais) até 2021, que ocorrerá principalmente nos países em desenvolvimento (MONTOSSI et al., 2013). Brasil é um exemplo de país com potencial para a produção ovina, uma vez que a produção é menor que o consumo, refletindo numa importação que fica próximo de 9% (ANUALPEC, 2011).

No Rio Grande do Sul (RS) a ovinocultura é uma atividade de grande importância econômica e cultural, principalmente na região da campanha do RS, região dominada pelo campo natural do Bioma Pampa. Nesses campos, a ovinocultura de corte tem um grande potencial, principalmente para pequenos e médios pecuaristas. Além do campo nativo, no Rio Grande do Sul as pastagens estivais apresentam grande potencial para a criação de ovinos (POLI et al., 2012; MONTEIRO et al., 2009; CARVALHO et al., 2004), auxiliando de forma significativa na redução da sazonalidade da produção de carne de cordeiro na região sul do Brasil (POLI et al., 2012).

Dentre as espécies tropicais, o capim aruana é uma das espécies de gramíneas tropicais mais utilizadas devido ao seu valor nutritivo, elevada produção, capacidade de suporte, desempenho animal, e aceitabilidade pelos animais, e por não apresentar compostos antinutricionais (DIFANTE et al., 2010, DIFANTE et al., 2009, SILVEIRA et al., 2015). Para incrementar a produção animal, além do uso das gramíneas tropicais, a introdução de leguminosas nos sistemas de produção pode melhorar a composição da dieta dos cordeiros. Além de melhorar o valor nutricional da dieta, devido ao maior aporte de proteína. Algumas leguminosas, tais como o feijão Guandu apresentam compostos, como os taninos, os quais podem favorecer a melhor utilização de proteínas e nutrientes pelos ruminantes (MOLLE et al., 2009), contribuindo para o desempenho animal e qualidade do produto final.

#### 2.1.1 Capim Aruana

O *Panicum maximum* Jacq., proveniente da África foi introduzido no Brasil acidentalmente por volta do século XVIII, por servir de cama para os escravos nas embarcações. Chegando ao Brasil, esta espécie adaptou-se muito bem, principalmente por ter encontrado solos férteis (CHASE, 1944). O capim Aruana é um cultivar do *Panicum maximum* melhorado geneticamente a partir do cv. Colonião, oriunda da África, que foi introduzida no Brasil em 1974 no Instituto de Zootecnia em Nova Odessa – SP, local onde foi melhorado e lançado em 1995. Tem como características porte médio, pode atingir aproximadamente 80 cm de altura; grande capacidade e rapidez de perfilhamento; excelente capacidade de

cobertura do solo que auxilia no controle da erosão e propagação por sementes, o que possibilita formação rápida da pastagem. Por se tratar de uma planta cespitosa, com crescimento em touceiras, possui uma arquitetura foliar ereta e aberta (possibilitando maior incidência de radiação solar), alta produtividade de forragem (18 a 21 t de MS/ha/ano; DUARTE, 2011). Gramíneas do gênero *Panicum* (Aruana, Áries, Massai e Tanzânia) apresentam valor nutritivo: PB (11 a 14%), FDN (70%), DIVMO (56- 65%), FDA (34 a 48%), lignina (5 a 8%; Brancio et al., 2002) adequados para a produção ovina (BRÂNCIO et al., 2003)

A utilização do capim Aruana para a terminação de cordeiros mostra resultados distintos. Menezes et al. (2010), trabalhando com cordeiros em pastejo rotativo obteve valores de 93 g de ganho de peso por animal por dia (GMD), enquanto Bueno (2001), obteve um GMD de 35g/dia. Já Fajardo et al. (2015) testaram o capim Aruana sob diferentes níveis de suplementação com concentrado (0, 1,5 e 2,5% PV) no desempenho de cordeiros lanados e encontraram GMD de 26,0; 76,0 e 143 g/dia.

### 2.1.2 Feijão Guandu

O feijão guandu *Cajanus cajan* (L.) Millspaugh pertence à família Fabaceae, subfamília Faboideae. É uma leguminosa arbustiva anual ou semiperene sendo uma cultura importante para diversos países dos trópicos e subtrópicos, principalmente os países asiáticos e africanos (AZEVEDO et al., 2007). É encontrada com frequência em todo o Brasil, apresenta utilização bastante diversificada, a cultura do feijão guandu pode ser usada para os mais diversos fins: como planta melhoradora de solos, na recuperação de áreas degradadas, como planta fitorremediadora, renovação de pastagens, na alimentação de animais domésticos e da pecuária e também na alimentação humana (AZEVEDO et al., 2007).

Desenvolve-se em clima quente e úmido, com temperatura média de 18 a 30°C e precipitação de 500 a 1700 m. Essa planta pode crescer até 4 m de altura (AZEVEDO et al., 2007), sendo a sua produtividade da forragem considerada elevada (até 12 toneladas por hectare ano). O feijão guandu apresenta alto valor nutritivo. As folhas e os ramos finos apresentam teores de proteína bruta entre 16 e 20% (MIZUBUTI et al.,2007), enquanto que a digestibilidade da matéria seca pode variar de 50 a 65% (COSTA et al., 2001. Shenkute et al. (2013) avaliaram níveis de inclusão de folhas secas de feijão Guandu na dieta de cabritos desmamados em pastagem nativa e encontraram GMD de 84,6 g/dia para um consumo de 132 g (MS) de folhas.

O feijão gundu também apresenta teor de taninos condensados considerado de moderado a baixo (< 5% / kg MS; LOUVANDINI et al., 2011)VITTI et al., 2005). Os Taninos condensados podem promover melhoria no desempenho dos animais devido a proteção da proteína da degradação ruminal, aumentando o fluxo de proteína metabolizável, resultando em maiores ganhos de peso (FRUTOS et al., 2004).

## 2.2. Compostos Antioxidantes nas Plantas

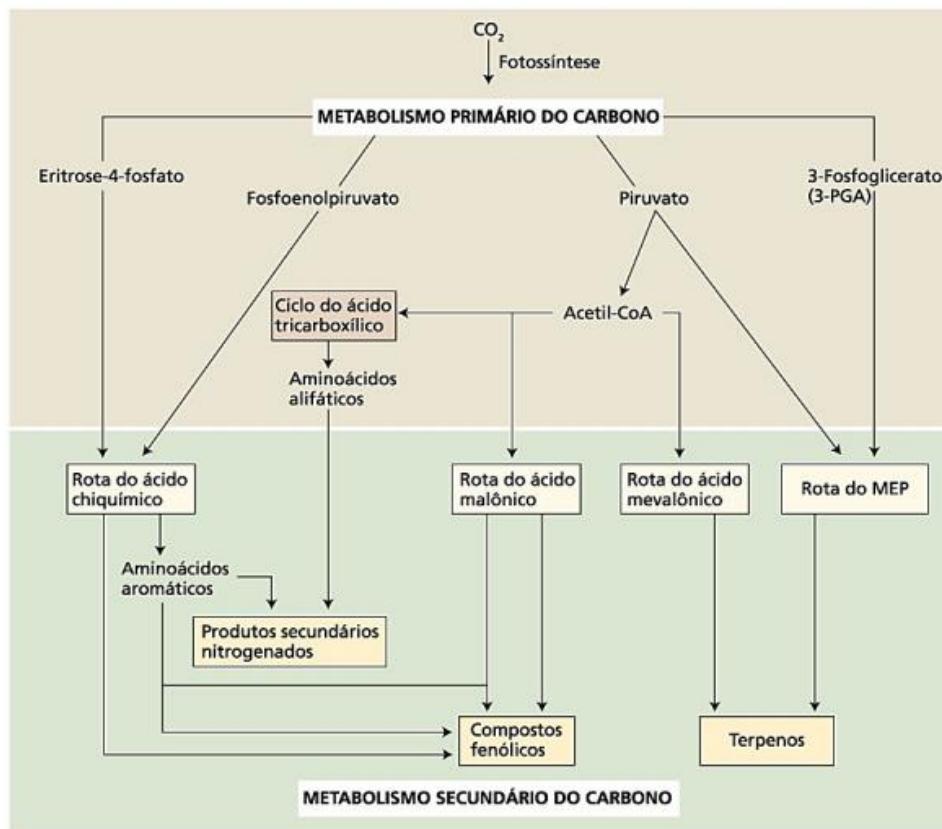


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Figura 1- Formação dos compostos secundários nas plantas

### 2.2.1 Tocoferol

O termo genérico “vitamina E” é utilizado para designar oito diferentes compostos, nomeados α-, β-, γ- e δ- (alfa, beta, gama e delta) tocoferóis e tocotrienois (AIN, 1979; CHUN et al., 2006). Os oito compostos são classificados em dois grupos: tocoferóis, derivados do tocol; e tocotrienóis, derivados do tocotrienol. Ambos os grupos possuem um anel 6-cromanol e uma cadeia lateral de natureza isoprênica constituída de dezesseis átomos de carbono, o que confere característica lipossolúvel ao tocoferol (RUPÉREZ et al., 2001). Os tocoferóis são compostos que contém o grupamentos metil-substituintes e cadeia lateral saturada, enquanto que os tocotrienóis apresentam estrutura idêntica, exceto pela presença de três duplas ligações na cadeia carbônica (Figura 2; BALL, 1998).

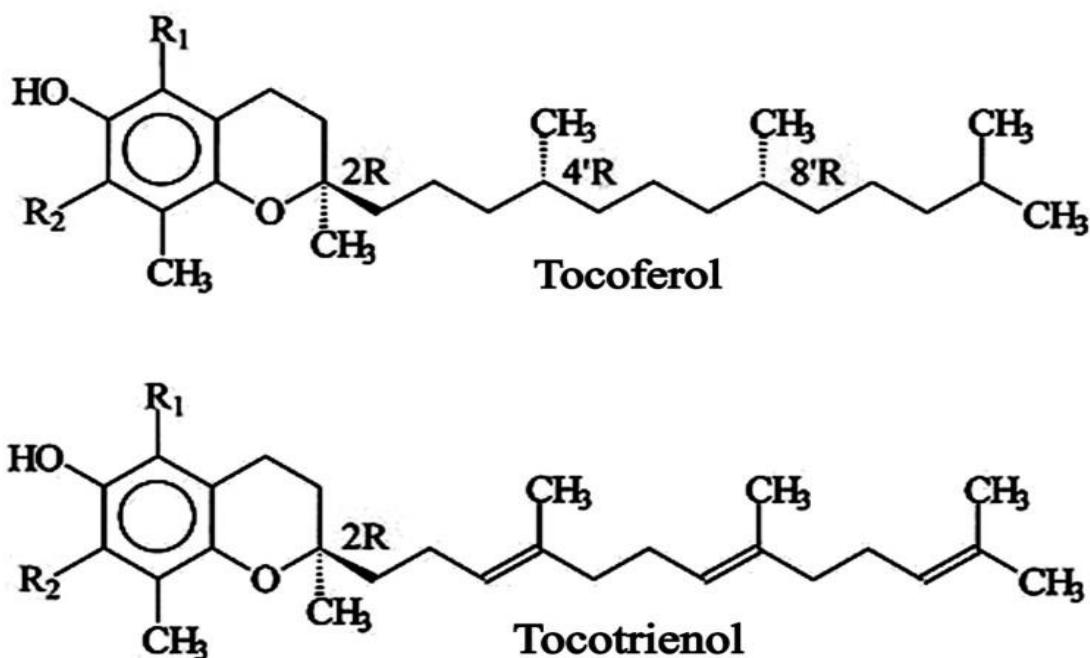


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Figura 2- Estrutura dos tocoferóis e tocotrienos

Os compostos dos tocoferóis são sintetizados pelo metabolismo secundário das plantas com objetivo de proteger a célula vegetal da oxidação e da peroxidação (Figura 3). No processo de fotossíntese, ocorre um constante equilíbrio entre a captura eficiente da energia solar e sua dissipação rápida quando capturados em excesso. O α -tocoferol pode contribuir para a preservação de um estado redox adequado nos cloroplastos, e manutenção da estrutura e da membrana dos tilacóides, em respostas ao estresse (MUNNE'-BOSCH, 2005; SATTLER et al., 2004). Tocoferóis têm sido encontrados em cloroplastos, mas também em vacúolos e núcleos de folhas (RAUTENKRANZ et al., 1994) e em cloroplastos e mitocôndrias de algas verdes (SHIGEOKA et al, 1986; KUSMIC et al, 1999).

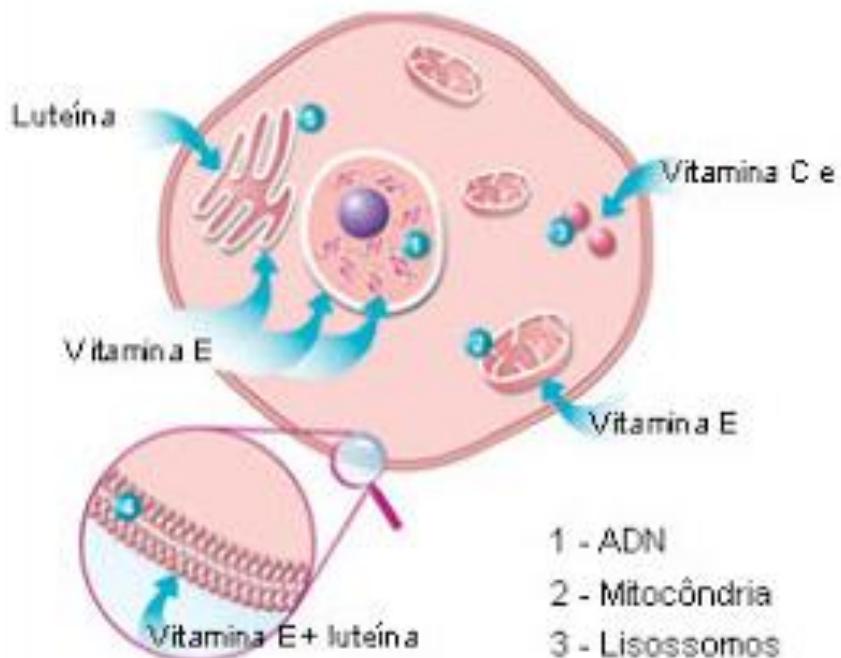


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Figura 3- Localização do tocoferol (vitamina E) nas plantas

O mecanismo que envolve a peroxidação lipídica depende de um iniciador, que pode ser a luz, calor, íons superóxido e peróxido, metais, radiação e enzimas, entre outros. Radicais livres são então formados a partir de ácidos graxos e desencadeiam a propagação das reações de oxidação em cadeia (PINCHUK & LICHTENBERG, 2002). A cadeia pode ser interrompida pela ação de substâncias que estabilizam esses radicais livres, impedindo a formação de novos radicais e a continuidade das reações. Os tocoferóis constituem um dos “bloqueadores” de cadeia mais eficientes, reagindo 200 vezes mais rapidamente, que um antioxidante sintético, como o butilhidroxitolueno (BHT) (PINCHUK & LICHTENBERG, 2002).

Os tocofeois atuam como doador de hidrogênio, interrompendo a cadeia de reações pois o radical tocoferoxil formado não apresenta reatividade sobre a estrutura lipídica. Esse papel antioxidante é desempenhado de forma única, uma vez que interage com o ambiente lipídico de forma acentuada devido a sua característica lipofílica. Além disso, a estrutura da vitamina E está localizada entre os componentes da membrana celular e assim, é uma das responsáveis pela linha de defesa primária das células contra o ataque de radicais livres (Figura 4; MACHLIN, 1991).

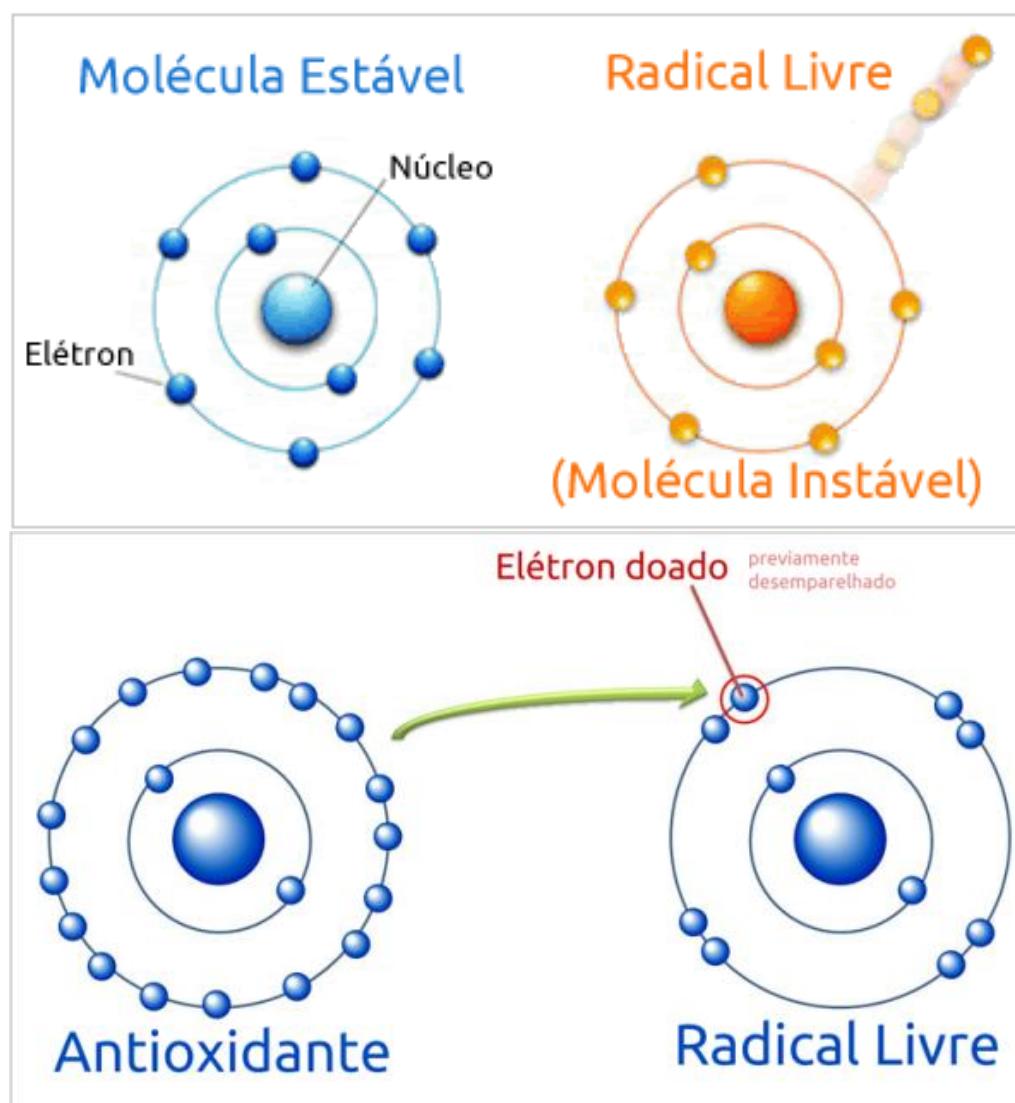


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Figura 4- Atuação dos tocoferóis (vitamina E) sobre os radicais livres

Os tocoferóis possuem ainda, a característica de ser o único antioxidante que tem habilidade de regenerar-se continuamente pela ação da vitamina C ou glutadiona (MACHLIN, 1991). Juntamente com a vitamina C, beta-caroteno e selênio, os tocoferóis compõem o grupo de nutrientes conhecidos como “antioxidantes alimentares”, sendo que a mesma é considerada o mais efetivo e mais abundantemente encontrada nas membranas celulares de mamíferos (PERCIVAL, 1998; RUPÉREZ et al., 2001).

Entre os compostos tocopheróis, o  $\alpha$ -tocoferol é apontado como sendo o mais potente em sua ação antioxidante (YOSHIDA et al., 2003). No entanto, alguns trabalhos indicam que outros isômeros como  $\gamma$ - e  $\delta$ -tocoferol são melhores antioxidantes (MASUCHI et al., 2008; FRANKEL, 1996).

## 2.2.2 Taninos

Além do tocoferol, os taninos condensados (Figura 5) também se destacam como importantes compostos antioxidantes das plantas. As forrageiras em geral são constantemente submetidas a estresses causados pela temperatura, déficit hídrico, radiação solar e deficiência de nutrientes que limitam a produção e a qualidade nutricional das mesmas. Além da limitação na quantidade de nutrientes, muitas espécies tropicais possuem pré-disponibilidade genética para produzir taninos em estágios particulares de desenvolvimento ou sob condição de estresse. Os metabólitos secundários constituem um meio de defesa contra bactérias, fungos, vírus, estresse ambiental e ataque de herbívoros, e podem proporcionar à planta características como gosto amargo, odor repulsivo e provocar intoxicações ou efeitos antinutricionais nos predadores (GINER-CHAVES, 1996).



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Figura 5- Formação dos taninos

Os taninos são polifenóis com peso molecular entre 500 e 3.000 daltons, fazem parte de um grupo de compostos secundários presente nas plantas, e podem ser encontrados em caules, cascas, folhas, flores ou sementes. Estão abrigados dentro dos vacúolos, principalmente em plantas dicotiledôneas (BARRY, 1989). Onde não interferem no metabolismo da planta, sendo liberados com a ruptura da célula, que pode ser causada pelo corte ou mastigação da forrageira (Figura 6; MIN et al., 2003).

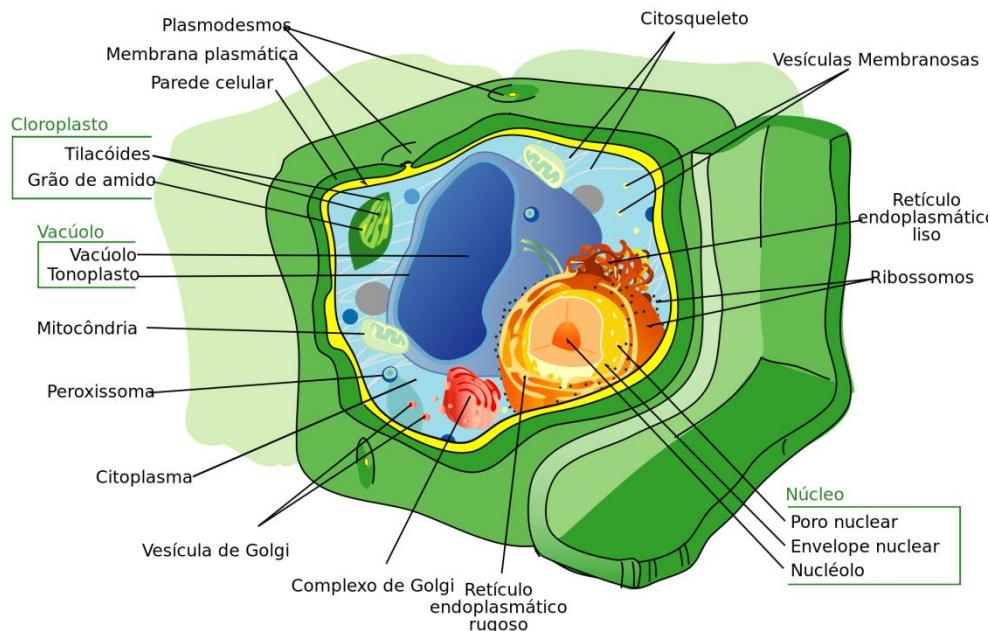


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Figura 6- Localização dos taninos nas plantas (Vacúolo)

Os taninos são divididos em dois grupos baseados nos tipos estruturais: hidrolisável e condensado (Figura 7). Taninos Hidrolisáveis (TH) são passíveis de serem degradados por hidrólise química ou enzimática nas várias unidades estruturais que os compõem. São constituídos por uma parte fenólica (ácido gálico e/ou ácido hexahidroxifênico) e uma unidade de hexose (normalmente a glucose) ligada através de um éster (MCMAHON et al., 1999; FRUTOS et al., 2004). O TH pode ser hidrolisado por aquecimento com ácido fraco. Em contraste, os taninos condensados podem sofrer uma degradação oxidativa somente pelo ácido mineral quente (MCMAHON et al., 1999).

Os TH estão normalmente presentes em baixa concentração nas plantas e podem sofrer facilmente hidrólise por bases, ácidos e esterases. O metabolismo microbiano e a digestão gástrica convertem esses taninos em metabólitos de baixo peso molecular. Alguns desses metabólitos são tóxicos e estão associados a hemorragias gastro-entéricas e necrose do fígado e rins, principalmente em monogástricos (CANNAS, 2001).

Os taninos condensados (TC), são polímeros de derivados fenólicos complexos, ligados por pontes carbono-carbono ou carbono-oxigênio-carbono, cuja ligações são mais resistentes à ruptura do que aquelas dos TH (LIMA FILHO & ABDALLA, 2011). São também denominados flavan-3-ol (por exemplo, catequina) ou flavan-3,4-diol (proantocianidinas), com variados pesos moleculares. As proantocianidinas são mais largamente distribuídas do que os taninos hidrolisáveis e são responsáveis pelos pigmentos vermelhos, roxos e azuis nas flores, frutos, sementes, caules e folhas (LIMA FILHO & ABDALLA, 2011).

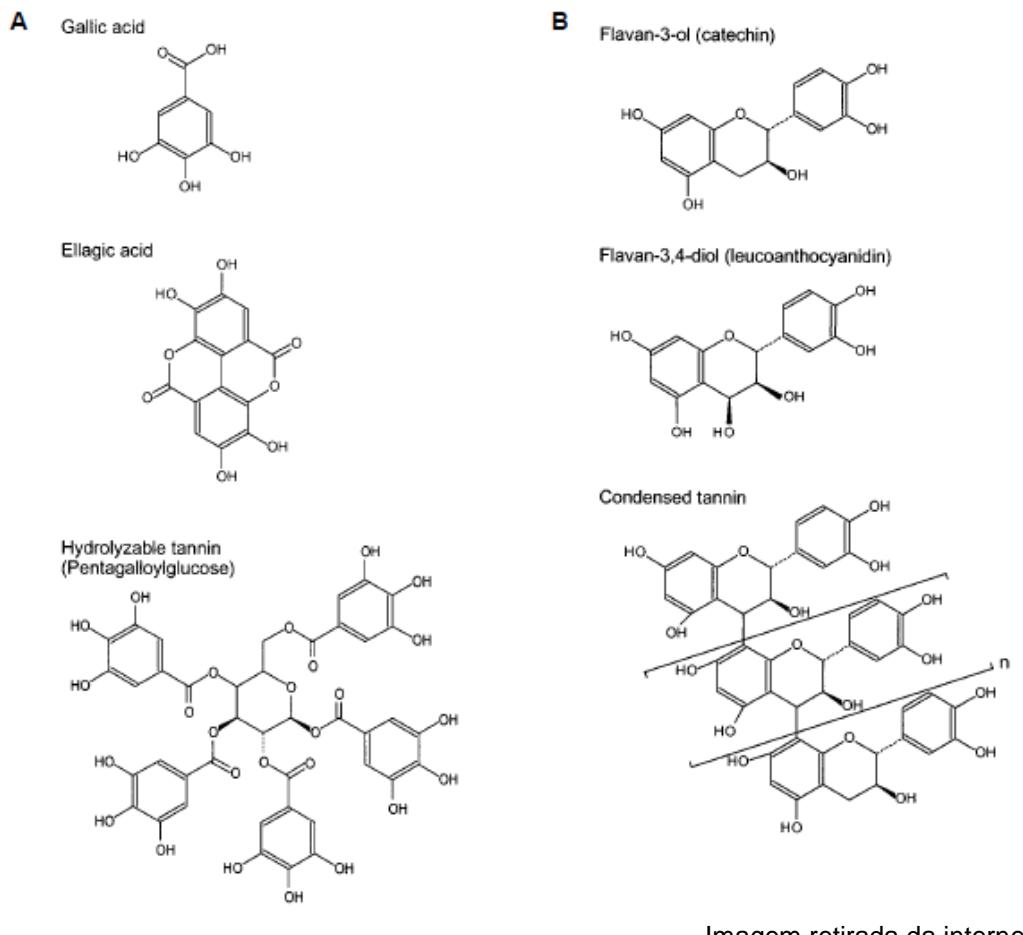


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Figura 7- Estrutura dos Taninos

A quantidade de taninos sintetizados pela planta depende da espécie, cultivar, tecido, estágio de desenvolvimento e condições ambientais. Esses fatores influenciam não somente a concentração, mas também a composição em monômeros e o peso molecular dos taninos (LASCANO et al., 2001), características que podem estar determinando a ação desses fenóis na qualidade nutricional das plantas.

### 2.3. Compostos antioxidantes das plantas e seus efeitos na produção animal

#### 2.3.1. Tocoferois

A dieta do animal tem uma forte relação com a qualidade do produto final, no entanto, muito pouco se sabe sobre o potencial efeito das pastagens em relação aos compostos bioquímicos. Sabe-se apenas que as pastagens são importantes fontes de tocoferóis, mas muito pouco explorados. As pastagens verdes são uma boa opção para aumentar o conteúdo muscular de  $\alpha$ -tocoferol (RIPOLL et al., 2013). O tocoferol não é degradado no rúmen (LEEDLE,

LEEDLE, & BUTINE, 1993), sendo depositado nas membranas das células musculares e nos depósitos de lipídios, funcionando como um antioxidante (LIU, LANARI, & SCHAEFER, 1995). Pastos frescos contêm naturalmente altas concentrações de  $\alpha$ -tocoferol. Cordeiros pastando forragem fresca podem ter concentrações elevadas de  $\alpha$ -tocoferol muscular, resultando na melhoria da estabilidade oxidativa e da cor da carne sem ter que usar suplementos de vitamina E (TURNER et al., 2002). Entretanto, ainda falta muita informação em relação as forrageiras (leguminosas e gramíneas), cultivadas no nosso país, no que diz respeito as concentrações de tocoferóis nas plantas e a sua retenção no músculo. Turner et al. (2002) encontraram uma concentração média de  $\alpha$ -tocoferol em plantas de alfafa de 137 mg / kg/MS e 169 mg / kg MS para azevém.

Concentrações de  $\alpha$ -tocoferol no músculo *longissimus* de cordeiro pastando alfafa e azevém foram semelhantes às concentrações de  $\alpha$ -tocoferol no longíssimos de ovinos alimentados com 15 e 150 UI de tocoferol suplementar / kg de concentrado (TURNER et al., 2002). A utilização de forrageiras como fonte de tocoferóis, ao invés de suplementação, se torna uma alternativa para o produtor, pois apesar dos benefícios da vitamina E, o concentrado enriquecido é caro e pode resultar em um aumento de mais do que 2,5% nos custos de produção (ALBERTI, 2012).

A concentração de tocoferol no músculo pode melhorar a vida útil da carne embalada. A vida útil é condicionada por processos oxidativos que são provocados por temperatura, exposição ao oxigênio, luz e crescimento microbiológico. Na indústria da carne a possibilidade de estender a vida de prateleira da carne por retardar a deterioração oxidativa, é um dos mais importantes objetivos (LUCIANO et al., 2009). A estabilidade da cor e a oxidação dos ácidos graxos de carne é influenciada pela composição dos tecidos musculares, o que é, por sua vez, regidos pela concentração e o tipo de gorduras, a capacidade antioxidante e de heme pigmentos (ferro heme), todas as quais estão firmemente ligadas com o alimento oferecido ao animal (PONNAMPALAM et al., 2012).

Os sistemas de produção de ruminantes em pastagem podem resultar em animais com elevadas concentrações de tocoferol muscular, resultando na melhoria da estabilidade oxidativa e de cor da carne sem ter que usar suplementos de vitamina E (RIPOLL et al., 2013). José et al. (2008), mostraram que a suplementação com vitamina E sintética (175,7 mg / kg de ração) pode resultar em melhor cor da carne (*músculo longissimus*) após 30 dias de prateleira. Ponnampalam et al. (2012) constatou que 3,46 mg de alfa-tocoferol nos tecidos musculares de cordeiros em pastagem anual (azevém e cevada), durante o final da primavera, levou a uma maior estabilidade de cor da carne, quando comparado a cor da carne de cordeiros alimentados com grãos de cevada, lentilha e feno, tendo apenas 1,69 mg  $\alpha$ -tocoferol/kg tecido muscular. A vitamina E, especialmente o  $\alpha$ -tocoferol, é largamente utilizada como um antioxidante, reduzindo a oxidação lipídica, as perdas por gotejamento e proporcionando estabilidade da cor (LÓPEZ-BOTE et al., 2001).

López-Bote et al. (2001) relataram que o nível ótimo para retardar a deterioração da carne estaria no intervalo 5,3-5,6 mg  $\alpha$ -tocoferol/kg muscular, o que corresponde a uma inclusão na dieta de 550-625 mg  $\alpha$ -tocoferol / kg de dieta. Turner et al, (2002) recomendaram que a concentração máxima de  $\alpha$ -tocoferol

no músculo *longissimus* foi resultado do consumo de aproximadamente 584 UI de vitamina E totais / dia, sugerindo que a suplementação com maior quantidade não afetou as concentrações α-tocoferol no muscular.

### 2.3.2. Taninos

Os taninos, sobretudo os taninos condensados, têm recebido crescente atenção na produção animal por possuírem a capacidade de proteger a proteína ingerida da degradação ruminal (*by pass*), quando em baixa concentração e por apresentarem importante ação antinutricional quando em alta concentração (acima de 5% da MS) na dieta. Os taninos condensados podem formar complexos com celulose, amido, pectinas, alcalóides, outros polifenóis e sais de metal pesado (GINER- CHAVES, 1996), entretanto, sua característica mais marcante, que explica a maioria de suas propriedades biológicas e antinutricionais, é a capacidade de formar complexos insolúveis com proteínas (JEAN-BAIN, 1998). A força desses complexos depende das propriedades dos taninos, porém também das proteínas. As proteínas com forte afinidade pelos taninos são caracterizadas por apresentarem uma grande proporção de prolina, uma relativa quantidade de aminoácidos de cadeia não polar, elevado peso molecular e estrutura terciária aberta (REED, 1995).

Geralmente, taninos induzem respostas negativas à nutrição de ruminantes (MANGAN, 1988). Esses efeitos podem ser instantâneos como a adstringência, reduzindo a palatabilidade e consequentemente o consumo, ou a longo prazo, agindo como fator antinutricional ou tóxico. Por outro lado, o consumo de taninos por ruminantes também tem sido relacionado a efeitos positivos sobre a nutrição (ANIMUT et al., 2008). Dentre os efeitos positivos, associados a concentrações por volta de 3-4% de tanino na MS, destacam-se a proteção da proteína alimentar contra a excessiva degradação ruminal; a diminuição do desperdício de amônia; o aumento da absorção de aminoácidos provenientes da dieta no intestino delgado, a prevenção do timpanismo e mais recentemente (ANIMUT et al., 2008), a redução da produção de gás metano no rúmen. O mecanismo de ação dos TC sobre a proteína ocorre com a formação de complexos estáveis em pH 3,5 e 7, mas se dissociam quando o pH cai abaixo de 3,5, como ocorre no abomaso (pH 2,5-3) ou é superior a 7, como ocorre no duodeno (pH 8) (VAN SOEST, 1994).

A ação antioxidante dos taninos condensados têm um efeito importante também na manutenção da cor e na redução da rancificação da carne, promovendo melhor conservação e maior tempo de prateleira do produto (LÓPEZ -BOTE et al., 2001; SOARES, 2002; LIU et al., 2012). Resultados de pesquisas mostraram que os sistemas em pastagens, comparados com sistemas com animais confinados, permitem obter carnes com menor conteúdo de gordura intramuscular e colesterol, melhor relação entre os ácidos graxos ômega-6: ômega-3, e maior concentração de CLA (ácido linoleico conjugado) (SANUDO & MONTOSSI, 2004), características benéficas à saúde humana. A carne ovina, bem como a carne de todos os ruminantes, é considerada rica em ácidos graxos saturados e monoinsaturados, com pequenas quantidades de poli-insaturados (SINCLAIR et al., 1982).

Além disso, os taninos podem modificar a composição dos ácidos graxos da carne (VASTA et al., 2007) e isso poderia afetar indiretamente sua

suscetibilidade a processos de oxidação. Luciano et al. (2009; 2011), relataram que a alimentação de cordeiros com dietas à base de concentrado ou com a inclusão de um extrato rico em polifenol de quebracho (*Schinopsis lorentii*) retardou a oxidação da mioglobina e estendeu a estabilidade da cor da carne armazenada tanto em ambiente de alto oxigênio modificado ou em condições aeróbias. Compostos fenólicos dietéticos têm sido frequentemente usados para reduzir fortemente a oxidação lipídica da carne ao longo do tempo de armazenagem ou exposição ao oxigênio. Zhong et al. (2009) encontraram maior estabilidade lipídica em carne de cabras que receberam catequinas complementares na dieta em comparação com a carne de animais no tratamento controle. Recentemente, Karami et al. (2011), verificaram que a inclusão de 0,5% de açafrão e *Andrographis paniculata* em uma dieta à base de concentrado para cabras reduziu a oxidação lipídica na carne armazenada e embalada-vácuo ao longo de 14 dias.

Priolo & Vasta (2007) concluíram a partir de um compilado de estudos, que animais alimentados com dietas ricas em taninos parecem ter a cor da carne mais clara em relação à carne de animais alimentados com as mesmas dietas, mas que foram suplementados com polietileno glicol. Eles relatam que uma possível explicação para esse resultado poderia ser a diminuição da produção de vitamina B12 pelos microorganismos do rúmen, como encontrado in vitro resultando numa produção reduzida de hemoglobina.

A adoção de estratégias de controle, alternativas integradas, como a incorporação do uso de compostos bioquímicos na criação animal, podem melhorar a qualidade da carne e incrementar a produção no sistema de produção. Além da possibilidade de ocorrer a presença de compostos na carne de interesse econômico, esses compostos podem trazer melhorias a saúde animal através da sua ação contra radicais livres.

## 2.4 Perfil de ácidos graxos no pasto

Nos vegetais, os lipídios podem ser divididos em dois grupos, os lipídios de reserva e os lipídios estruturais. Os lipídios são compostos principalmente por glicolipídios e fosfolipídios. Os principais ácidos graxos são mirístico (C14:0), palmítico (C16:0), esteárico (C18:0), linoleico (C18:2) e o linolênico (C18:3), os ácidos graxos com 20 e 22 carbonos são encontrados em menor quantidade (VAN SOEST, 1994).

O teor e a composição de ácidos graxos podem ser afetados por vários fatores, tais como espécies e variedades de plantas, clima, comprimento do dia, precipitação, fertilização e estádio de crescimento. Os lipídios das plantas estão principalmente associados às membranas de tilacoídes dos cloroplastos (KALAČ et al., 2010).

As plantas forrageiras apresentam alta concentração de ácidos graxos poli insaturados (PUFA), especificamente C18: 2n-6 e C18: 3n-3. A forragem fresca contém uma proporção elevada (50-75%) do seu teor total de ácidos graxos na forma de C18: 3n-3 (DEWHURST et al., 2001). Dierking et al (2010),

encontraram maiores concentrações de C16:0 nas leguminosas quando comparada com as gramíneas ou com mistura de gramíneas e leguminosas.

Quando colhido no mesmo estágio de desenvolvimento, Boufaïed et al. (2003), encontraram diferenças significativas, tanto nas espécies do mesmo grupo funcional da planta (gramínea ou leguminosa) quanto entre os dois grupos. As leguminosas apresentaram maiores concentrações de C14: 0, C16: 0, C18: 0, C18: 1 e C18: 2n-6 e ácidos graxos totais e menores concentrações de 18: 3n-3, mas também foram observadas grandes variações entre as espécies encontradas em cada grupo funcional.

Glasser et al. (2013), afirmaram que as diferenças entre gramíneas e leguminosas são menores quando temos a interferência do estádio fenológico na concentração de ácidos graxos nas plantas. Os PUFAs diminuem com a avanço do estádio fenológico das plantas (GLASSER et al., 2013). O intervalo de corte também pode alterar a concentração de ácidos graxos, segundo Dewhurst et al. (2001), quando eles diminuíram o intervalo de corte de 38 dias para 20 dias, a concentração de ácidos graxos aumentou.

A composição química das forragens também influencia na concentração de ácidos graxos nas plantas, Glasser et al. (2013), relatam que a proporção de ácido linolênico estava positivamente relacionada com o teor de proteína bruta e negativamente relacionado com o conteúdo de fibras. Quando ocorre uma diminuição de proteína bruta, diminui ácidos graxos e consequentemente C13:3, quando aumenta FDN aumentou as concentrações de C18:0, C16:0, C18:1 e C18:2.

## 2.5 Perfil de ácidos graxos na carne

Os lipídios na carne se apresentam como triglicerídeos, uma composição de glicerol e três ácidos graxos. Os ácidos graxos são definidos pela quantidade de átomos de carbono que formam uma cadeia, com ou sem a presença de duplas ligações. Os ácidos graxos podem ser classificados em saturados (sem dupla ligação) ou insaturado (com dupla ligação). Esses ácidos graxos ainda são divididos monoinsaturado, polinsaturado e saturado. Os ácidos graxos ômega 6 e ômega 3 são ácidos graxos com mais de uma ligação e são considerados essenciais porque o organismo não tem a capacidade de sintetizá-los, temos que obter esses ácidos graxos da dieta.

A carne dos ruminantes, quando comparada à dos monogástricos, possui maior concentração de ácidos graxos saturados e menor relação poliinsaturados:saturados, sendo resultado do processo de biohidrogenação dos ácidos graxos insaturados pela ação de microrganismos ruminais (FRENCH et al., 2000). Vários fatores podem afetar o processo de biohidrogenação e a composição dos ácidos graxos. Dentre esses fatores, estão o sistema de alimentação, a composição das dietas, a relação volumoso:concentrado e o tipo de volumoso utilizado (DEMIREL et al., 2006; NUERNBERG et al., 2008).

A carne de cordeiro contém aproximadamente 4% de gordura (Prata, 1999), é rica em ácidos graxos saturados e monoinsaturados, com menores quantidades de poliinsaturados (MONTEIRO et al., 2009). Os ácidos saturados mais encontrados nesta carne são o mirístico (C14:0), palmítico (C16:0) e

esteárico (C18:0); os monoinsaturados palmitoleico (C16:1 n7) e oleico (C18:1 n9) e os poliinsaturados linoleico (C18:2 n6), linolênico (C18:3 n3) e araquidônico (C20:4 n6).

O perfil dos ácidos graxos pode estar relacionado com a genética dos animais, idade, gênero, raça, mas o efeito mais significativo é determinado pela nutrição do animal (DE SMET et al., 2004). Em ruminantes em geral há uma grande diferença na concentração de ácidos graxos ingeridos pelo animal e o perfil lipídico encontrados na carne. Isso ocorre porque os ruminantes consomem dietas ricas em ácidos graxos poliinsaturados advindos das pastagens, mas no rúmen as bactérias por meio do processo da biohidrogenação os transformam em ácidos graxos saturados.

Os consumidores estão cada dia mais preocupados com a qualidade de sua alimentação. Atenção especial tem sido dada ao perfil de ácido graxos (FA) e sua partição em ácidos graxos saturados (SFA) e ácidos graxos poliinsaturados (PUFA), especialmente n-3 em detrimento de n-6 (HAJJI et al., 2016). A carne a pasto fornece uma maior concentração de ácido trans-vacênico (TVA) (C18: 1 t11), um importante ácido graxo monoinsaturado para a síntese de ácido linoléico conjugado (CLA: C18: 2 c-9, t-11), o qual é sintetizado nos tecidos corporais (BAUMAN et al., 2006). O ácido linoleico conjugado é um ácido graxo encontrado apenas em produtos de origem animal, não sendo sintetizado pelo organismo humano. O CLA é o único ácido graxo anti-carcinogênico, sendo capaz de evitar e combater o câncer (HA et al., 1987). Além disso, os animais alimentados a pasto tendem a ter mais baixo teor de gordura, uma consideração importante para os consumidores interessados na diminuição do consumo geral de gordura (DALEY et al., 2010).

Daley et al. (2010), realizaram uma revisão que abrangeu três décadas de pesquisa e concluíram que a carne de animais alimentados com pastagem apresentam um perfil lipídico mais desejável, quando comparado aos animais que se alimentavam com grãos. A carne de animais criados a pasto também apresenta maior concentração de isómeros de CLA, e melhor proporção de n6:n3, que é mais desejável devido aos seus benefícios para a saúde. A literatura, portanto, tem evidenciado o efeito benéfico do pasto no perfil lipídico do músculo quando comparados à animais confinados. Por outro lado, mesmo em animais terminados a pasto podem ocorrer diferenças na concentração de certos ácidos graxos (LOURENÇO et al., 2007; PRACHE et al., 2011), sendo tais diferenças muitas vezes atribuídas à presença de leguminosas na pastagem.

### 3. HIPÓTESES E OBJETIVOS

A hipótese central do trabalho é de que o tipo de pasto tropical utilizado em sistemas de produção de cordeiros altera a qualidade da carne de cordeiros produzida.

Neste sentido, o objetivo geral foi mensurar as características produtivas do capim aruana e do feijão guandu, e características qualitativas das dietas disponibilizadas aos cordeiros, e seus reflexos sobre a qualidade da carne de cordeiro.

Os objetivos específicos foram:

1. Determinar e avaliar, as forragens tropicais (capim aruana, feijão guandu e mistura), quanto seu potencial produtivo.
2. Determinar a qualidade, perfil lipídico e os teores de taninos e tocoferóis presentes nas dietas dos cordeiros, provenientes das forragens tropicais (capim aruana, feijão guandu e mistura);
3. Avaliar os efeitos dos tipos de pastagem (capim aruana, feijão guandu e consórcio) e dos taninos, sobre as características da carcaça dos cordeiros;
4. Avaliar os efeitos dos tipos de pastagen (capim aruana, feijão guandu e mistura), e dos taninos, sobre o perfil lipídico e concentração de tocoferol na carne dos cordeiros;
5. Correlacionar a presença de antioxidantes (taninos e tocoferóis) com a coloração da carne e oxidação lipídica.

## **CAPÍTULO II**

**FEEDING SYSTEMS AND TOCOPHEROL CONCENTRATION IN THE DIET  
AND THEIR EFFECTS ON THE QUALITY OF LAMB MEAT: META-ANALYSIS**

Este capítulo é apresentado de acordo com as normas de publicação do jornal  
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**FEEDING SYSTEMS AND TOCOPHEROL CONCENTRATION IN THE DIET  
AND THEIR EFFECTS ON THE QUALITY OF LAMB MEAT: META-  
ANALYSIS**

**SISTEMAS DE ALIMENTACIÓN Y CONCENTRACIÓN DE TOCOFEROL EN LA  
DIETA Y SUS EFECTOS EN LA CALIDAD DE LA CARNE DE CORDERO:  
META-ANALISIS**

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Short title: Effect of food and antioxidants on meat quality

**ABSTRACT**

The presence of antioxidants in forage plants can modify the composition and quality of ruminant meat. It is known, for example, that the concentrations of some fatty acids in the diets of animals can alter the ratio of n-6: n-3, fatty acids and the concentration of conjugated linoleic acid (CLA) in the meat, yielding meat of better dietary quality. The objective of the present study was to evaluate, through a meta-analysis, the effects of the alimentary systems and the tocopherol levels found in the diet of lambs on the qualitative characteristics of their meat. A search of the computerized literature in Science Direct databases, PubMed, Scopus, and the Scielo virtual library was carried out to select works that evaluated the quality of lamb meat. As a first requirement for inclusion in the meta-analysis, articles were selected with the keywords "tocopherol", "meat" and "lamb". The data were classified according to the type of food system and the

level of tocopherol in the diet of lambs. Production systems alter the qualitative characteristics of meat. Lambs raised exclusively on pasture present a higher concentration of tocopherol in their meat, a lower ratio of omega 6: omega 3, a lower concentration of omega 6 and a higher concentration of CLA. Regardless of the dietary systems, when we classify the tocopherol levels of the lambs' diet, the concentration of tocopherol and the level of fatty acids are altered by the levels of tocopherol in the diet. The lower level of tocopherol in the diet generated a meat with lower concentration of tocopherol and with greater propensity to lipid oxidation.

**Key words:** antioxidant, concentrated, omega 3, oxidative stress, pasture

## RESUMEN

El uso de antioxidantes de las plantas puede modificar la composición y la calidad de la carne de rumiantes. Se sabe, por ejemplo, que las concentraciones de algunos ácidos grasos en las dietas de los animales pueden alterar la relación de n-6: n-3 y la concentración de ácido linoleico conjugado (CLA) en la carne, produciendo carne de mejor calidad dietética. El objetivo del presente estudio fue evaluar, mediante un metanálisis, los efectos de los sistemas alimentarios y los niveles de tocoferol encontrados en la dieta de los corderos sobre las características cualitativas de su carne. Se realizó una búsqueda de la literatura computarizada en las bases de datos Science Direct, PubMed, Scopus y la biblioteca virtual Scielo para seleccionar trabajos que evaluaran la calidad de la carne de cordero. Como primer requisito para la inclusión en el metanálisis, los artículos se seleccionaron con las palabras clave "tocoferol", "carne" y "cordero". Los datos se clasificaron según el tipo de sistema alimentario y el nivel de tocoferol en la dieta. Los sistemas de producción alteran las características cualitativas de la carne. Los corderos criados exclusivamente en pastos presentan una mayor concentración de tocoferol en su carne, una proporción menor de omega 6: omega 3, una menor concentración de omega 6 y una mayor concentración de CLA. Independientemente de los sistemas dietéticos, cuando clasificamos los niveles de tocoferol de la dieta de los corderos, la concentración de tocoferol y el nivel de ácidos grasos se ven alterados por los niveles de tocoferol. El nivel más bajo de tocoferol en la dieta generó una carne con

una menor concentración de tocoferol y con una mayor propensión a la oxidación de los lípidos.

**Palabras claves:** antioxidante, concentrado, estrés oxidativo, omega 3, pasto

## INTRODUCTION

The search for healthier foods with a longer shelf-life has attracted the interest of both consumers and the food industry. Due to the behavioural modifications of the consumer population, the offering of products considered to be healthy has led several researchers to try to manipulate the composition of foods, especially elements of foods that are related to cardiac problems (Itavo et al., 2016). Extending the shelf life of meat by delaying oxidative deterioration is an important goal in the meat supply chain (Luciano et al., 2009).

The search for healthier foods has led researchers in meat science to characterize the lipid profile of these foods. Shelf life attributes, such as colour stability and oxidation of meat fatty acids, are influenced by the composition of muscle tissues, which, in turn, are governed by antioxidant capacity, the concentration of haem pigments (haem iron) and the concentration and lipid composition found in muscles. All these characteristics are linked to the feed given to animals (Papuc et al., 2017; Luciano et al., 2009).

Tocopherol is an antioxidant that plays an important role as an inhibitor of the oxidation of free radicals, and it reacts with oxygen and makes it impossible to convert unsaturated fatty acids into aldehydes (Lobo et al., 2010). The most frequent cause of deterioration of meat quality is lipid oxidation (Papuc et al., 2017). These reactions result in changes in colour, loss of taste and nutritional value and thus limit the shelf-life of the meat (Luzia and Jorge, 2009). The addition of tocopherol to a lamb diet, via either pasture or supplementation, may maintain the desirable red colour of the meat in natura on display at a retail store. The protective effect of tocopherol is exerted by retarding the oxidation of the oxymyoglobin pigment and inhibiting the oxidation of polyunsaturated fatty acids (PUFAs) (Gonzalez-Calvo et al., 2015).

Previous studies have reported the benefits of tocopherol in the quality and maintenance of lamb meat quality. However, not all studies evaluate meat quality-related variables related to oxidation and fatty acid composition due to the complexity and limitations of each study. Therefore, it is important to carry out a study that compiles these studies to obtain novel information concerning the effects of tocopherol on various aspects of meat quality. In such cases, meta-analysis is a valuable tool. Thus, the objective of the present study was to evaluate the effects of food systems as well as tocopherol levels in the diet on the qualitative characteristics of lamb meat using a meta-analysis.

## MATERIAL AND METHODS

### Bibliographical research and selection of studies

A literature search was conducted in multiple databases, including Science Direct, PubMed, Scopus and the Scielo virtual library, to select studies that evaluated the quality of the lamb regarding the tocopherol content in the diet of the animals and the type of food offered to the animal. This review was conducted in four stages: identification, selection, evaluation of eligibility and inclusion, as recommended in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009).

Combinations of the search terms "tocopherol", "meat" and "lamb" were used in the research. The selected articles were published between 2007 and February/2018. After the search, 272 articles were found, and 25 articles were repeated in some databases. After this first selection, the abstracts of these 242 articles were read, in which the first requirement for inclusion in the meta-analysis was to find the keywords: "tocopherol", "meat" and "lamb". The selected papers were read in full for a second stage of selection, according to pre-defined eligibility criteria, and after this stage, 31 studies were selected for the meta-analysis. The work of Turner et al., 2002, which aided in the classification of levels, described below, was also added to the base.

The information extracted from articles was organized in a spreadsheet. The concentrations of tocopherol in the diet and the types of food offered to the lambs were included in the analysis. The variable responses extracted from the articles and used in

the meta-analysis are described in Table 1. Among them are the concentrations of tocopherol and fatty acids, as well as characteristics of the lamb meat, including 1) meat tocopherol content (Tocopherol); 2) total fatty acid content (FA); 3) monounsaturated fatty acid content (MUFAs); 4) polyunsaturated fatty acid content (PUFAs); 5) saturated fatty acid content (SFAs); 6) fatty acid content of omega 3 (*n*-3) and omega 6 (*n*-6) groups; 7) ratio of *n*-6: *n*-3; 8) conjugated linoleic acid content (CLA); 9) thiobarbituric acid reactive substances, six days of storage (TBARS); 10) brightness (L \*); 11) redness (a\*), 12) yellowness (b\*), 13) hue angle (H°) and 14) chroma(C\*).

**Table 1.** Description of the primary studies included in the meta-analysis

Studies <sup>1</sup>	Country	Type of food	Observed variables
Álvarez et al., 2014	Spain	Concentrated Pasture	L*; a*; b*; C*
Bellés et al., 2018	Spain	Concentrated -	Tocopherol; MUFA; PUFA; SFA; n-6; n-3; n-6:n-3
Berthelot et al., 2014	France	Concentrated -	FA; MUFA; SFA; n-6; n-3; n-6:n-3; CLA
Bhatt et al., 2015	India	Concentrated Pasture	MUFA; PUFA; SFA; n-6:n-3; CLA; TBARS
Brito et al., 2017	Australia	Pasture	Tocopherol; n-6:n-3; TBARS
González-Calvo et al., 2014	Spain	Concentrated -	Tocopherol; MUFA; PUFA; SFA; n-6; n-3; n-6:n-3; TBARS; CLA; L*; a*, b*
D'Alessandro et al., 2012	Italy	Concentrated Pasture	Tocopherol; TBARS
Hopkins et al., 2013	Australia	Pasture	Tocopherol
Jose et al., 2016	Australia	Concentrated Pasture	Tocopherol
Kasapidou et al., 2012	United Kingdom	Concentrated Pasture	Tocopherol; n-6; n-3; n-6:n-3; TBARS; CLA
Lee et al., 2007	United States	Concentrated -	Tocopherol
Liu et al., 2013	China	Concentrated -	Tocopherol; MUFA; PUFA; SFA; n-6; n-3; n-6:n-3
Lobón et al., 2017	Spain	Concentrated Pasture	Tocopherol; L*; a*; b*, H°, C*
Milewski et al., 2014	Poland	Pasture	Tocopherol; FA; MUFA; PUFA; SFA; n-6; n-3; n-6:n-3; CLA

Morán et al., 2012 a	Spain	Concentrated -	L*; a*;b*
Morán et al., 2012 b	Spain	Concentrated -	TBARS
Morán et al., 2013	Spain	Concentrated -	MUFA; PUFA; SFA; n-6; n-3; n-6:n-3; CLA
Muela et al., 2014	Spain	Concentrated -	TBARS; L*; H°, C*
Muíño et al., 2014	Spain	Concentrated -	Tocopherol; MUFA; PUFA; SFA; n-6; n-3; n-6:n-3; TBARS
Ortuño et al., 2015	Spain	Concentrated -	TBARS; L*; a*; H°; C*
Petron et al., 2007	Belgium	Pasture	Tocopherol; TBARS; L*; a*;b*
Ponnampalam et al., 2012 a	Australia	-	Tocopherol; FA; PUFA; n-6; n-3; n-6:n-3; TBARS
Ponnampalam et al., 2012 b	Australia	Concentrated Pasture	Tocopherol; PUFA; n-6; n-3; n-6:n-3; a*
Ponnampalam et al., 2016	Australia	Concentrated Pasture	Tocopherol; n-6:n-3
Ripoll et al., 2011	Spain	Concentrated Pasture	TBARS; L*;H°
Ripoll et al., 2013	Spain	Concentrated Pasture	Tocopherol; TBARS; L*, a*; b*, H°; C*
Sales et al., 2013	Brazil	Concentrated -	L*; a*;b*
Simitzis et al., 2013	Greece	Concentrated -	FA; L*;a*
Turner et al., 2012	United States	Concentrated Pasture	Tocopherol
Vieira et al., 2012	Spain	Concentrated -	Tocopherol; MUFA; PUFA; SFA; n-6; n-3; n-6:n-3; TBARS; L*; a*;b*
Yagoubia et al., 2018	Tunisia	Concentrated -	Tocopherol; n-6:n-3; TBARS; L*, a*;b*; H°,C*

<sup>1</sup> The list of references used for the meta-analysis and the variable responses extracted from the articles

## Data analysis

The data from the different articles were classified according to the type of food consumed by the lambs and the level of tocopherol found in the diet. In the first

classification, the data were grouped according to the three types of food systems: Pasture (animals kept exclusively on pasture); Concentrated (animals kept exclusively receiving some type of concentrate with little added of bulky to the feeding); Pasture + Concentrated (animals kept in the pasture or silage receiving some type of concentrate). In the second classification, the data were grouped according to the levels of tocopherol found in the diet of lambs: Level 1 (animals receiving in the diet up to 200 mg kg<sup>-1</sup> DM); Level 2 (animals receiving 201 to 400 mg kg<sup>-1</sup> DM) and Level 3 (animals receiving more than 400 mg kg<sup>-1</sup> DM tocopherol in their diet). The classification of tocopherol levels in their diet was based on the work of Turner et al. (2002), who reported that tocopherol levels above 401 mg kg<sup>-1</sup> reach a plateau, and more tocopherol is not accumulated in the meat. Therefore, we used levels up to 400 mg kg<sup>-1</sup> as an intermediate level and included a level above (more than 400 mg kg<sup>-1</sup>) and another below (up to 200 mg kg<sup>-1</sup>).

After the normality test of the residues, a variance analysis was performed using the MIXED procedure in the SAS statistical program. A test of selection of structures was made using the Bayesian information criterion (BIC), from which the structure "variance component (VC)" was selected. When differences were observed, the means were compared using the LSmeans test de Tukey at 5% the tendencies when  $0.05 \leq P < 0.10$  probability test. Variables were also submitted to Pearson correlation analysis and multiple regression using the STEPWISE procedure (Forward = 0.05). The variables, conjugated linoleic acid (CLA) and omega n-6: omega n-3 ratio were transformed via log<sup>10</sup> because they did not present normality. For the variables b \*, H ° and C \* of colour, when compared to foods, we only have the comparison between pasture x concentrate, by absence of values for the mixed classification (concentrate + pasture). For the variable b \* of colour, when compared to tocopherol levels, we only have the classification between level 1 x level 3, due to the absence of values for the intermediate level (level 2).

## RESULTS AND DISCUSSION

### **Effects of food systems on the quality of lamb meat**

The tocopherol content in the muscle of lambs kept exclusively on pasture is 38.04% higher compared to the other food systems ( $2.54 \text{ mg kg}^{-1}$ ; Table 2). The proportion of forage in the diet of lambs receiving concentrate + pasture was not sufficient to alter the deposition of tocopherol in the lambs' meat (Table 2). The grazing is a option for increasing  $\alpha$ -tocopherol muscle content, as green forages are rich in this compound (Ripoll et al., 2013; Jose et al., 2016).

The type of tocopherol that the food system made available may be been related to the concentration of tocopherol in lamb meat because according to Ponnampalam et al. (2012a), who kept the lambs in a perennial pasture or in an annual pasture and fed a concentrated diet, a higher concentration of alpha-tocopherol was found in the muscle of lambs kept in the perennial pasture. This result is because animals in perennial pastures, ingested greater amounts of alpha-tocopherol and lambs who were kept in the annual pasture in senescence and received the concentrated diet ingested more gamma-tocopherol. The alpha-tocopherol form is most prevalent in different tissues of animals and humans. In reality, there is a preference for the absorption of alpha-tocopherol in relation to gamma-tocopherol in the peripheral system of the body (Ponnampalam et al., 2012a).

**Table 2. Attributes of lamb meat from different feeding systems.**

Variables	Food Systems			P <sup>1</sup>	SEM <sup>2</sup>	n <sup>3</sup>
	Pasture	Concentrated	Pasture+Concentrated			
Tocopherol <sup>4</sup>	4.10 <sup>a</sup>	2.45 <sup>b</sup>	2.63 <sup>b</sup>	0.0003	0.25	67
FAs <sup>5</sup>	43.85	37.88	44.17	0.6828	4.47	14
SFAs <sup>5</sup>	51.98	46.27	42.01	0.4400	4.53	31
MUFAs <sup>5</sup>	41.33 <sup>b</sup>	41.55 <sup>b</sup>	48.34 <sup>a</sup>	0.0260	2.95	31
PUFAs <sup>5</sup>	5.70	6.23	7.65	0.5040	1.35	34
<i>n</i> -6 <sup>5</sup>	2.52 <sup>b</sup>	6.14 <sup>a</sup>	4.02 <sup>ab</sup>	0.0431	1.15	41
<i>n</i> -3 <sup>5</sup>	1.23	1.52	0.83	0.2904	0.35	41
<i>n</i> -6: <i>n</i> -3 <sup>5</sup>	1.88 <sup>b</sup>	5.71 <sup>a</sup>	4.84 <sup>a</sup>	0.0003	0.74	53
CLA <sup>5</sup>	0.85 <sup>a</sup>	0.24 <sup>b</sup>	0.76 <sup>a</sup>	0.0118	0.15	22

TBARS <sup>6</sup>	0.23	0.94	0.29	01241	0.35	51
Luminosity	58.77 <sup>a</sup>	43.16 <sup>b</sup>	31.32 <sup>c</sup>	0.0005	4.65	38
Redness	10.33	9.34	12.40	0.1553	1.54	30
Yellowness	15.04 <sup>a</sup>	10.90 <sup>b</sup>	-	0.0001	0.51	21
Hue angle	51.70	35.18	-	0.3666	12.31	18
Chroma	15.63	13.13	-	0.0840	0.91	16

<sup>1</sup>probability; <sup>2</sup> standard deviation of the mean; <sup>3</sup> number of repetitions; <sup>4</sup> mg kg<sup>-1</sup>; <sup>5</sup> % ; <sup>6</sup> mg of malonaldehyde kg<sup>-1</sup>; <sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ . For the variables b \*, H ° and C \* of colour when compared to foods, we only have the comparison between pasture x concentrate, by absence of values for the mixed classification (concentrate + pasture).

Type of food systems only affected the MUFA, *n*-6 and *n*-6:*n*-3 ( $P < 0.05$ ; Table 2). The Pasture+Concentrated food systems resulted in a meat with greater MUFA, there may have been a higher rate of passage of these fatty acids through the rumen with the mixed feed, and also because they are less prone to lipid oxidation (Díaz et al., 2011).

The Pasture food systems resulted in a meat with less *n*-6 and lower *n*-6: *n*-3 ratio. The lower values of *n*-6 fatty acids in the meat of fed animals in pasture systems may be related to a lower concentration of this lipid in plants and, consequently, lower accumulation in the intramuscular lipids of animals (Popova et al., 2015). The ability of polyunsaturated fatty acids to be incorporated into phospholipids is limited, and *n*-6 fatty acids compete more efficiently than *n*-3 fatty acids (Wood et al., 2008). The *n*-6: *n*-3 ratio the meat of lambs raised exclusively on pasture (1.88) is within the limits recommended by Simopoulos (2002), 4: 1 to be beneficial to human health.

In this meta-analysis, it was found that the concentration of *n*-6 in the diet was the main cause of the variation of the *n*-6: *n*-3 ratio in lamb meat. There was no effect of dietary systems on the concentration of omega-3 (*n*-3) family of fatty acids (Table 2), which contradicts trends that have been reported in the literature and indicates that there is a higher concentration *n*-3 in meat from ruminant animals when the animals are fed exclusively in a pasture (Cividini et al., 2014). A possible explanation for lambs kept exclusively on pasture has not differed with regards to the *n*-3 concentration of the other

systems, and it may be related to the variety of pasture types, their different phenological stages, the composition of the pasture, and the presentation and conservation of this pasture (Garcia et al., 2016). According to Castagnara et al. (2011) and Garcia et al. (2016), growth rate and age of the plant influences the concentration of fatty acids. The proportion of leaves decreases over time, and the stem is half to one-third the concentration of fatty acids in the leaves.

The participation of pasture in food systems influence the concentration of conjugated linoleic acids (CLA) in the meat of lambs (Table 2). Lambs consuming Pasture or Pasture + Concentrated showed a proportion CLA that was three times higher ( $P < 0.05$ ; Table 2) than lambs consuming exclusively Concentrated. Boughalmi and Araba (2016), working with three food systems, also found that when animals are grazing, or not receiving supplement, the concentration of CLA ( $0.88 \text{ g } 100 \text{ g}^{-1}$ ) is higher than animals receiving exclusively concentrate ( $0.51 \text{ g } 100 \text{ g}^{-1}$ ). This result can be explained by the fact that when ruminants consume grass, it includes polyunsaturated fatty acids (PUFAs), which are biohydrogenated in the rumen and transformed into vaccenic acids, which are the precursor to CLA (Hajji et al., 2016). One of the best known forms of CLA is rumenic acid (C18: 2 cis9, trans11), which provides benefits to human health (Salter and Tapper, 2013).

Lipid peroxidation of meat is measured by the content of thiobarbituric acid reactive substances (TBARS). Food systems did not show a significant effect on TBARS, with a mean of  $0.48 \text{ mg of malonaldehyde kg}^{-1}$  (Table 2). The stability of fatty acid oxidation of meat is influenced by the composition of muscle tissues, of which are tightly related to feed offered (Daley et al., 2010). The results found in this meta-analysis contradict what is described in the literature which report that grazing animals consume a greater amount of antioxidants by decreasing lipid oxidation of the meat. Hajji et al. (2016) found an important effect of the food system on lipid oxidation: the animals kept on pasture showed lower concentrations of TBARS. In the data found in this meta-analysis, we observed that the results present values with great variation among themselves, but not enough to detect a significant difference (Table 2). Popova et al. (2015) worked with data from a meta-analysis, and they also did not find a significant effect of the food system on the total lipid content.

The lambs the Pasture food systems present the greater L\*, b\* value ( $P<0.05$ ) and tendency of greater C\* value ( $P=0.0840$ ) in the meat, when compared to the other food systems (Table 2). Atti et al. (2013), working with lambs also found higher values of b \* in the coloration of meat from lambs they grazed compared to those with concentrate. Animals with grazing as part of their diet consume a greater proportion of carotenoid pigments and antioxidants that contribute to increases in the haeminic pigments, changing the colour and brightness of the lamb meat (Costa et al., 2011). The darker coloration of the meat of the animals on pasture is related to the increase of myoglobin in the muscle, justified by the greater physical activity of these animals (Perlo et al., 2008).

### **Effect of tocopherol levels of lambs' diet on the quality of lamb meat**

Meta-analysis shows that the tocopherol content of the diet has an influence on the tocopherol content in lamb meat. This influence may be affected by the different levels of tocopherol found in the food . As expected, the tocopherol content in the lamb meat is lower when the dietary content is less than  $200 \text{ mg kg}^{-1}$  DM (level 1 - Table 3). However, when tocopherol levels in the diet are above  $400 \text{ mg kg}^{-1}$  DM, there is no significant increase in the concentration of tocopherol in the meat. The highest concentration of tocopherol in the diet (above  $400 \text{ mg kg}^{-1}$  DM - level 3) corresponds to a lower meat concentration than when it is  $200$  to  $400 \text{ mg kg}^{-1}$  DM (level 2;Table 3).This result suggests that there is a limit to the inclusion of tocopherol in the diet with an effect on the concentration of tocopherol in the muscle. This result was also found by Turner et al. (2002) who, working with different levels of alpha-tocopherol in the diet, found a quadratic equation regarding dose and muscle concentration. They reported that a plateau occurs where the highest concentration of tocopherol in the diet occurred when the animals received  $403 \text{ mg kg}^{-1}$  MS. However, the duration of dietary supplementation may be a factor that influences the deposition of alpha-tocopherol. In general, higher tocopherol content in the diet or longer supplementation time are associated with higher tocopherol concentrations in the meat (Ripoll et al., 2013).

**Table 3.** Attributes of lamb meat for three levels of tocopherol in the diet.

Variables	Leve's							
	Level 2							
	Level 1		201 - 400	Level 3				
	0- 200 mg	kg <sup>-1</sup> MS	mg kg <sup>-1</sup>	> 400 mg	kg <sup>-1</sup> MS	P <sup>1</sup>	SEM <sup>2</sup>	n <sup>3</sup>
Tocopherol <sup>4</sup>	1.97 <sup>b</sup>	4.35 <sup>a</sup>	2.85 <sup>c</sup>	0.0003	0.27	67		
FAs <sup>5</sup>	27.38 <sup>b</sup>	35.13 <sup>ab</sup>	68.38 <sup>a</sup>	0.0446	8.49	14		
MUFAs <sup>5</sup>	42.38 <sup>ab</sup>	47.83 <sup>a</sup>	41.02 <sup>b</sup>	0.1196	2.06	31		
PUFAs <sup>5</sup>	6.05	6.10	7.43	0.6538	1.44	34		
SFAs <sup>5</sup>	48.01	47.39	44.52	0.8312	5.05	31		
<i>n</i> 6 <sup>5</sup>	4.50	4.56	3.62	0.7926	1.23	41		
<i>n</i> 3 <sup>5</sup>	1.31	1.53	0.73	0.2736	0.34	41		
<i>n</i> 6: <i>n</i> 3 <sup>5</sup>	4.04	2.69	5.70	0.0905	0.85	53		
CLA <sup>5</sup>	0.52	0.66	0.66	0.7447	0.16	22		
TBARS <sup>6</sup>	1.26 <sup>a</sup>	0.54 <sup>ab</sup>	0.27 <sup>b</sup>	0.0018	0.38	51		
Luminosity	45.05	41.79	46.41	0.7641	3.91	38		
Redness	10.14	12.24	9.70	0.7306	1.69	30		
Yellowness	9.22	-	8.83	0.5777	0.47	21		
Hue angle	40.11	34.41	55.85	0.1948	13.04	18		
Chroma	14.69	13.05	15.76	0.2779	1.07	16		

<sup>1</sup>probability; <sup>2</sup> standard deviation of the mean; <sup>3</sup> number of repetitions; <sup>4</sup> mg kg<sup>-1</sup>; <sup>5</sup> %; <sup>6</sup> mg of malonaldehyde kg<sup>-1</sup>; <sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ . For the variable b \* of colour, when compared to tocopherol levels, we only have the classification between level 1x level 3, due to the absence of values for the intermediate level (level 2).

Most of the groups of FA according the degree of saturation were not affected by level of tocopherol (Table 3). The level of tocopherols only affected the FA and MUFA ( $P < 0.05$ ; Table 3). The FA content in lamb meat increases as the tocopherol levels ingested by lambs in the diet increase, the highest FA content was observed when the

animals were supplemented with more than 400 mg kg<sup>-1</sup> DM (level 3 - Table 3). Berthelot et al. (2014), working at levels similar to those found in this analysis, reported that when lambs received the highest levels of tocopherol in the diet, they tended to have a higher FA concentration in the muscle.

The level 2 (201 - 400mgkg<sup>-1</sup> MS) resulted in a meat with greater MUFA ( $P<0.05$ ; Table 3). That could be related to the higher tocopherol theory in meat (Table 3), which could prevent oxidation of two MUFAs, resulting in a higher concentration of meat. Liu et al. (2013) Working with different levels of tocopherol supplementation in the diet, they state that there is an effect of tocopherol levels on the diet of lambs in the concentrations of MUFAs, PUFAs and SFAs in the meat. In our review, meta-analysis shows that dietary tocopherol levels do not influence PUFAs, and SFAs concentrations.

Meta-analysis shows that it does not have a significant effect of levels tocopherol on the omega-6, omega-3 and the n-6 / n-3 ratio in meat ( $P> 0.05$ ; Table 3). Because tocopherol is mainly present in green, pastures and that grass-fed animal have a lower n-6: n-3 ratio than animals fed with concentrate, it was expected that the tocopherol in the diet could influence the levels the omega-6 and omega -3 (Mapiye et al., 2012). However, this variation of the unsaturated fatty acids is influenced by different reasons, among them the finishing of the animal. Due to the different variations that occurred between the studies, it was not possible to determine the differences between the levels of tocopherol.

Although the CLA content was influenced by the feeding system to which the lambs were submitted (Table 2), the CLA content is not associated with the tocopherol concentration in the diet ( $P>0.05$ ; Table 3). Although there was a higher concentration of tocopherol in the pasture, this did not materialize as an instrument to improve the amount of CLA in the meat. Since CLA is primarily a product of ruminant animals, produced from the bacteria present in the ruminal environment of these animals, and since the level of tocopherol does not alter ruminal bacteria, the CLA content was similar between levels (Table 3).

Lipid oxidation, measured as TBARS, was significantly altered by the level of tocopherol in the diet ( $P<0.05$ ; Table 3). We observed a correlation between decreasing TBARS and increasing tocopherol dose in the diet ( $P = 0.0022$ ;  $R^2 = -0.41$ ). Kasapidou et al. (2009), working with levels also found a decrease in lipid oxidation with increasing

dose of tocopherol. Even though the level of lipid oxidation at level 1, which showed the highest concentration of TBARS, this value exceeded slightly the threshold accepted value of 1 mg MDA kg<sup>-1</sup> meat (Ripoll et al., 2011).

The level of tocopherol in diet did not affect the colour variables of meat ( $P>0.05$ ; Table 3). Ripoll et al. (2013), used different doses on different days with 500 mg tocopherol day<sup>-1</sup>, and they found no difference in the colour. This result shows that tocopherol in the diet does not affect the colouring of the meat. All lambs displayed average L\* values of 40, indicating a light-coloured and acceptable meat (Hajji et al., 2016).

## CONCLUSION

Food systems alter the qualitative characteristics of meat. Lambs exclusively raised on pasture present higher concentrations of tocopherol in meat, lower relation omega 6: omega 3, lower omega 6 and higher concentration of CLA. Regardless of dietary systems, tocopherol levels altered the concentration of tocopherol in meat. The lower level of tocopherol in the diet generated a meat with lower concentration of tocopherol and with greater propensity to lipid oxidation.

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## **CAPÍTULO III**

### **FORAGE PHYSICAL CHARACTERISTICS AND LAMBS DIET BIOCHEMICAL CONTENT IN TROPICAL PASTURES**

Este capítulo é apresentado de acordo com as normas de publicação do **Food Research International**.

## Forage physical characteristics and lambs diet biochemical content in tropical pastures

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**Abstract:** The aim of the study was to evaluate tropical pastures: aruana grass (*Panicum maximum* cv. IZ-5), pigeon pea (*Cajanus cajan*) and mixed (half of the area with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*), in relation to forage physical characteristics, and chemical composition, lipid profile and antioxidants of lambs diet, along the growing periods of forage. Lambs grazed all pastures continually during 92 days. Forage was sampled for simulating grazing every 21 days, except for the last sampling that was done at 29 days of interval. Forage production and bromatological composition of diet of lambs were evaluated. The pasture species presented variation of forage mass, stem production, leaf production and height over time of the evaluation. The insertion of legume in the diet of lambs does not alter the concentration of tocopherol, but over the evaluation periods the levels of condensed tannin and total tannin increase in the diet containing legumes. The majority of fatty acids studied were related to the neutral detergent fiber content of the diet. The participation of the legume in the diet of the lambs increases the content of omegas 3 and 6 available in the diet of lambs.

**Keywords:** Aruana grass, n6: n3, NDF, Pigeon pea, PUFA, tocopherol

### 1. Introduction

All over the world, pastures are the main part of ruminants diet. According to the Brazilian Census of Agriculture, 2006 (IBGE, 2010), the total pasture area (natural and cultivated) in Brazil was 172.3 million hectares, therefore, tropical forage species presents great potential for ruminant feeding (Castagnara et al., 2011; Euclides et al., 2008). Tropical

pastures besides the high potential of forage production, presents composition of fatty acids and of antioxidants (Paulino et al., 2015; Louvandini et al, 2011), which can have a significant impact on meat quality, and also repercussions on human health.

Among the biochemical compounds of tropical plants, the presence of tannins and tocopherols stands out. These compounds are not related to the metabolism and maintenance of the plant directly, but in its ability to survive and adapt, promoting its maintenance and permanence in the environment. Tropical forages can vary their chemical composition and their production with the advancement of pasture use, due to changes in structure occurred by the advancement of the phenological stage. Tropical grass such as aruana (*Panicum maximum* cv. Aruana) has been shown to have high nutritional quality, with intense grazing tolerance and appropriate forage production (Gerdes et al., 2005 and Paulino et al., 2015). Besides that, the use of tropical legumes can potentially increase the sward quality, especially related to a greater supply of crude protein and condensed tannins (Vitti et al., 2005 and Louvandini et al, 2011).

As the plants grow several changes can happen, as forage growth rate (Castagnara et al., 2011), chemical composition (Pahkala and Pihala, 2000), antioxidants levels (Lynch et al., 2001) and lipid profile (Khan et al., 2012). Sources of variation of forage lipid concentration, for example, are plant species, growth stage, temperature and light intensity (Garcia et al., 2016).

There is a growing interest in the lipid composition of ruminant products, specially the fatty acids composition. The current levels of lipids ingested by humans are considered too high, and the overall fatty acid composition imbalanced. Animal diet is an important factor to modulate the meat fatty acid profile, and fresh pastures are an important source for beneficial fatty acids, but its composition can change according to the plant species and also the phenological state.

It is essential to find a balance between the forage production, chemical composition and lipid profile of the diet, in aim to produce a high quality final product (meat or milk), with pleasant characteristics to consumers. Therefore, the objective of this study was to evaluate tropical pastures: Aruana grass (*Panicum maximum* cv. IZ-5), Pigeon pea (*Cajanus cajan*) and Mixed (half of the area with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*), in relation to forage physical characteristics, and chemical composition, lipid profile and antioxidants of lambs diet, along the growing periods of forage.

## **2. Material and methods**

The experimental protocol, which involved finishing lambs, were reviewed and approved by the Ethics Committee on the Use of Animals of Universidade Federal do Rio Grande do Sul (ECUA-UFRGS), Project No: 27830 - Lambs sustainable production systems.

### *2.1 Experimental site: Location and climatology*

The study was conducted at the Agronomic Experimental Station (EEA) of Universidade Federal do Rio Grande do Sul (UFRGS), Brazil (Latitude  $29^{\circ} 13' 26''$  S, Longitude  $53^{\circ} 40' 45''$  W). The experiment was carried out from 11<sup>th</sup>January, 2016 to 12<sup>th</sup>April, 2016, being 92 days of experimental period. The climatic conditions during the experimental period are shown on Figure 1.

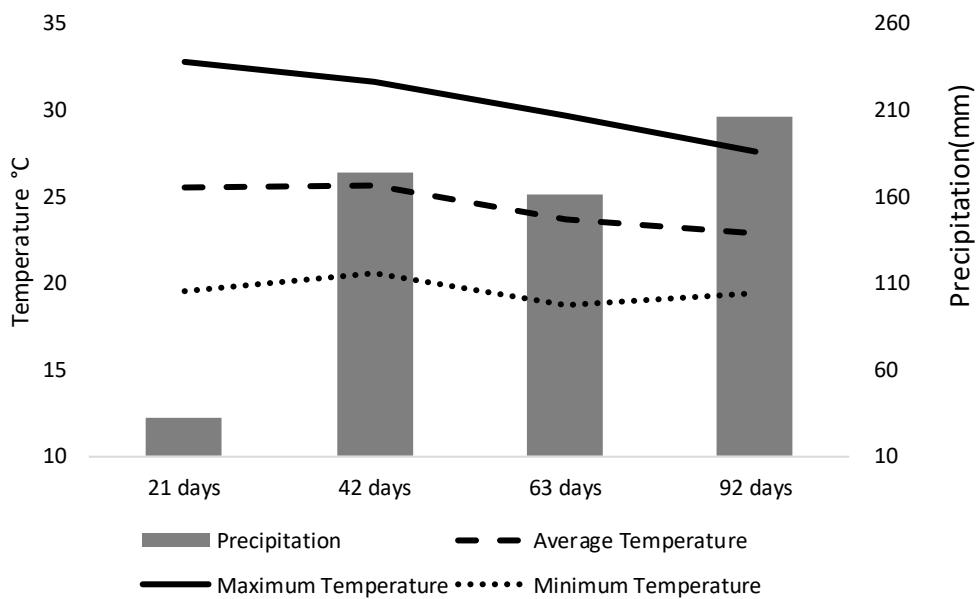


Figure 1- Average precipitation, Average temperature, Maximum temperature and Minimum temperature data for the experimental period (from 11<sup>th</sup>January, 2016 to 12<sup>th</sup>April, 2016)

### *2.2 Experimental design*

The experiment was set out in a randomized block design. Three blocks were used to control the effects of soil type and slope of the land, and the three pastures were randomly distributed within each block. Each experimental unit was formed by a sward of a 0.2 ha paddock, each sward had three replications with 6 lambs in each. Three pastures were

compared: 1) Aruana grass (*Panicum maximum* cv. IZ-5; AG); 2) Pigeon pea (*Cajanus cajan*; PP) and 3) Mixed (half of the area with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*) grass forage.

During 92 days of assessment, lambs grazed continually the paddocks with variable number of regulating animals in order to maintain the same herbage allowance in all treatments (10-11 kg dry matter.100 kg<sup>-1</sup> of body weight ha. day<sup>-1</sup>). This herbage allowance was adjusted every 21 days, using the put-and-take technique (Mott and Lucas, 1952).

### *2.3 Pasture production*

Since the first day of experiment, forage sampling was applied every 21 days, except the last sampling, which was done at 29 days. Fifty random height measurements were recorded from each paddock using a sward stick (Bircham, 1981).

The forage mass (FM) and daily accumulation rate (DAR) were obtained by cutting, at the ground level, the herbage contained in a 0.25 m<sup>2</sup> quadrat, taking 9 sampling points per paddock, three cuts were random inside the piquet, three cuts were inside the cage and the other three cuts were outside the cageOne sub-sample was used to determine the dry matter (DM) and the second one to assess the botanical study (leaf blade, stem and senescent material). With leaf and stem percentage it was obtained the leaf: stem ratio (L: S). With the data obtained from forage production and with the percentage of leaf blade and stem, the values of leaf production (LP; kg DM ha<sup>-1</sup>) and stem production (SP; kg DMha<sup>-1</sup>) were calculated.

### *2.4 Sampling of the chemical composition of the diet*

Forages samples was collected according to the Hand-plucking technique (Euclides et al. 1992), the lambs were followed for grazing, the trained evaluators, similar samples collected harvested by lambs. Samplings were carried out in two shifts, obeying the grazing peaks of the lambs (at dawn and at dusk of the day). Care was taken to protect the samples from light and high temperatures. Soon after the morning and afternoon collection a sub-sample was taken for the determination of alpha-tocopherol of the lambs diet. At the end of the day of collection the samples were homogenized, a sub sample was removed for further lyophilization and tannin determination, and the remainder of the sample was oven-heated at 65 ° C for 72 hours, milled in Wiley type mill at 1 mm for the determinations of its chemical composition, fatty acid profile and dry matter *in situ* digestibility (ISDMD) of the lambs diet.

## 2.5 Analysis

### 2.5.1 Tocopherol and tannin

The alpha-tocopherol content was determined in samples of two daily periods (morning and afternoon), representing the grazing peaks of the animals in the pasture following the methodology of Val et al. (1994). Samples were chopped with a scissors into 0,9 cm<sup>2</sup> particles and then homogenized. Two subsamples of 0.25g were put in a 15 ml Falcon flask, containing 5ml of acetone and a pinch of ascorbic acid. The subsamples were homogenized in a TURRAX for 30 seconds at 13000 rpm and then agitated for 20 minutes. Samples were centrifuged for 4 minutes at 2000 rpm. The supernatant collected and filtered into a 1.5 ml vial. The determination was done with an HPLC (Waters Acquity UPLC CLASS), with mobile phase MeOH: H<sub>2</sub>O 93: 7 (v: v), and NovaPak4 µm 30cm column. Oven temperature, flow rate, pressure and injection volume were 40°C, 1ml/min, 2620 psi and 10 µl respectively. The eluate was detected using a Waters 474 scanning fluorescence detector set for the emission at 322 nm and excitation at 293 nm. The α-tocopherols was identified by comparing their retention times with those of the corresponding standard.

The tannin contents of the forages were determined in lyophilized forage samples and grounded in a Wiley mill at 0.5 mm. The total tannin (TT), hydrolyzable (TH) and condensed (TC) tannin contents were determined by adapting the methodologies of Grabber et al. (2013), Makkar (2000), Porter et al. (1986) and Saura-Calixto et al. (2007), expressed as gram equivalent (eq-g) of leucocyanidine kg<sup>-1</sup> of DM. For the calculations, the following formula was used: eq -g Leucocyanidine (L) / kg DM = {absorbance x [10x (dilution volume in ml) / (460 x sample weight)] / (DM, in kg)} X 10.

### 2.5.2 Chemical composition

Chemical composition of forages was analyzed according to official methods reported by AOAC, 1995. Forage samples were dried in a forced air circulation oven at 65°C for 72 hours and milled in a Willey type mill at 1 mm. Dry matter (DM), ash, crude protein (CP) and (EE) ether extract contents were determined. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were carried out following the sequential procedure of Van Soest et al. (1991). The dry matter *in situ* digestibility (ISDMD) determined by the technique described by Ørskov and McDonald (1979).

### *2.5.3 Fatty acid profile*

Fatty acids from the feedstuffs were extracted and derivatized according to the method described by Sukhija and Palmquist (1988; extracted with heptane instead of benzene) and by Lee et al. (2012) in IMF. Fatty acids methyl ester determination was performed using gas chromatography (SCION 436-GC; Bruker, Billerica, MA) equipped with a cyanopropyl capillary column (BR-2560, 100 m × 0.25 mm.i.d. × 0.20 µm thickness; Bruker), flame ionization detector, and Compass CDS software (Bruker). Fatty acid quantification was performed as described in the ISO 12966-4 (2015), and identification was performed using the GLC 538 and GLC 463 standard references (Nu-Chek Prep Inc., Elysian, MN). Fatty acids were expressed as a percentage of the total amount of the identified fatty acids. Groups of FA based on the saturation level were estimate: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated Fatty acids (PUFA), PUFA omega 6, PUFA omega 3; and the ratio n6: n3.

### *2.6 Statistical Analysis*

Data were analyzed using SAS statistical software. Results were analyzed as a randomized block design, with repeated measures in time. Statistical analyses were performed using mixed model (MIXED procedure) with fixed effects of pasture species or type of diet, period and their interaction, the other effects were random. Period was considered to be the repeated measure over time, where each period represented the measurement of the same experimental unit under a different condition.

In order to adjust the temporal autocorrelation observed in data measured on time, covariance structures were tested to fit the models, based on Bayesian information criterion (BIC), from which the structure "component of variance (VC)" was selected. The normal distribution of data was tested. When differences were observed, the averages were compared using the Tukey 10% probability test. Interactions involving pasture species or type of diet and period sampling were significant at 10%. The variables were also submitted to Pearson correlation analysis. Multiple regressions were tested considering the productive variables of the pasture and dietary bromatological variables (explanatory variables), on the fatty acid profile (response variables) of the lambs diet.

## **3. Results**

### *Forage production*

The effects of pasture species, sampling periods and their interaction related to the pasture production variables are shown in Table 1. There was a significant interaction between pasture species and sampling periods ( $P<0.05$ ) for most of the pasture production parameters. However, the L: S ratio showed to be similar among forages with a mean of 0.46 ( $P>0.10$ ).

Pasture production parameters evolution is shown in Figure 2. There were, in general, significant interaction between pasture species and sampling period. Most of variables were different only at 63 d and 92 d. Nevertheless, no significant differences were found between the pasture species at 21 d and 42 d sampling periods with regard to grass height, FM and LP (Figure 2A, 2B, 2D). The DAR was not different among the pasture species at 63 d sampling periods (Figure 2C). The SP was not significant different among the different pasture species at 21 d assessment periods (Figure 2E).

In the 63d sampling periods, the Pigeon pea showed the greatest height and the Aruana grass and Mixed pasture were not significant different. In relation to the FM, LP and SP characteristics, Aruana grass had the greatest values, mixed sward the lowest, and Pigeon pea the intermediate. The Pigeon pea did not differ from Aruana grass and Mixed sward in relation to the FM, LP and SP. The DAR was similar among pasture species in the 63 d sampling periods.

In the 92 d sampling period, Pigeon pea continue to be the tallest pasture. The greatest FM was found for Pigeon pea and Aruana grass. The Pigeon pea had the largest DAR and SP, and mixed sward had the lowest. Aruana grass did not differ between the other pastures in relation to the DAR and SP. The LP was similar among pasture species in the 92 d sampling periods.

Table 1- Average pasture production and physical characteristics according to the pasture species: aruana grass (*Panicum maximum* cv. IZ-5), Pigeon pea (*Cajanus cajan*), mixed (half of the area with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*) under grazing of lambs

Variables <sup>1</sup>	Pasture species <sup>2</sup>			Probability		
	Aruana grass	Pigeon pea	Mixed	MSE <sup>3</sup>	Pasture	Interaction
Height	56.34	94.51	60.23	3.34	0.0049	0.0001
FM	7098.23	6192.05	5197.65	334.67	0.0299	0.0389
DAR	58.29	64.4	55.36	5.78	0.5282	0.0289
LP	1766.66	1340.32	1193.42	128.1	0.0299	0.0389

SP	4436.12	3703.57	2964.10	286.99	0.0071	0.0001
L: S	0.54	0.43	0.42	0.047	0.1377	0.3226

Height(cm): forage height; FM ( $\text{Kg ha}^{-1}$ ): Forage mass; DAR ( $\text{Kg ha}^{-1}$ ): Daily Accumulation Rate; LP ( $\text{Kg ha}^{-1}$ ): Leaf production per ha; SP( $\text{Kg ha}^{-1}$ ): Stem production; DM: Ratio L: S:Leaf: steam ratio; <sup>2</sup>Pasture species: Aruana grass (*Panicum maximum*); Pigeon pea (*Cajanus cajan*) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>3</sup>MSE: mean square error

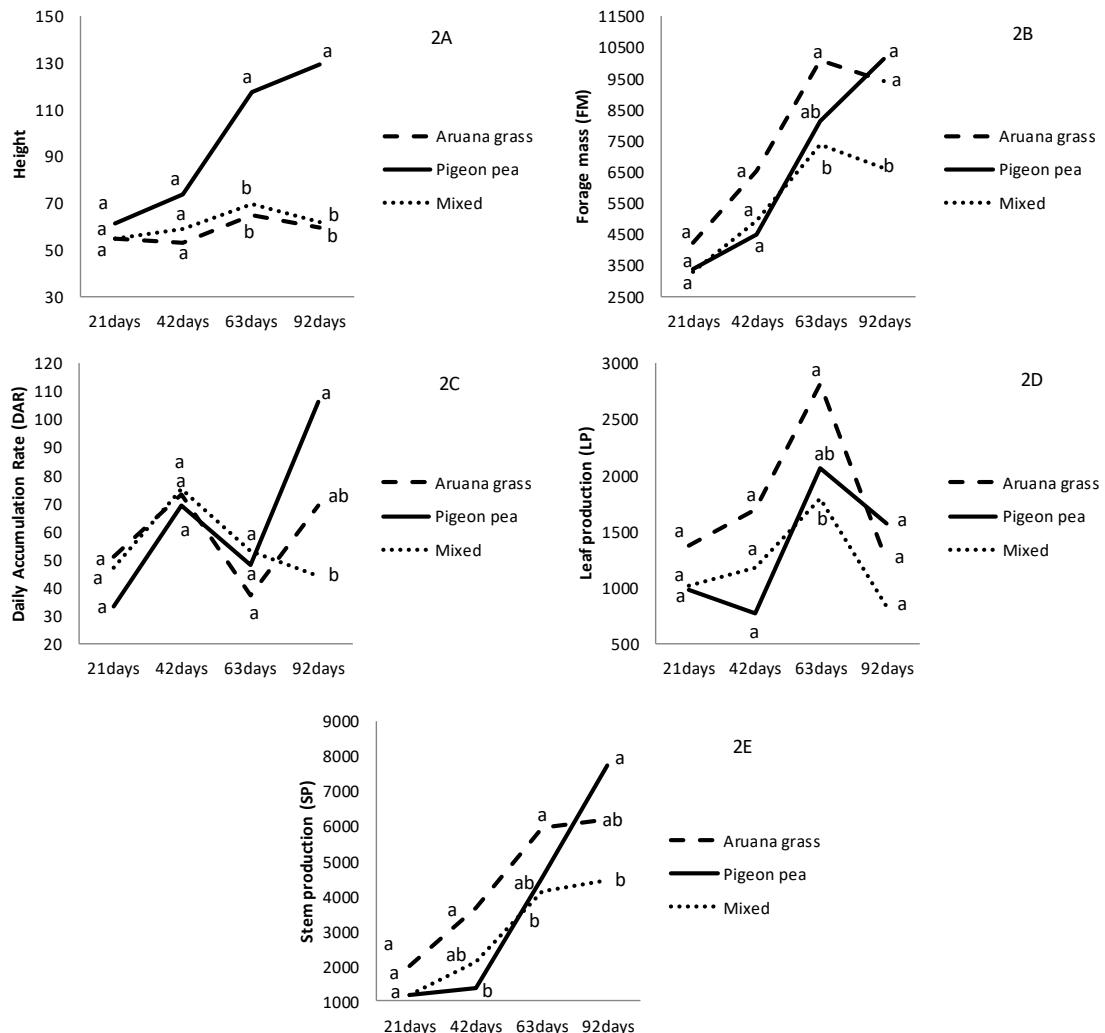


Figure 2- Interaction between species of tropical forages under grazing of lambs and sampling period for production forages variables

a, b Letters different within figure for each of pasture species differ significantly at  $P < 0.10$

#### *Chemical composition of the diet*

The effects of type of diet, sampling periods and their interaction in relationship to the chemical variables from the presumable diets of lambs are shown in Table 2. There was no significant interaction between type of diet and periods ( $P>0.10$ ) for DM, ISDMD and EE

parameters. However, there were significant interaction for OM, ash, ADL, CP and NDF between type of diet and periods ( $P<0.10$ ; Table 2).

The grazed sward for all three types of forage showed to be similar ( $P>0.10$ ) in DM, ISDMD and EE. The greater fiber (ADF) and ash levels were found in the Aruana grass, lower in Pigeon pea and intermediary in the Mixed. The last one did not differ from the other two pasture (Table 2;  $P<0.10$ ).

Chemical parameters evolution of diet is shown in Figure 3. There were, in general, significant interaction between type of diet and sampling period. During sampling periods, it was observed that most of variables of the diet were different only at 63 d and 92 d. Nevertheless, no significant differences were found between type of diet at 21 d and 42 d sampling periods with regard to OM, ashes, ADL and CP (Figure 3A, 3B and 3C).

In the 63 d and 92 d sampling periods, the diets of Pigeon pea showed the greatest OM, Aruana grass diet lowest, and Mixed was intermediate. The Mixed diet did not differ from Pigeon pea and Mixed. The ashes content was inverse to OM values found in these sampling periods. In the 63 d sampling period, the diet of Pigeon pea showed greatest ADL at diet, Aruana grass lowest and Mixed was intermediate, without differing from Pigeon pea and Aruana grass. In the 92 d sampling period, diet of Pigeon pea continued to present the greatest ADL, and Aruana grass and Mixed diets were significantly the lower.

The NDF content in the diet, differ between the 21 d, 63 d and 92 d sampling periods (Figure 3D). The diet of Aruana grass showed the greatest content, and Pigeon pea the lowest and Mixed diet was intermediate, without differing from the other treatments. The CP content the diet, differ in 92 d the sampling period (Figure 3E). The Pigeon pea diet showed the greatest content, Aruana grass the lowest and Mixed diet was intermediate, without differing from types of diet.

Table 2- Average chemical composition of according to the type of diet: Aruana grass (*Panicum maximum* cv. IZ-5), Pigeon pea (*Cajanus cajan*), Mixed (half of the area with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*) under grazing of lambs

Variables <sup>1</sup>	Type of diet <sup>2</sup>			MSE <sup>3</sup>	Probality	
	Aruana grass	Pigeon pea	Mixed		Diet	Interaction
DM	22.51	24.17	23.5	0.88	0.4878	0.6628
OM	88.35	90.51	88.92	0.44	0.0433	0.0067
ISDMD	58.24	58.85	53.76	2.4	0.4076	0.2929
Ashes	11.33	9.09	10.80	0.35	0.0247	0.0122

CP	14	20	16.18	1.27	0.0973	0.0946
EE	2.52	3.04	3.22	0.4	0.29	0.8914
NDF	62.54	47.83	56.49	1.89	0.0254	0.0045
ADF	31.55 a	27.70 b	29.99 ab	0.66	0.0482	0.2317
ADL	3.60	7.73	5.02	0.48	0.0142	0.0001

<sup>1</sup>DM (%): Dry matter content; OM (%): Organic matter content; ISDMD (%): In situ dry matter digestibility (ISDMD) content of digestible organic matter; CP (%): Crude Protein; ES (%): Ether extract; NDF (%): Neutral detergent fiber; ADF (%): Acid detergent fiber; ADL(%): acid detergent lignin; <sup>2</sup>type of diet: Aruana grass (Panicum maximum); Pigeon pea (Cajanus cajan) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>3</sup>MSE: mean square error; a, b Values within a row with different letters differ significantly at P < 0.10.

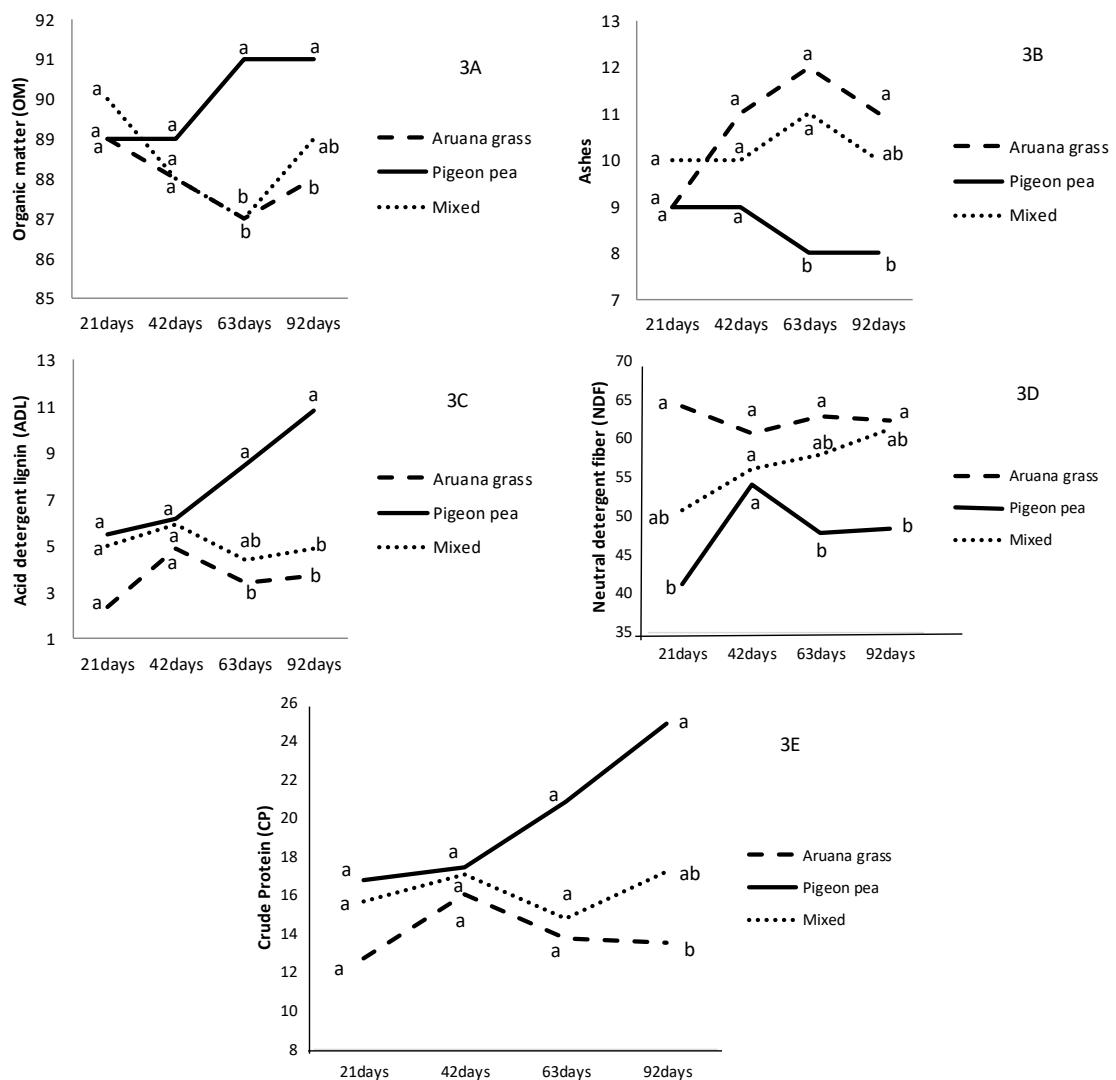


Figure 3: Interaction between type of diet of lambs and sampling period for chemical composition variables

a, b Letters different within figure for each of pasture species differ significantly at P < 0.10

#### *Antioxidants composition of the diet*

The effects of type of diet, sampling periods and their interaction related to the antioxidants composition variables are shown in Table 3. There was significant interaction between type of diet and sampling periods ( $P>0.05$ ) regarding TT and TC antioxidants composition. There was no significant interaction between type of diet and sampling periods ( $P>0.10$ ) for alpha-tocopherols and TH (Table 3). The alpha-tocopherol and TH showed to be similar among type of diet with a mean of  $137.22 \text{ mg kg}^{-1}$  and  $2.34 \text{ (eq-g)}$  of leucocyanidine  $\text{kg}^{-1}$  of DM respectively.

Antioxidant compositions of type of diet evolution are shown in Figure 4. There were no significant differences between the type of diet at 21 d and 42 d sampling periods regarding to TT and TC (Figure 4a, 5b). At 63 d and 92 d sampling periods, diet of Pigeon pea showed the greatest TT and TC the diet Aruana grass and Mixed lowest. The content of TC was related to the pasture height (H) according to the multiple regression:  $\text{TC} = -8.50 + 0.19 \text{ H}$  ( $P = 0.0001$ ), the height of pasture influenced in 76.2% of the TC concentration in lambs diet in our experimental situation.

Table 3- Antioxidants composition according to the diet: Aruana grass (*Panicum maximum* cv. IZ-5), Pigeon pea (*Cajanus cajan*), Mixed (half of the area with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*) under grazing of lambs

Variables	Type of diet <sup>1</sup>			MSE <sup>2</sup>	Probability	
	Aruana Grass	Pigeon pea	Mixed		Diet	Interaction
$\alpha$ -Tocopherols <sup>3</sup>	138.77	136.89	136.00	14.37	0.9819	0.7165
TT <sup>4</sup>	2.44	15.10	7.51	1.41	0.0001	0.0005
TH <sup>5</sup>	1.67	3.55	1.82	0.37	0.3054	0.4680
TC <sup>6</sup>	1.17	9.87	4.49	1.00	0.0001	0.0018

<sup>1</sup>type of diet: Aruana grass (*Panicum maximum*); Pigeon pea (*Cajanus cajan*) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>2</sup>MSE: mean square error; <sup>3</sup> alfa-Tocopherol:extracted from fresh grass  $\text{mgKg}^{-1}$ , <sup>4</sup> TT: Total tannins (eq-g) of leucocyanidine  $\text{kg}^{-1}$  of DM ; <sup>5</sup> TH: Hydrolysable tannins (eq-g) of leucocyanidine  $\text{kg}^{-1}$  of DM ; <sup>6</sup> TC: Condensed tannins (eq-g) of leucocyanidine  $\text{kg}^{-1}$  of DM.

a, b Values within a row with different letters differ significantly at  $P < 0.10$ .

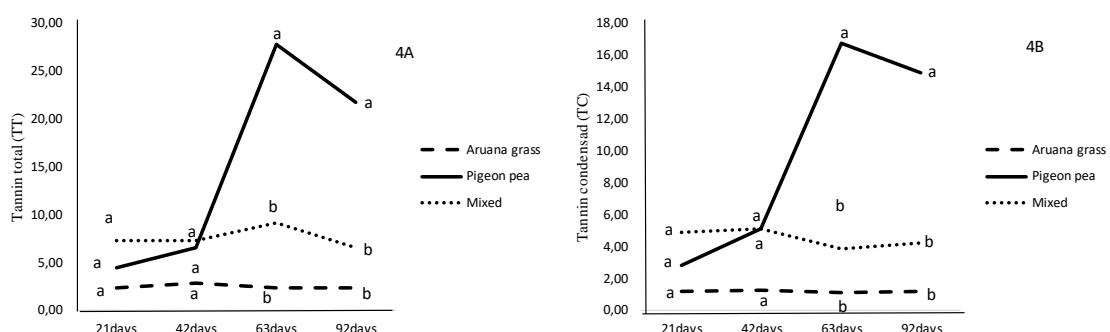


Figure 4: Interaction between type of diet of lambs and sampling period the tannins of composition

a, b Letters different within figure for each of pasture species differ significantly at P < 0.10

#### *Lipid profile of the diet*

The effects of type of diet, sampling periods and their interaction related to the lipid profile are shown in Table 4. There was significant interaction between type of diet and sampling periods ( $P>0.05$ ) for C18:2 n6, C20:0 and C23:0 in the lipid profile (Figure 5A, 5B and 5C). The C15:0, C16:0 and C18:1 cis9 also present interaction between type of diet and sampling periods ( $P<0.10$ ; Figure 5D, 5E and 5F).

The Pigeon pea diet showed highest C18:2n6 concentration, independent of the sampling period. In the 21 d sampling period, Pigeon pea and Mixed showed greatest C18:2n6 concentrations. In the 42 d and 92 d sampling periods, Pigeon pea showed greatest concentration, Aruana grass the lowest and Mixed was similar among the other diets. In the 63d the C18:2n6 concentration was similar between the types of diet. The C18:2 n6 content was negatively correlated to the ashes, NDF and ADF, content of the lambs diet ( $r^2= -0.50$ ;  $r^2= -0.58$ ;  $r^2= -0.51$ ;  $P<0.001$ ), respectively.

The Aruana grass adjusted to a linear regression along the period for C20:0 concentration, where each day of pasture use is to decrease 0.023% in the content of C20:0 in diet ( $\hat{y}= 4.00 - 0.023x$ ;  $R^2=81\%$ ;  $P<0.05$ ). There were no significant differences between the type of diet at 42 d, 63 d and 92 d sampling periods. At 21 d however, Aruana grass showed the greatest C20:0 concentration, Pigeon pea the lowest and the Mixed did not differ between Aruana grass and Pigeon pea.

The C23:0 concentration was not different between the type of diets at 21 d, 63 d and 92 d sampling periods. Interaction was significant at 42 d, where Pigeon pea diet showed the highest concentration, Aruana grass the lowest and the Mixed did not differ between Pigeon pea and Aruana grass.

The C15:0 concentration differ at 63 d and 92 d sampling period (Figure 5D). The diet of Aruana grass showed the greatest concentration of C15:0, the Pigeon pea the lowest. The C16:0 differ in all sampling periods (Figure 5E). At 21 d and 42 d, the Aruana grass showed the greatest C16:0 concentration, Pigeon pea the lowest and Mixed was intermediate. At 63 d and 92 d, the Aruana grass and Mixed showed the greatest C16:0 concentration and Pigeon pea

the lowest. The C16:0 content was positively correlated to the content of NDF and negatively to ADL content of the lambs diet ( $r^2= 0.64$ ;  $r^2= -0.65$ ;  $P<0.001$ ), respectively.

The C18:1 cis9 concentration tended to differ at 92 d (Figure 5F). Pigeon pea diet showed the higher concentration than Aruana grass, being Mixed treatment intermediate, without differing from the other diets. The content of C18: 1 cis9 was related to the NDF according to the multiple regression:  $y = 8.49 - 0.073 \text{ NDF}$  ( $P = 0.0002$ ). The NDF content in the diet of lambs influenced in 34.3% the concentration of C18: 1 cis9.

The diet of Aruana grass presented the higher SFA concentration, Pigeon pea the lower and Mixed the intermediate (Table 5). The C12:0, C10:0; C14:0; C18:0; C20:0 and C17:0 had the highest concentration in the diet of Aruana grass. The C18:0 content, is negatively correlated to the content of CP and ADL of the lambs diet ( $r^2= -0.65$ ;  $r^2= -0.65$ ;  $P<0.001$ ), respectively.

The concentration of MUFA was similar among the type of studied diet (Table 4). The C16:1 n7concentration was greatest in the diet of Aruana grass, Pigeon pea the lowest and intermediate in the Mixed diet (Table 4).

The greatest concentration of PUFA in the diet was found in the Pigeon pea, and Aruana grass the lowest, being the Mixed diet intermediate. The PUFA content is positively correlated by the ADL and negatively NDF content of the lambs diet ( $r^2= 0.63$ ;  $r^2= -0.67$ ;  $P<0.001$ ), respectively.

The C18:3 n3 concentration was greater in the diet of Pigeon pea and Mixed, and Aruana grass the lowest. The C18:3 n3 content, is positively correlated by the content of CP and ADL and negatively by NDF content of the lambs diet ( $r^2= 0.63$ ;  $r^2= 0.67$ ;  $r^2= -0.66$ ;  $P<0.001$ ), respectively. The n6:n3 concentration did not differ between type of diet ( $P>0.10$ ; Table 4).

Table 4- Lipid profile of diet according to the pasture: Aruana grass (*Panicum maximum* cv. IZ-5), Pigeon pea (*Cajanus cajan*), Mixed (half of the area with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*) under grazing of lambs

Variables <sup>1</sup>	Type of diet <sup>2</sup>			MSE <sup>3</sup>	Probality	
	Aruana grass	Pigeon pea	Mixed		Diet	Interaction
C 10:0	0.89 a	0.39 b	0.61 ab	0.06	0.0233	0.632
C12:0	6.07 a	2.85 c	4.35 b	0.200	0.0009	0.8602
C14:0	2.23 a	1.53 b	1.81 ab	0.130	0.0403	0.2865
C15:0	5.11	3.49	4.21	0.155	0.0031	0.0818

C16:0	39.97	32.50	36.56	0.744	0.0006	0.0520
C16:1 n7	5.88 a	3.35 c	4.34 b	0.198	0.0019	0.5513
C17:0	1.01 a	0.67 ab	0.66 b	0.065	0.0359	0.5143
C18:0	7.95 a	6.30 b	6.90 ab	0.195	0.0142	0.2419
C18:1 cis9	3.76	5.27	4.19	0.264	0.0213	0.0963
C20:0	2.73	1.39	2.10	0.187	0.0304	0.0140
C22:0	1.63	1.54	1.67	0.084	0.6534	0.2913
C23:0	0.42	0.56	0.50	0.035	0.1812	0.0001
C18: 3n3 (n3)	11.50 b	23.12 a	18.03 a	1.050	0.0053	0.6182
C18: 2 n6( n6)	10.79	17.00	13.99	0.602	0.0062	0.0204
SFA	68.04 a	51.23 c	59.43 b	1.230	0.0014	0.2767
MUFA	9.65	8.64	8.54	0.330	0.1723	0.0958
PUFA	22.29 c	40.15 c	32.03 a	1.611	0.009	0.7110
<i>n6:n3</i>	0.95	0.76	0.79	0.045	0.1466	0.2695

<sup>1</sup>FAME: Fatty acids methyl esters (%); SFA (%): Sum of saturated fatty acids; MUFA (%): Sum of monounsaturated fatty acids; PUFA (%): Sum of polyunsaturated fatty acids; n3: omega 3; n6:omega 6; n6:n3: ratio omega 3/ omega 6; <sup>2</sup>pasture species: Aruana grass (*Panicum maximum*); Pigeon pea (*Cajanus cajan*) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>3</sup>MSE: mean square error  
a, b Values within a row with different letters differ significantly at P < 0.10.

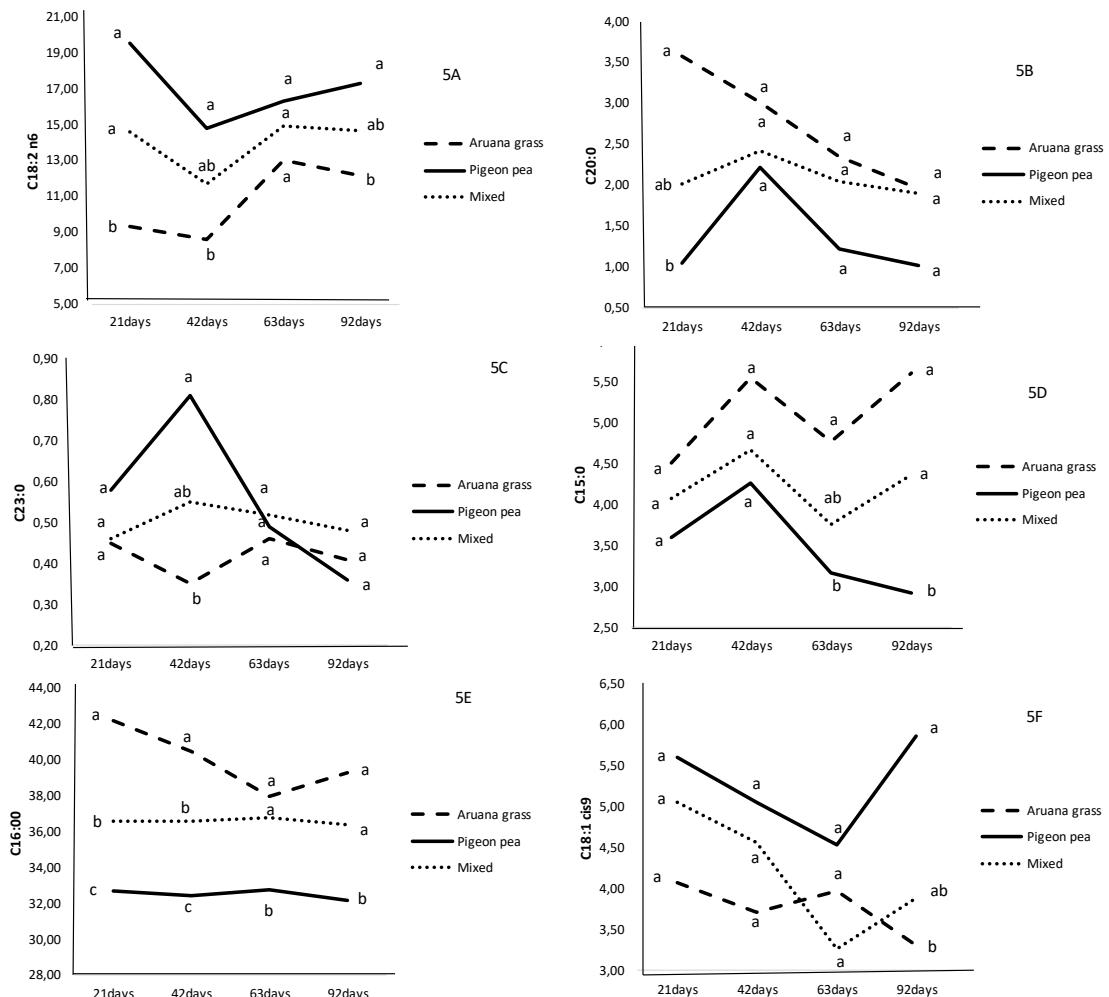


Figure 5: Interaction between type of diet of lambs and sampling period the for lipid profile variables

a, b Letters differents within figure for each of pasture species differ significantly at  $P < 0.10$

#### **4.Discussion**

The present study tested the hypothesis that different periods along the pasture growth affect the pasture physical characteristics, the chemical composition, tannins and tocopherol content, and lipid profile of the lambs diet. This hypothesis was based on the expectation of a changing along the pasture growth period the pastures characteristics and the lambs diet from distinct species of tropical pastures as Aruana grass, Pigeon pea legume and the presence of this two pasture species at the same area (Mixed).

Most of the variables related to pasture production, behaved differently from the 63d of samplings periods. Only the L: S ratio was similar between pasture species. The L: S ratio is a variable of great importance for animal nutrition and for the management of pasture plants because it is associated with the facility that animals harvest the preferred fodder (leaves) (Brâncio et al., 2003).

The Pigeon pea, a leguminous plant, presented the greatest height of pasture during all the experimental period. Because it is a shrub species (Ramos, 1994), there must have occurred elongation of stem, causing a greater increase of the height.

The pasture species when used in a monoculture system produced more FM. There could have been interaction between the species when found in consortium (Mixed), because legumes are able to fix N amounts that contribute to improved soil fertility and increased pasture production (Paciullo et al., 2003). Nevertheless, interaction was not observed in our study probably because the pastures were sown in separated special bands within the same paddock. Therefore, the grasses did not take advantage of the greater N arrangement in the soil.

The DAR was different among pasture species in the last sampling period. The Pigeon pea presented greatest accumulation rate, Mixed lowest, and Aruana grass did not differ from the other species. According to Castagnara et al. (2011), the rate of pasture accumulation varies largely due to management and climatic conditions, which in our study were similar among pasture species. Which may have contributed to the higher accumulation rate in pigeon pea, it was because they were shrub species and because they had the highest stem prodution during the sampling period.

The LP differs between pasture species in 63 d sampling. The Aruana grass produces a greater amount of leaf blades when compared to mixed, the Pigeon did not differ from the other pastures. In this same period the FM and SP presents similar behavior. Analyzing the structural separation spreadsheets we found a higher proportion of inflorescence at day 63 d in the Aruana grass. According to Miller (1979), there is a high growth of aerial biomass before flowering. SP presented differences between pasture species after 42d. In this period, Aruana grass showed the greatest SP, and Pigeon pea the lowest, due to the greater population density of the grass, ends up producing a greater quantity of stems. Over time the legume ends up increasing its production of stems due to its growing habit, producing heavier stems compared to grass.

The structure of pastures produced in monoculture did not interfere in pasture production, but showed direct reflexes in the production of stems and in the height of pastures in the last period sampling. The Pigeon pea, at the end of the assessment period showed the highest height and the highest production of stems. Which can be bad for animals grazing, mainly sheep.

The modification of the pasture structure over time were not enough to alter the DM, ISDMD, and EE the chemical analysis parameters the diet of lambs, likely due to the continue grazing that kept quality pasture similar. Contradicting Van Soest (1994), who stated that pasture plants differ widely in their chemical composition, even when they grow under the same environmental conditions.

The level of ADL were lower in diet of Pigeon pea, these data show that the diet of Pigeon pea have greatest amounts of digestible fiber than that contained in the Aruana grass and Mixed. However the lignin content present in this diet makes the quality of the legume diet inferior to other diets. The diet of legume loses quality over time and might be attributed to the wall lignification which increase with plant maturity (Kozloski et al., 2005). The increase in the lignin content and a marked decrease in the mineral content in our study, is related to the low ash content found in the diet of legume.

The OM and Ashes of diet, have opposite behavior over time presenting negative correlation among these variables ( $P = 0.0001$ ,  $r^2 = -0.89$ ). Whereas the increase of OM in diet of Pigeon pea, the ashes content is lower. The OM in diet of Pigeon pea presented the same statistical behavior as for pasture height, and found a positive correlation between these variables ( $P = 0.0007$ ,  $r^2 = 0.53$ ). Generally, when a larger process of plant nutrient dilution

and translocation occurs for plant growth, it normally contains lower ash concentrations due to increased stem tissue (Pahkala and Pihala, 2000).

With the advancement of the sampling periods it was observed that Mixed diet tended to present intermediary nutritional quality due to content of NDF and CP in de diet. This is because with the advancement of the pasture cycle, grasses and legumes are changing their quality and mixed ends up giving the animal a slightly more balanced diet on the issue of nutrient quality.

The animal diets presented similar concentrations of alpha-tocopherol. The leaf:stem ratio (Livingston et al., 1968), pasture species (Danielsson et al., 2008) and sampling period (Lynch et al., 2001), could alter tocopherol concentration, but in our study these factors were not sufficient to alter tocopherol concentration. Because the leaf: stem ratio was similar between pasture species, and the plants were maintained under continuous grazing, and this may have delayed the action of the phenological stage on the conentration of tocopherol, providing to the animal a diet with similar concentration of alpha-tocopherol. The alpha-tocopherol values found in types of diet are more than sufficient to suppress the daily needs of lambs (NRC, 2007) and to maintain staining and decrease the impacts of lipid oxidation (Ripoll et al., 2011; Ripoll at al., 2013).

The type of diets presented similar concentrations of TH. We can observe that TC varies over time, causing the concentration of TT to vary in a similar way in the diet. This variation occurs due to the participation of the legume in the diets (Barry, 1989). The legume over time increase its deposition of lignin and concentrated higher levels of TC and TT, this deposition of phenolic compounds increases during the development of plants (Cabiddu et al., 2010). The diet mixed shows intermediate values of TC and TT due to the participation of the legume in the diet.

The height of pasture influenced in 76.2% the concentration of TC in the diet of lambs. In our study, this relationship of height and TC content may be related to the lignin content, which was higher in the diet composed of legume. Because tannins and lignin belong to the same group of polymers, like this the legumes produced greater lignin and TC.

The Aruana grass diet presented greatest SFA concentration. The C15:0, C16:0 and C20: 0 FA of lambs diet, showed variation over time within each diet. Even though there was interaction between diet and sampling period, the diet composed of Aruana grass presented the highest values of these FA. We found a positive correlation between C16: 0 and NDF ( $r^2 =$

0.64), and the NDF values of the diet. We also observed that the diet composed of Aruana grass always had the highest values of NDF. According to Glasser et al. (2013), when there is an increase in NDF levels, over the time of pasture use, there is an increase in the saturated FAs mainly C16:0, therefore we confirm the findings from this author.

The Pigeon pea diet tended to accumulate higher concentration of C18:1 cis9 in the last sampling period. The NDF content of diet influenced in 34.3% the concentration of C18: 1 cis9. The lower NDF content in the legume may have influenced the higher accumulation of C18: 1 cis9 diet of Pigeon pea. When harvested at the same stage of development, grass and legume present significant differences in FA, the vegetables had higher concentrations C18: 1 (Boufaïed et al., 2003). Garcia et al. (2016) also found a higher concentration of C18: 1 in the leguminous plants, but these authors found no difference in concentration due to the time of cutting.

The PUFA concentration was greatly impacted by the addition of legumes. For instance, Pigeon pea and mixed diet contained PUFA greater than Aruana grass. Additionally, C18:3n3 was similar between Pigeon pea and mixed diet. The higher concentration of C18: 3 n3 content in the diet containing legumes may be related to the fact that this fatty acid was positively correlated with content of CP and ADL ( $r^2=0.63$  and  $r^2=0.67$ , respectively) and negatively correlated of NDF ( $r^2= -0.66$ ), which presented lower content in Pigeon pea diet. Our findings contradict Boufaïed et al. (2003) and Garcia et al. (2016) which finds a higher C18:3n3 concentration in the grasses.

The C18:2 n6 content of diet differed over time of pasture use. The diet of Pigeon pea always presented the highest values, the Aruana grass diet the lowers, and the mixed diet intermediate values. Boufaïed et al. (2003) and Garcia et al. (2016) also found a higher concentration of C18:2 n6 in the studied leguminous plants. We found a negative correlation between C18:2 n6 and NDF ( $r^2= -0.58$ ), which are in accordance to the founds of Glasser et al. (2013) therefore, the concentration of C18:2n6 in diet can be influenced by the advancement of phenologycal state of the plant and the concentration of CP and NDF.

## **Conclusion**

The pasture species presented variation of forage mass, stem production, leaf production and height over time of the evaluation of the pastures. The insertion of legume in the diet of lambs does not alter the concentration of tocopherol, but over the evaluation periods the levels

of condensed tannin and total tannin increase in the diet containing legumes. The increase in neutral detergent fiber content in lambs diet results in the lower concentration of C18: 1 cis 9, omega 6 and polyunsaturated fatty acid available to lambs. The participation of the legume in the diet of the lambs increases the content of omegas 3 and 6 available improving the n6: n3 ratio in the diet of lambs.

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## CAPÍTULO IV

### CARCASS TRAITS AND MEAT QUALITY OF LAMBS GRAZING TROPICAL PASTURE

Este capítulo é apresentado de acordo com as normas de publicação do **Food Research International**.

## Carcass traits and meat quality of lambs grazing tropical pasture

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**Abstract:** The aim of the study was to assess the influence of type of pasture (Aruana grass, Pigeon pea and Mixed) on carcass traits and meat quality of lambs. We used 54 lambs distributed on three grazing treatments. i. Aruana grass pasture (AG), ii. Pigeon pea (PP), and iii. Mixed pasture of AG and PP (MIXED). To test the tannin effect, a half part of the lambs from each treatment received Polietileneglycol (PEG) twice a day. After 92 days of experiment, the lambs were submitted to a fasting period of 24 hours for solids and 12 hours for liquids and slaughtered. Twenty-four hours post mortem, carcass were evaluated and longissimus muscle was sampled to determine the fatty acid profile, tocopherols, color and lipid oxidation. Results showed that type of pasture had no effect on carcass traits and meat quality. The inclusion of PEG in the lambs' diets increased the value of hue angle and decreased the value of redness of subcutaneous fat color. The inclusion of the leguminous plants in the pasture resulted in a meat with lesser concentration of saturated fatty acids and greater of polyunsaturated fatty acids concentration. The meat of lambs raised exclusively in Aruana grass presents lower n6: n3 ratio in the meat and lower lipid oxidation after 6 days of storage. We concluded that grazing Aruana grass produce a meat with a lower n6: n3 ratio and lower lipid oxidation, which are important both for the meat industry and for human health.

**Keywords:** Aruana grass, Pigeon pea, fatty acids, lipid oxidation, tannin, tocopherol

### 1. Introduction

Pasture production systems improves meat quality. Production systems based exclusively in pasture can contribute to improving the meat quality characteristics, producing, in addition at low cost. Tropical pastures have a high potential of production with an appropriate chemical composition for the nutrition requirements of ruminants (Paulino et al., 2015), with a good fatty acid profile (FA) for human health (Khan et al., 2012) and with the presence of important amounts of antioxidants compounds, such as condensed tannins and tocopherols, which, can improve the animal performance and the carcass and meat quality.

Due to its relation with human health, there is a growing interest in the lipid composition of ruminant products, mainly the fatty acid profile (Glasser et al., 2008). Ruminant meat is an important source of conjugated isomers of linoleic acid (CLA) and of n3 polyunsaturated fatty acids (PUFA; Bessa et al., 2007). Besides, consumers appreciate the visual and sensory aspects of meat, which are related to the lipid content and pigment oxidation, and, then they can be the major causes to damage the meat (Hajji et al., 2016).

The meat color and lipid oxidation are influenced by the composition of the muscular tissues, mainly amount of fat, FA profile, and antioxidant action, which are related to diet to the animal (Ponnampalam et al., 2012a; 2012b; Luciano et al., 2009). To reduce the oxidative process, and thus, its effects on meat, mainly there are two ways: adding antioxidant to the meat or feed the ruminant with diets rich in antioxidants as pasture. The inclusion of additives in the meat produces rejection of the consumers (Resconi, 2007), whereas feeding fresh pasture is highly appreciate for consumers.

Meat from grazing ruminants present concentration of tocopherol, resulting an improvement of the oxidative process and of the color stability of the meat (Ponnampalam et al., 2012a; Descalzo and Sancho, 2008). The antioxidant action of condensed tannins, as well as tocopherols, also has an important effect on the maintenance of color and meat rancification promoting better conservation and longer product shelf life (López -Bote et al., 2001; Liu et al., 2011). Although there are numerous studies related to the condensed tannins (Vasta and Luciano, 2011; Luciano et al., 2009; Priolo et al 2000) and tocopherols (Ripoll et al., 2012; 2011; Liu et al., 2009; Turner et al., 2002) there are few information about the effect of the concentration of tannins and tocopherols in tropical plants on the quality of lamb. The aim of the study was to assess the influence of type of pasture (Aruana grass, Pigeon pea and Mixed) on carcass traits and meat quality of lambs

## 2. Material and methods

The experimental protocol evolving finishing and slaughtering lambs were reviewed and approved by the Ethics Committee on the Use of Animals of Universidade Federal do Rio Grande do Sul (ECUA-UFRGS), Project No: 27830 - Lambs sustainable production systems.

### 2.1 Experimental site: localization and climate

The study was conducted at the Agricultural Experiment Station (EEA) of Universidade Federal do Rio Grande do Sul (UFRGS), Brazil (Latitude 29° 13' 26" S, Longitude 53° 40' 45" W). The experiment was carried out from 11<sup>th</sup> January, 2016 until 12<sup>th</sup> April, 2016, being 92 days of experimental period. The climate of the region is classified as Cfa 2 - subtropical climate, with 23 C° average temperature, 29 C° maximum temperature, 19 C° minimum temperature and 195 mm average precipitation, data for the experimental period .

### 2.2 Experimental animals and design

Fifty-four crossbred lambs (Corriedale x Texel), male, castrated, weaned with age and average weight of  $4.11 \pm 0.16$  months and  $20.4 \pm 3.97$  kg, respectively, were distributed in three types of pastures: 1) Aruana grass (*Panicum maximum*; AG); 2) Pigeon pea (*Cajanus cajan*; PP) and 3), and Mixture of Aruana grass and Pigeon pea (MIXED). The experimental pastures were set up in a randomized block design. Three blocks were used to control the effects of soil type and slope of the land, and the three pastures were randomly distributed within each block as paddocks. Each pasture had 3 paddock of 0.2 ha with 6 tester in each (n=18). In each paddock, 3 lambs received 60g day<sup>-1</sup> of polyethylene glycol (PEG) and the other 3 lambs received water (WATER). PEG and WATER were dosed orally twice a day (at 9 and 16 h). PEG dosing was performed to determine the effect of condensed tannin (Makar et al., 1995).

### 2.3 Grazing management and animals weighings

Grazing was managed in a continuous put and-take stocking to maintain a constant allowance of 10-11% (10-11 kg dry matter 100 kg<sup>-1</sup> of live weight ha day<sup>-1</sup>) of green leaf in all treatments. Herbage allowance was adjusted every 21 days, using the put-and-take technique (Mott and Lucas, 1952). All lambs had free access to water and mineral salt. The lambs were

weighed every 21 days, and with the difference between the weighing the average daily gain (ADG) was obtained.

#### *2.4 Pasture sampling, pasture accumulation and feeding intake*

Pasture sampling was performed at every 21 days, except the last sampling which was done at 29 days. In each collection it was evaluated parameters of pasture production and chemical composition.

The forage mass (FM) and daily accumulation rate (DAR) were obtained by cutting, at the ground level, the herbage contained in 0.25 m<sup>2</sup> quadrat, taking 9 sampling points per paddock. Samples were weighed and the vegetal material were separated in leaf blade, stem and senescent material than and oven-dried at 60 °C for 72 h. The offer of leaf blade (OLB) was calculated by the formula: OLB = (LP/n + DAR) \*100/SR). Where OLB = offer of leaf blade (% BW); LP = total leaf production (kg DM ha<sup>-1</sup>); n = 21 days; DAR= daily pasture accumulation rate (kg DM ha<sup>-1</sup> day<sup>-1</sup>). Pastures samples were collected according to the Hand-plucking technique (Euclides et al. 1992) and they are bulked by sampling period for the determinations of its nutritive value, tannin, tocopherol, fatty acid profile of pastures and dry matter *in situ* digestibility (ISDMD).

Total feeding intake was estimated every 21 days, using chromium oxide (Cr<sub>2</sub>O<sub>3</sub>). The lambs were manually dosed at 9 a.m. with 1.0 g Cr<sub>2</sub>O<sub>3</sub>. The dosing period was 11 days and from the eighth day the fecal collection was performed straight into the rectum, according to the methodology of Kozloski et al. (2006).

#### *2.5 Slaughter procedures and carcass measurements*

After 92 days of feeding regime, lambs were weighed (BW) and slaughtered in a commercial slaughterhouse. Previous to slaughter, the lambs were subjected to a fasting period, 24 hours for solids and 12 hours for liquids, in accordance with Brazilian regulation 47, March 19<sup>th</sup>, 2013. Electrical stunned was applied to lambs, and then they were exsanguinated and eviscerated. Immediately, the carcasses were weighed to obtain the hot carcass weight (HCW), and stored at 4 °C for 24 h, reweighed to obtain cold carcass weight (CCW). The carcass yield (CY) was estimate as HCW / BW \* 100.

Thereafter, the carcass conformation (CC), fatness degree (FD), and subcutaneous fat thickness (SFT) were evaluated. For the conformation, indices from 1 to 5 (very poor to

excellent) were attributed with a scale of 0.5, as well as for the fatness degree (excessively lean to excessively fat) (Osório and Osório, 2003). The SFT was measured between the 12<sup>th</sup> and 13<sup>th</sup> ribs (Cañeque and Sanudo, 2005), using the pachymeter.

The subcutaneous fat color was assessed in the lumbar region with a portable colorimeter Chroma Meter Cr-400 (Minolta Camera Co., Ltda., Osaka, Japan), iluminante D65, 10° for standard observation, calibrated for a white pattern. CIE L\*, a\*, and b\* color space. The lightness (L\*), redness (a\*), and yellowness (b\*) were recorded. The hue angle (H°) was calculated as  $H^{\circ} = \tan^{-1}(b^*/a^*) \times 57.29$ , and chroma (C\*) was calculated as  $C^* = (a^*2 + B^*2)^{1/2}$ .

## *2.6 Meat sampling, pH and color measurements and storage time study*

The pH was measured 24 h *post mortem* (pH 24 hours), in the *longissimus* muscle, in the space between the fourth and fifth lumbar vertebra. A peagram *Lutron* PH-208, previously calibrated and equipped with a cutting blade tip was used for muscle penetration.

The carcasses were split along the dorsal line and the *longissimus thoracis et lumborum* (LTL) muscles from both half carcasses were collected. On the right side of LTL, from 6<sup>th</sup> to 10<sup>th</sup> thoracic vertebrae were sampled, vacuum-packed and stored at -20°C until the physical-chemical analysis. The left LTL from 6<sup>th</sup> to 12<sup>th</sup> thoracic vertebra, were sliced into 2.5-cm-thick. The two samples were used to determine the color and water retention capacity (WRC). Other two samples were assigned to one of the display time studied (1 and 6 days). From the left side of the carcass two samples of 2.5 cm thickness of the LTL muscle were taken between the 12<sup>th</sup> and 13<sup>th</sup> thoracic vertebra. They were air-free packed and stored at -80°C for the determination of tocopherol and fatty acid profile. The lumbar portion was vacuum packed and was frozen at -20 °C until the determination of shear force (WBSF).

Two samples of LTL muscle of 1 cm thickness each, were used for the storage time study. In first samples (1d) pH was measured, than the 1d sample were packed in aluminum foil and then in plastic and stored at -80°C until future analysis of lipid oxidation (TBARS). The other sample (T6), were placed in trays, wrapped with oxygen permeable polyvinyl chloride film and kept in darkness at 4°C for 6 days and then pH was measured, were packed in aluminum foil and then in plastic and stored at -80°C until future analysis of lipid oxidation (TBARS).

## 2.7 Pasture analysis

### 2.7.1 Chemical composition of the pastures

Chemical composition of pastures was analyzed according to official methods reported by AOAC, 1995. Pasture samples were dried in a forced air circulation oven at 65°C for 72 hours and milled in a Willey type mill at 1 mm. Dry matter (DM), ash, crude protein (CP) and (EE) ether extract contents were determined. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were carried out following the sequential procedure of Van Soest et al. (1991). The dry matter *in situ* digestibility (ISDMD) determined by the technique described by Ørskov and McDonald (1979).

### 2.7.2 Tocopherol, tannin and fatty acid profile

The alpha-tocopherol content was determined in fresh pasture following the methodology of Val et al. (1994). The extraction was performed with acetone and addition of ascorbic acid, and the determination was done with an *High performance liquid chromatography* (HPLC) (Waters Acquity UPLC CLASS), with mobile phase MeOH: H<sub>2</sub>O 93: 7 (v: v), and NovaPak4 µm 30cm column, using the chromatographic procedure described by Górnas et al. (2014).

The tannin contents of the pastures were determined in lyophilized pasture samples grounded in a Wiley mill at 0.5 mm. The total tannin (TT), hydrolyzable (TH) and condensed (TC) contents were determined by adapting the methodologies of Grabber et al. (2013), Makkar (2000), Porter et al. (1986) and Saura-Calixto et al. (2007), expressed as gram equivalent (eq-g) of leucocyanidine kg<sup>-1</sup> of DM. For the calculations, the following formula was used: eq -g leucocyanidine (L) / kg DM = {absorbance x [10x (dilution volume in ml) / (460 x sample weight)] / (DM, in kg)} X 10.

Fatty acids from the feedstuffs were extracted and derivatized according to the method described by Sukhija and Palmquist (1988) and by Lee et al. (2012) in IMF. Fatty acids methyl ester determination was performed using gas chromatography (SCION 436-GC; Bruker, Billerica, MA) equipped with a cyanopropyl capillary column (BR-2560, 100 m × 0.25 mm i.d. × 0.20 µm thickness; Bruker), flame ionization detector, and Compass CDS software (Bruker). Fatty acid quantification was performed as described in the ISO 12966-4 (2015), and identification was performed using the GLC 538 and GLC 463 standard references (Nu-Chek Prep Inc., Elysian, MN). Fatty acids were expressed as a percentage of the total amount of the

identified fatty acids. Groups of FA based on the saturation level were estimate: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA omega 6, PUFA omega 3; and the ratio n6: n3.

### *2.7.3 Intake and Dry Matter in situ Digestibility*

The OM intake (OMI, %, 100 BW) was calculated by the formula: OMI = ((fecal production / (1-digestibility of the pasture)\ BW\*100. Based on OMI for lambs was calculating of alpha-tocopherol intake(ATI): ((OMI \* 100) /% DM) \* (mg kg<sup>-1</sup>, alpha-tocopherol in pastures). The concentration of chromium in dry feces was determined by atomic absorption spectrophotometry (Kozloski et al., 1998). To estimate fecal production (FP), the following formula was used: FP = chromium administered (g day<sup>-1</sup>) / chromium in feces (g kg<sup>-1</sup> DM; POND et al., 1989). Dry matter in situ digestibility (ISDMD) was determined using a rumen fistulated bovine, the dried samples being weighed and incubated in polyester filter bags (porosity 41 µm) for 48 hours.

## *2.8 Meat Analysis*

### *2.8.1 Color*

The color parameters of the LTL muscle was measured in a thawed sample after 30 minutes blooming using a portable colorimeter Chroma Meter Cr-400 (Minolta Camera Co., Ltda., Osaka, Japan), iluminante D65, 10° for standard observation . Colour coordinates were expressed as lightness (L\*), redness (a\*), yellowness (b\*), chroma (C\*) and hue angle (H°).

### *2.8.2 Meat physicochemical characteristics*

Dry matter (DM) was determinate in a dryer at 105 °C, ether extract (EE) and water retention capacity (WRC) following the methodology of Osório et al. (1998). Shear Force (WBSF) was evaluated by the maximum shear force in kgf/cm<sup>2</sup> in a Warner-Bratzler cell with a 1.016-mm blade coupled in Texture Analyzer TA-500 (Lloyd Instruments) using NEXYGEN software. Maximum shear force was logged for each sub-sample in NEXYGEN software curve. The average of seven sub-samples was used for each steak in the statistics analysis.

### *2.8.3 Lipid oxidation at storage*

The lipid oxidation was determined using the Thiobarbituric Acid-Reactive Substances method (TBARS), according to the method described by Pikul et al. (1989). The TBARS values are expressed as mg of MDA per kg of meat.

#### *2.8.4 Tocopherol and Intramuscular fatty acid profile*

Meat samples were lyophilized to determine intramuscular fat and tocopherol contents. To determine the tocopherol samples were submitted to a saponification process with 10% KOH ( $H_2O$ : Ethanol 50:50 v: v), in inert nitrogen atmosphere overnight at room temperature. The extraction of tocopherols was carried out in 9:1 of hexane: ethyl acetate (v:v) with addition of BTH antioxidant, as established by Prates et al. (2006). The determination was done with a *High performance liquid chromatography* (HPLC) (Waters Acquity UPLC CLASS) as had been described above.

Intramuscular FA profile was determinate according to Lee et al. (2012). The *Gas chromatography* (GC) condition to determine the FA were the same of those explained for pasture analysis. As in the feed FA, groups of FA based on the saturation level were estimate: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated Fatty acids (PUFA), PUFA omega 6, PUFA omega 3; and the ratio n6: n3 were done. To facilitate the interpretation of the results, the sum of the fatty acids corresponding to **C18:1** (C18:1 *trans*11; C18:1 *cis*-9; C18:1 *trans*-15; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13, C18:1 *trans*-16 e C18:1 *cis*-15), **CLA** (C18:2 *cis*, *trans*-11; C18:2 *trans*-9, *cis*-11; C18:2 *trans*-10, *cis*-12), **Omega 3** (n3: C22:6 n3; C22:5 n3; C20:5 n3; C22:3 n3; C18:3 n3), **Omega 6** (n6: C22:4 n6; C20:4 n6; C20:3 n6; C20:2 n6; C18:3 n6; C18:2 n6; C18:2 n6 *trans*-9,12), **SFA** (C24:0; C22:0; C20:0; C18:0; *i*-C18:0; DMA-C18:0; C17:0; C16:0; *a*-C18:0; *i*-C16:0; DMA-C16:0; C15:0; *a*-C15:0; *i*-C15:0; C14:0; *i*-C14:0; C13:0; *a*-C13:0; C12:0; C11:0; C10:0), **MUFA** (C24:1 n9; C22:1; C20:1 n9; C18:1 *cis*-15; C18:1 *trans*-16; C18:1 *cis*-13; C18:1 *cis*-12; C18:1 *cis*-11; C18:1 *trans*-15; C18:1 *cis*-9; C18:1 *trans*-11; C17:1 *cis*-9; C16:1 *cis*-9; C16:1 *cis*-7; C16:1 *cis*-7; C16:1 *trans*-9; C15:1; C14:1 *cis*-9; C12:1), **PUFA** (C22:6 n3; C22:5 n3; C22:4 n6; C20:5 n3; C22:3 n3; C20:4 n6; C20:3 n6; C20:3 n9; C20:2 n6; C18:2 *trans*-10, *cis*-12 cla; C18:2 *trans*-9, *cis*-11 cla; C18:2 *cis*-9, *trans*-11 cla; C18:3 n3; C18:3 n6; C18:2 n6; C18:2 n6 *trans* 9,12).

#### *2.9-Statistical Analysis*

Data were analyzed using SAS statistical software. For the meat quality data, the design was of randomized blocks with subdivided parcel structure, where the type pasture will constitute the main plots and the use of polyethylene glycol the subplots. Were analyzed with a mixed model (MIXED procedure) with the type pasture, the inclusion PEG and their interaction, as fixed effects and the other effects were random.

For data on pasture production, compost antioxidants (Tannin and Tocopherol) of pasture and quality (chemical analysis), the design was randomized blocks, with repeated measures in time. Were analyzed with a mixed model (MIXED procedure) for repeated measurements with the type of pasture and sampling period as fixed effects and the other effects were random.

After the normality test of the residues, covariance structures were tested to fit the models, based on Bayesian information criterion (BIC), from which the structure "component of variance (VC)" was selected. The normal distribution of data was tested. When differences were observed, the averages were compared using the Tukey 10% probability test. Interactions involving type pasture and polyethylene glycol were significant at 10%. The variables were also submitted to Pearson correlation analysis.

### **3. Results**

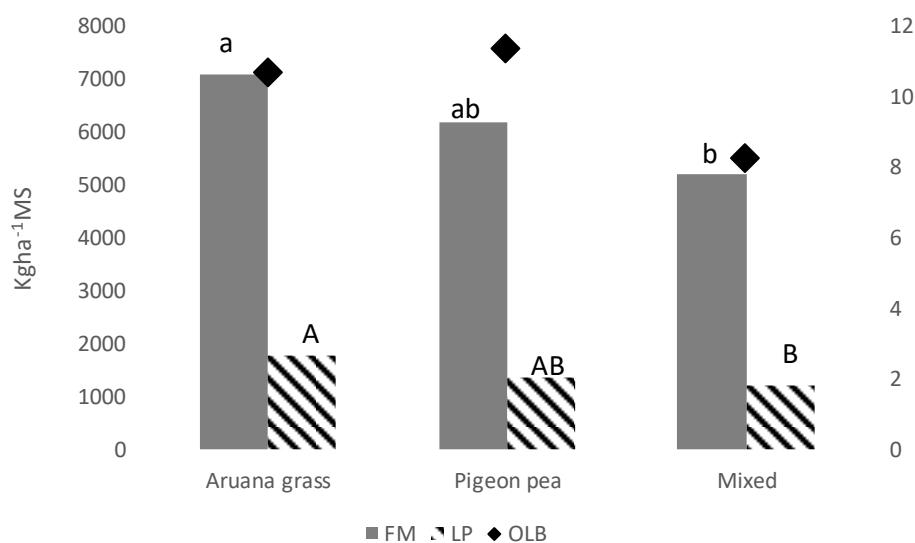
All data related to animal production and meat quality did not show any interaction between type of pasture and PEG. Therefore, all results were presented separately for the two effects studied.

#### *3.1 Pasture accumulation, chemical composition of pastures and lambs average daily gain*

Type of pasture presented different production of FM and LP ( $P>0.05$ ; Figure 1). The FM and LP was greater in Aruana grass and lower in the Mixed ( $P<0.05$ ), whereas Pigeon pea did not differ between both pastures evaluated. Despite the differences observed in FM and LP, the OLB (Figure 1) was similar in all three treatments. The chemical composition of the diets is shown in Table 1. The studied type of pasture presented similar CP, EE and ISDMD ( $P > 0.10$ ). The ash, NDF and ADF were higher in Aruana grass than in Pigeon pea pasture, whereas Mixed pasture presented intermediate contents (Table 1;  $P<0.05$ ). The OM and ADL contents were lower in Aruana grass than in Pigeon pea pasture, whereas Mixed pasture presented intermediate contents (Table 1;  $P<0.05$ ).

There were no significant differences among the type of pasture in alpha-tocopherol content ( $P>0.10$ ; Table 1), with an average value of  $137\text{mg}^{-1}\text{kg}$ . The TT and TC were higher in Pigeon pea pasture, intermediated in the Mixed pasture and lower Aruana grass ( $P=0.001$ ; Table 1). Hydrolyzable tannins concentration was similar among the type of pasture and ranged from 1.6 to 3.5(eq-g) of leucocyanidine  $\text{kg}^{-1}$  of DM ( $P>0.10$ ; Table 1).

Fatty acid profile of the pastures is described in Table 1. The Aruana grass presented greater  $\sum\text{SFA}$  concentration and lower  $\sum\text{PUFAs}$ ,  $n$ -3 and  $n$ -6 than Pigeon pea ( $P<0.05$ ). Mixed treatment presented intermediate contents of  $\sum\text{SFA}$  and  $\sum\text{PUFA}$  ( $P<0.05$ ) being similar to both pastures for PUFA  $n$ -3, similar to Pigeon pea ( $P>0.10$ ) and for PUFA  $n$ -6, which was similar to the other pastures ( $P>0.10$ ). The  $n$ -6: $n$ -3 ratio was similar among the type of pasture( $P>0.10$ ; Table 1).



a, b Values within FM differ at  $P<0.10$

A, B Values within LP differ at  $P<0.10$

Figure 1- Mean forage mass (FM), leaf production (LP) and offer of leaf blade (OLB) on different type of pasture under grazing of lambs

Table 1- Pastures chemical composition and fatty acids profile (% FAMEs / total identified FAMES) pasture under grazing of lambs

Variables <sup>1</sup>	Type of pasture <sup>2</sup>			Probability	
	Aruana grass	Pigeon pea	Mixed	MSE <sup>3</sup>	P-Pasture
DM (%)	22.51	24.17	23.50	0.89	0.4878
OM (%)	88.35 b	90.51 a	88.92 ab	0.49	0.0433
Ashes (%)	11.33 a	9.09 b	10.80 ab	0.38	0.0247
ISDMD (%)	58.24	58.85	53.76	2.60	0.4076
CP (%)	14.00b	20.00 a	16.18 ab	1.47	0.0973
EE (%)	2.52	3.04	3.22	0.39	0.2900
NDF (%)	62.54 a	47.83 b	56.49 ab	2.27	0.0359
ADF (%)	31.55 a	27.70 b	29.99 ab	0.72	0.0482
ADL (%)	3.60 b	7.73 a	5.02 ab	0.54	0.0142
Alpha-Tocopherol	138.77	136.89	136	14.37	0.9819
Total Tannin	2.44 c	15.10 a	7.51 b	1.41	0.0001
Hydrolyzed tannin	1.67	3.55	1.82	0.37	0.3054
Condensed tannin	1.17 c	9.87 a	4.49 b	1.00	0.0001
<i>FA profile (%/FA total)</i>					
C14:0	2.23 a	1.53 b	1.81 ab	0.12	0.0403
C16:0	39.97 a	32.50 c	36.56 b	0.75	0.0006
C18:0	7.95 a	6.30 b	6.90 ab	0.21	0.0142
C18:1 cis9	3.76 b	5.27 a	4.19 ab	0.27	0.0213
ΣSFA	68.04 a	51.23 c	59.43 b	1.29	0.0014
ΣMUFA	9.65	8.64	8.54	0.36	0.1723
ΣPUFA	22.29 c	40.15 a	32.03 b	1.18	0.0090
n-3	11.50 b	23.12 a	18.03 a	1.15	0.0053
n-6	10.79 b	17.00 a	13.99 ab	0.66	0.0062
n6:n3	0.95	0.76	0.79	0.05	0.1466

<sup>1</sup> DM (%): Dry matter content; OM (%): Organic matter content; ISDMD (%): content of digestible organic matter; CP (%): Crude Protein; EE (%): Ether extract ; NDF (%): Neutral detergent fiber; ADF (%): Acid detergent fiber; ADL(%): lignin; Alpha-Tocopherol: mgkg<sup>-1</sup>; Total Tannin: (eq-g) of leucocianidine kg<sup>-1</sup> of DM); Hydrolyzed tannin: (eq-g) of leucocianidine kg<sup>-1</sup> of DM) ; Condensed tannin: (eq-g) of leucocianidine kg<sup>-1</sup> of DM); FAME: Fatty acids methyl esters; n3(%): omega 3; n6(%):omega 6; ΣSFA (%): Sum of saturated fatty acids; ΣMUFA (%): Sum of monounsaturated fatty acids; ΣPUFA (%): Sum of polyunsaturated fatty acids; n6:n3: ratio omega 3/ omega 6; <sup>2</sup>type of pasture: Aruana grass (*Panicum maximum*); Pigeon pea (*Cajanus cajan*) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>3</sup>MSE: mean square error

a, b Values within a row with different letters differ significantly at P < 0.10.

Regarding the ADG of lambs was similar among the type of pasture (P>0.10; Table 2). Intake of pasture and alpha-tocopherol is shown in Table 2. The lambs grazing Mixed pasture consumed 13% less than the pure pastures (P <0.05; Table 2). Lambs grazing Aruana grass and Pigeon pea presented a 14.17% greater intake of alpha-tocopherol than that registered in the lambs of Mixed pasture (Table 2).

### 3.2 Carcass characteristics and on subcutaneous fat colour

The carcass characteristics are shown in Table 2. The lambs were slaughtered with an average weight of  $25.7 \pm 4.36$  kg, regardless of the type of pasture. The type of pasture had not effect in any studied carcass parameter (Table 2;  $P>0.10$ ), whereas the inclusion of PEG to improve the carcass fatness degree, carcass conformation and carcass yield ( $P<0.10$ ; Table 2).

There were no significant differences ( $P>0.10$ ) among type of pasture on the subcutaneous fat color parameters (Table 2). The lambs from the inclusion of PEG had greater  $H^\circ$  and lower  $a^*$  ( $P < 0.05$ ), and to greater  $L^*$  ( $P<0.10$ ; Table 2) on subcutaneous fat color.

Table 2– Effect of type of pasture and the inclusion of PEG on the intake organic matter (% $,100\text{kg}^{-1}$  BW), intake alfa-tocopherol fresh matter ( $\text{mg kg}^{-1}$  FM) by lambs, average daily gain ( $\text{kg day}^{-1}$ ), carcass characteristics and on subcutaneous fat colour

Variables <sup>1</sup>	Type of pasture <sup>2</sup>			PEG <sup>3</sup>			Probability	
	Aruana grass	Pigeon pea	Mixed	Water	Peg	MSE <sup>4</sup>	p-Pasture	p-PEG
OMI (% $,100\text{kg}^{-1}$ BW)	1.67 a	1.68 a	1.45 b	1.63	1.57	0.062	0.0167	0.4684
ATI ( $\text{mg kg}^{-1}$ FM)	253.84 a	242.78 a	213.11b	250.21a	222.94b	13.18	0.0144	0.0192
ADG ( $\text{g day}^{-1}$ )	0.07	0.05	0.05	0.05	0.06	0.001	0.4124	0.5103
HCW (kg)	10.5	10.0	9.7	9.8	10.4	0.62	0.4251	0.1129
CCW (kg)	10.2	9.8	9.5	9.5	10.1	0.61	0.4170	0.1099
CY (%)	38.1	38.3	37.5	37.4 b	38.5 a	0.84	0.7764	0.0986
FD	2.7	2.7	2.6	2.5 b	2.8 a	0.21	0.8587	0.0626
CC	2.8	2.7	2.5	2.5 b	2.8 a	0.17	0.2339	0.0602
SFT (mm)	2.3	2.0	1.6	1.9	2.1	0.37	0.3689	0.3810
<i>Fat Colour</i>								
Luminosity	74.56	73.59	73.23	72.83 b	74.76 a	1.04	0.5822	0.0805
Redness	6.71	8.16	6.92	8.13 a	6.40 b	0.61	0.3691	0.0420
Yellowness	9.26	9.9	8.24	9.28	8.98	0.79	0.3363	0.7145
Hue angle	55.28	51.29	48.9	48.05 b	55.60 a	2.78	0.3610	0.0382
Chroma	11.69	12.98	10.94	12.51	11.24	0.85	0.3270	0.2031

<sup>1</sup> ADG( $\text{kg day}^{-1}$ ): Average daily gain; OMI(% $,100\text{kg}^{-1}$  BW): intake organic matter; ATI ( $\text{mg kg}^{-1}$  FM): intake alfa-tocopherol fresh matter (FM), BWS: BW slaughter; HCW: Hot carcass weight; CCW: Cold carcass weight; CY: carcass yield ; FD: Fatness degree; CC: Carcass conformation; SFT Subcutaneous fat thickness; <sup>2</sup>type of pasture: Aruana grass (*Panicum maximum*); Pigeon pea (*Cajanus cajan*) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>3</sup>PEG: WATER: lambs received water; PEG: lambs received  $60\text{g day}^{-1}$  of polyethylene glycol;<sup>4</sup>MSE: mean square error

a, b Values within a row with different letters differ significantly at  $P < 0.10$ .

### 3. 3 Meat physicochemical characteristics

There was not effect of the type of pasture on the *longissimus muscle* characteristics ( $P>0.10$ ). The inclusion of PEG to improve the pH 24 hours ( $P = 0.0516$ ; Table 3). The chemical composition, WRC and shear force were not affected by type of pasture or PEG ( $P>0.10$ ; Table 3). The L\*, b\*, H°, C\* values of LTL did not differ between type of pasture and inclusion of PEG ( $P>0.10$ ; Table 3). The type of pasture to affect the a\* colour meat ( $P=0.08$ ; Table 3), being lower in Arauna grass.

Table 3 –Effect of type of pasture and inclusion of PEG on *longissimus muscle* physicochemical characteristics

Variables <sup>1</sup>	Type of pasture <sup>2</sup>			PEG <sup>3</sup>		Probability		
	Aruana grass	Pigeon pea	Mixed	Water	Peg	MSE <sup>4</sup>	p-Pasture	p-PEG
pH 24 hours	5.66	5.87	5.85	5.68 b	5.91 a	0.09	0.3726	0.0516
DM (%)	24.25	23.99	23.71	24.14	23.83	0.67	0.5013	0.4097
EE (%)	2.10	1.94	1.80	1.95	1.95	0.24	0.5142	0.9939
WRC (gkg <sup>-1</sup> )	625.9	633.9	646.6	634.0	636.9	7.50	0.2564	0.7449
WBSF (kg fcm <sup>-1</sup> )	3.06	3.09	3.29	3.18	3.12	0.21	0.7502	0.8340
<i>Muscle Colour</i>								
Luminosity	43.87	41.99	42.77	42.68	43.07	0.67	0.2626	0.6322
Redness	23.67 b	24.61 a	24.04 ab	24.26	24.19	0.24	0.0886	0.8134
Yellowness	9.20	8.77	8.90	9.10	8.82	0.23	0.4720	0.3073
Hue angle	21.23	19.59	19.98	20.5	20.03	0.43	0.1304	0.3832
Chroma	25.41	26.14	25.99	25.93	25.76	0.27	0.2030	0.5692

<sup>1</sup>DM: Dry matter content; EE: Ether extract; WRC: Water retention capacity; WBSF: shear force; <sup>2</sup>type of pasture: Aruana grass (*Panicum maximum*); Pigeon pea (*Cajanus cajan*) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>3</sup>PEG: WATER: lambs received water; PEG: lambs received 60g day<sup>-1</sup> of polyethylene glycol; <sup>4</sup>MSE: mean square error

a, b Values within a row with different letters differ significantly at  $P < 0.10$ .

### 3.4 Tocopherol and Intramuscular fatty acid profile

The concentration of alpha-tocopherol in lamb meat (LTL muscle) was not affected by neither the type of pasture or PEG ( $P>0.10$ ; Table 4). Gamma-tocopherol was greater in Aruana grass, intermediated in Mixed and lower Pigeon pea ( $P=0.06$ ).

Meat fatty acids content (mg 100<sup>-1</sup> g of meat) was not affected by the type of pasture or inclusion of PEG ( $P>0.10$ ; Table 4). Most of FA was not affected by type of pasture, except the SFA i-C14:0 ( $P<0.01$ ), C15:0 ( $P>0.001$ ), i-C16:0 ( $P<0.01$ ), C17:0 ( $P<0.05$ ), i-C18:0 ( $P<0.05$ ), C18:0 ( $P<0.05$ ) C24:0 ( $P<0.05$ ), and the C16:1 trans9 ( $P<0.01$ ) and C24:1n9 ( $P<0.05$ ; Table 4).

Most of the groups of FA according the degree of saturation were not affected by any of the studied factors. Type of pasture affected the  $\sum$ SFA,  $\sum n\text{-}6$  and  $n\text{-}6/n\text{-}3$  ratio ( $P<0.05$ ). Meat from Aruana grass presented greater SFA and lower  $\sum n\text{-}6$  and  $n\text{-}6/n\text{-}3$  ratio than Pigeon pea ( $P>0.05$ ). Meat from Mixed pasture presented an intermediate values ( $P>0.05$ ), except for the  $n\text{-}6/n\text{-}3$  ratio, which were significantly different between the three types pasture ( $P<0.05$ ). The  $\sum$ PUFAs was higher in the meat of lambs grazing exclusively Pigeon pea or Mixed pasture ( $P=0.07$ ). There other groups of FA were similar among types pasture and inclusion of PEG ( $P>0.10$ ).

Table 4- Effect of type of pasture and the inclusion of PEG on lamb meat Tocopherol concentration and fatty acid profile (% FAMEs /FAMES total)

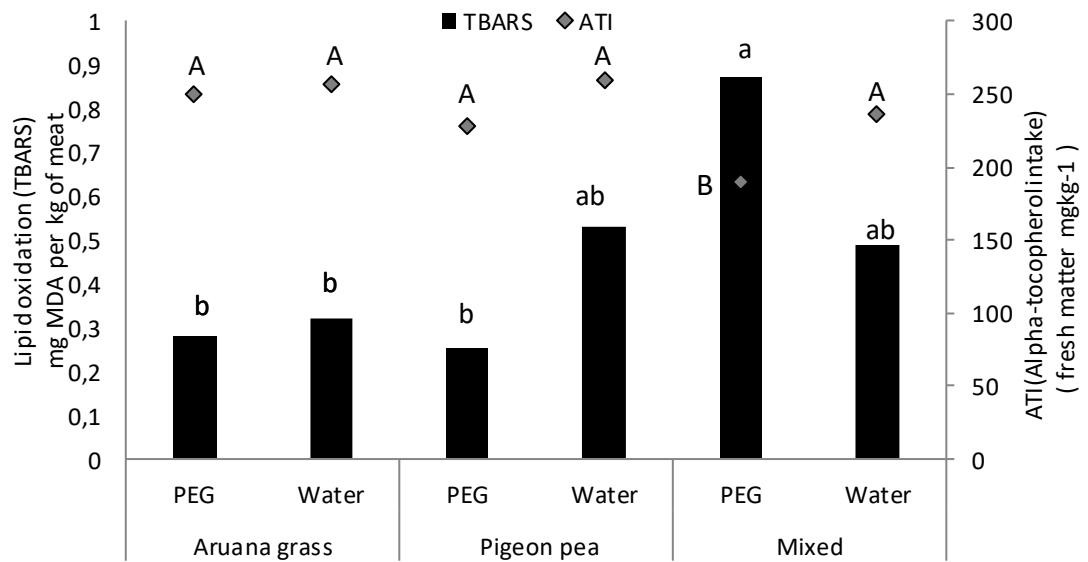
Variables	Type of pasture <sup>11</sup>			PEG <sup>12</sup>		Probability		
	Aruana grass	Pigeon pea	Mixed	Water	Peg	MSE <sup>13</sup>	p-Pasture	p-PEG
$\alpha$ -Tocopherol <sup>1</sup>	3.95	4.62	3.98	4.00	3.90	0.23	0.1392	0.528
$\gamma$ -Tocopherol <sup>1</sup>	0.16 a	0.13 b	0.15 a	0.14	0.151	0.006	0.0598	0.1957
FA mg/100g <sup>2</sup>	156.59	152.94	152.37	156.94	151.00	6.02	0.8857	0.4363
C11:0 <sup>3</sup>	0.40	0.25	0.43	0.38	0.34	0.03	0.1017	0.3037
C12:0 <sup>3</sup>	0.21	0.16	0.22	0.23 a	0.16 b	0.02	0.2975	0.0362
C13:0 <sup>3</sup>	0.42	0.31	0.31	0.35	0.34	0.05	0.3908	0.7189
i-C14:0 <sup>3</sup>	0.17 b	0.33 a	0.44 a	0.37 a	0.27 b	0.02	0.0086	0.0009
C14:0 <sup>3</sup>	1.72	1.49	1.80	1.89 a	1.49 b	0.16	0.5118	0.0896
C14:1 cis 9 <sup>3</sup>	0.10	0.10	0.08	0.08	0.10	0.03	0.8942	0.4741
a-C15:0 <sup>3</sup>	0.24	0.34	0.33	0.33 a	0.27 b	0.37	0.2474	0.0354
C15:0 <sup>3</sup>	0.48 a	0.16 c	0.28 b	0.32	0.30	0.02	0.0006	0.3260
C15:1 <sup>3</sup>	0.18	0.29	0.28	0.27	0.23	0.04	0.3014	0.3879
i-C16:0 <sup>3</sup>	0.28 a	0.15 b	0.17 b	0.19	0.18	0.01	0.0077	0.1505
a-C16:0 <sup>3</sup>	0.36 b	0.38 b	0.50a	0.42	0.40	0.04	0.0574	0.6450
DMA- C16:0 <sup>3</sup>	4.13	5.88	5.37	5.22	5.02	0.41	0.1002	0.6214
C16:0 <sup>3</sup>	19.97	19.02	18.96	19.28	19.36	0.53	0.4024	0.8646
C16:1 trans 9 <sup>3</sup>	0.45 a	0.29 b	0.44 a	0.40	0.40	0.01	0.0027	0.7955
C16:1 cis 7 <sup>3</sup>	0.22	0.21	0.22	0.22	0.21	0.01	0.9591	0.6019
C16:1 cis 9 <sup>3</sup>	1.22	0.98	1.10	1.12	1.08	0.06	0.1498	0.6758
C17:0 <sup>3</sup>	0.69 a	0.51b	0.58 ab	0.60	0.60	0.02	0.0216	0.9964
C17:1 cis 9 <sup>3</sup>	1.41	1.43	1.54	1.48	1.45	0.04	0.3169	0.6744
DMA-C18:0 <sup>3</sup>	3.22	3.83	3.78	3.60	3.62	0.20	0.2152	0.9295
i-C18:0 <sup>3</sup>	0.10 b	0.13 ab	0.15 a	0.13	0.12	0.01	0.0442	0.8199
C18:0 <sup>3</sup>	19.65 a	16.77 b	17.72 b	17.95	19.14	0.38	0.0140	0.5956
$\Sigma$ C18:1 <sup>3-4</sup>	26.64	24.72	24.04	24.71	25.56	1.12	0.3040	0.4979
C20:0 <sup>3</sup>	0.14	0.11	0.13	0.13	0.12	0.01	0.1461	0.3361
C20:1 n9 <sup>3</sup>	0.01	0.01	0.01	0.01	0.01	0.002	0.4799	0.8694
C20:3 n9 <sup>3</sup>	0.27	0.42	0.36	0.36	0.34	0.002	0.1010	0.5077

C22:0 <sup>3</sup>	0.04 b	0.07 a	0.06 a	0.05	0.06	0.006	0.0566	0.5445
C22:1 <sup>3</sup>	0.03	0.02	0.02	0.02	0.02	0.004	0.5148	0.4723
C24:0 <sup>3</sup>	0.04 a	0.01 b	0.01 b	0.02	0.02	0.01	0.0301	0.8893
C24:1 n9 <sup>3</sup>	0.05 a	0.02 b	0.03 b	0.04	0.03	0.01	0.0114	0.4641
Σ SFA <sup>3-5</sup>	52.45 a	50.47 b	51.64 ab	51.82 a	51.19 b	0.27	0.0243	0.0677
Σ MUFA <sup>3-6</sup>	30.35	28.12	27.81	28.98	29.14	1.13	0.2939	0.5472
Σ PUFA <sup>3-7</sup>	16.20 b	20.44 a	19.51 a	18.77	18.67	1.00	0.0732	0.9346
Σ CLA <sup>3-8</sup>	0.68	0.52	0.64	0.64	0.58	0.04	0.1748	0.2903
Σ n6 <sup>3-9</sup>	8.32 b	12.03 a	10.74 ab	10.45	10.27	0.59	0.0257	0.7902
Σ n3 <sup>3-10</sup>	6.92	7.46	7.77	7.31	7.47	0.47	0.4201	0.7468
n6:n3 <sup>3</sup>	1.12 c	1.62 a	1.40 b	1.43	1.39	0.04	0.0021	0.2342

<sup>1</sup> mgkg<sup>-1</sup> of fresh meat; <sup>2</sup> grams of fatty acids 100g of fresh meat<sup>-1</sup>; <sup>3</sup> % ; <sup>4</sup>(C18:1 *trans*-11; C18:1 *cis*-9; C18:1 *trans*-15; C18:1 *cis*-11; C18:1 *cis*-12; C18:1 *cis*-13, C18:1 *trans*-16 e C18:1 *cis*-15); <sup>5</sup> (C24:0; C22:0; C20:0; C18:0; i-C18:0; DMA-C18:0; C17:0; C16:0; a-C18:0; i-C16:0; DMA-C16:0; C15:0; a-C15:0; i-C15:0; C14:0; i-C14:0; C13:0; a-C13:0; C12:0; C11:0 e C10:0); <sup>6</sup> (C24:1 n9; C22:1; C20:1 n9; C18:1 *cis*-15; C18:1 *trans*-16; C18:1 *cis*-13; C18:1 *cis*-12; C18:1 *cis*-11; C18:1 *trans*-15; C18:1 *cis*-9; C18:1 *trans*-11; C17:1 *cis*-9; C16:1 *cis*-9; C16:1 *cis*-7; C16:1 *cis*-7; C16:1 *trans*-9; C15:1; C14:1 *cis*-9 e C12:1); <sup>7</sup> (C22:6 n3; C22:5 n3; C22:4 n6; C20:5 n3; C22:3 n3; C20:4 n6; C20:3 n6; C20:3 n9; C20:2 n6; C18:2 *trans*-10, *cis*-12 cla; C18:2 *trans*-9, *cis*-11 cla; C18:2 *cis*-9, *trans*-11 cla; C18:3 n3; C18:3 n6; C18:2 n6 e C18:2 n6 *trans*-9,12); <sup>8</sup>(C18:2 *cis*, *trans*-11; C18:2 *trans*-9, *cis*-11 ; C18:2 *trans*-10, *cis*-12); <sup>9</sup>(C22:4 n6; C20:4 n6; C20:3 n6; C20:2 n6; C18:3 n6; C18:2 n6; C18:2 n6 *trans*-9,12); <sup>10</sup>(C22:6 n3; C22:5 n3; C20:5 n3; C22:3 n3; C18:3 n3); <sup>11</sup>type of pasture: Aruana grass (*Panicum maximum*); Pigeon pea (*Cajanus cajan*) and mixture of Aruana grass and Pigeon pea (MIXED); <sup>12</sup>PEG: WATER: lambs received water; PEG: lambs received 60g day<sup>-1</sup> of polyethylene glycol; <sup>13</sup>MSE: mean square error  
a, b Values within a row with different letters differ significantly at P < 0.10.

### 3.5 Storage time study: pH and lipid oxidation

The type of pasture and inclusion of PEG did not affect the pH at 6d, drip losses 6d and TBARS 1d (P> 0.10) with mean values of 5.24, 6.04% and 0.10 mg MDA per kg of meat; respectively). TBARS at 6d storage showed an interaction between the type of pasture and the inclusion of PEG (P = 0.0352; Figure 2). The addition of PEG in Mixed pasture increased the lipid oxidation of meat (P<0.05), whereas did not affect the other treatments. Aruana grass produced a meat with lower TBARS at 6d of storage, regardless of the inclusion of the PEG (Figure 2; P>0.05). The intake of alpha-tocopherol of lamb grazing Mixed+PEG was lower (Figure 2; P<0.05) than on the other of treatments.



a, b Values within TBARS differ significantly at  $P<0.10$

A, B Values within ATI differ significantly at  $P<0.10$

Figure 2- Lipid oxidation (TBARS) after 6 days of storage and alpha-tocopherol intake (ATI;  $\text{mg kg}^{-1}$ fresh matter) in type of pasture

#### 4. Discussion

Even the types of pasture producing different FM and LP, the supply of OLP was similar between the types of pasture, reflecting a similar performance of lambs. According to Hodgon (1990), in grazing system, pasture should be offered at a level of three times of animal's requirements, which according to NRC (2007), is around 2.7 to 3.5 % BW. However, in the present study the pasture intake was only 1.6% BW, which caused a low ADG ( $0.056 \text{ g day}^{-1}$ ), and our results are in line to those found by Fajardo et al. (2016), who found mean  $0.026 \text{ g day}^{-1}$  in lambs grazing Aruana pastures. Tropical pastures, depending on the management to which they are imposed, can present special dispersion of the leaf blades which could increase the time of grazing (Carvalho et al., 2001) and therefore reducing food intake and animal performance.

The difference in chemical composition and FA profile of the three pastures was not sufficient to affect pasture intake, not changing the ADG of lambs, enabling similar BW at slaughter. The FA profile of the pastures depends on several factors such as species, degree of senescence (Boufaied et al., 2003), phenological stage and degree of fertilization. In the present

study we assessed two different species Grasses vs Leguminous, and their Mixture, which presented intermediate FA contents between grass and leguminous plants.

Carcass parameters and subcutaneous fat were unaffected by type of pasture, which is a consequence of the pasture intake and similar ADG on the feeding regimes, resulting in carcasses with same BW at slaughter. Carcass characteristics are mostly unaffected by tocopherol on the type of pasture. Kasapidou et al. (2012) and Turner et al. (2002) working with increasing doses of tocopherol in the diet also did not find differences in the carcass characteristics.

In the present study, PEG inclusion to improve the CY, FD and CC. This result was unexpected, as TC may alter rumen protein degradability and increase the CP digestion in the intestinal portions (Ishlak et al., 2015), it would lead to a more efficient digestion of nutrients and consequently would alter the carcass characteristics. Priolo et al. (2000), working with a diet containing 2.5% TC with or without PEG, also reported lambs fed the tannin diet had a lower carcass yield and had less fat. The addition of PEG in the diet may have caused a better-fractionated absorption of the amino acids in the intestine (Waghorn, 1996; Waghorn et al., 1994), altering some characteristics of the carcass. Regarding the subcutaneous fat colour, the inclusion of PEG may have favored a greater accumulation of lutein, resulting in a higher content of H° (Priolo et al., 2002; Ripoll et al., 2012) and lower content of a\* and a tendency of higher L\*.

The pH at 24 hours was not affected by the type of pasture, in contrast to Brito et al. (2016), who found effect of the type of pasture on the pH at 24 hours. The inclusion of PEG in the diet presents a higher pH 24 hours. The TC can increase pH 24 hours because it could have an effect on muscle glycogen, altering lactic acid formation during storage, and may modify pH (Priolo et al., 2000), contradicting the findings of this study.

The lambs that grazed Pigeon pea, present the greater value of a\* in the LTL, when compared to the others of pastures, which may be related to the greater content of condensed tannins in Pigeon pea. The tannins could alter the ruminal microorganisms, mainly those related to the biosynthesis of vitamin B12, which is related with synthesis of heme pigments, and thus can affect the color of meat (Priolo and Vasta 2007).

Lambs grazing Pigeon pea tended to accumulate less gamma-tocopherol in the muscle. Ponnampalam et al. (2012a) when evaluating different feeding systems (perennial pasture, annual pasture, hay, lucerne hay, oat grain, flaxmeal, flaxseed) found different concentrations

of gamma and alpha tocopherol in pastures. But they only analyzed the concentration of alpha tocopherol in the meat. Among the compounds of vitamin E, alpha-tocopherol is reported to be the most potent in its antioxidant action (Yoshida et al., 2003).

Although alpha-tocopherol consumption by lambs between types of pasture was different, the concentration of alpha-tocopherol in meat was similar, showing an average of 4.18 mg of alpha-tocopherol kg<sup>-1</sup>. The prolonged intake of tocopherol, may have resulted in saturation of muscle, involving that all lambs presented similar level in the meat. Ponnampalam et al. (2012a), Turner et al. (2002) e López-Bote et al., (2001) concluded that low amount of alpha-tocopherol kg<sup>-1</sup> of meat may cause and improvement of the lipid oxidation and of the color stability.

Type of pasture and inclusion of PEG did not affect the content of fat in the muscle. In grazing system, lambs had a great energy expenditure on pasture (Moron- Fuenmayor and Clavero, 1999), what can prevent the fat deposition.

Grazing legume-rich pasture resulted in a meat with less  $\Sigma$ SFA, less stearic acid and greater  $\Sigma$ PUFA concentration. Our results are in line to those from Vasta et al. (2009) and Ponnampalam et al. (2017), who found that the animals that grazed legume showed higher concentration of PUFA than grazing annual ryegrass. In contrast, Dierking et al. (2010) observed that esteric acid content was greater when animals received legumes. The  $\Sigma$ MUFA, mainly the C18:1, were similar among treatments, in agreement with Ponnampalam et al. (2012b; 2017). Turner et al. (2014) reported that when lambs select legumes, impacts may occur in ruminal fermentation and consequently there is an increase of PUFAs in meat. The TC present in the leguminous plants also may have an influence on ruminal bio-hydrogenation (Vasta et al., 2009), favoring the greater passage, and increase PUFA in meat (Lee et al., 2004). In our study, even the highest *n*6: *n*3 ratios (1.62: 1 for lambs grazing Pigeon-pea pasture) were largely below to those 4:1 recommended by Simopoulos (2002) to be beneficial to human health.

The CLA are mainly originated, directly or indirectly, from ruminal biohydrogenation of linoleic and linolenic acids (Bessa et al., 2005). Type of pasture provided distinct concentrations of these acids (Table 1), but not sufficient to alter the concentration of CLA in the lambs' meat, the opposite reported by Vasta et al (2007), who found lower concentration of CLA when lambs received a tannin-rich diet supplemented with PEG.

Lambs consuming Aruana grass showed lower lipid oxidation (Figure 2) at day 6, which may be directly related to the tendency to have lower concentration of PUFAs in the

meat. The lower consumption of alpha tocopherol by lambs in Mixed + PEG treatment (Figure 2), can have influenced the oxidation of the meat. The condensed tannin concentration was not enough to affect the oxidation, which may be related to Pigeon pea be considered a plant with a moderate to low degree (<5% kg DM<sup>-1</sup>) of tannin concentration (Vitti et al. 2005; Godoy, 2007; Louvandini et al., 2011).

## Conclusion

The type of pasture had no effect on animal performance, carcass traits and meat physicochemical characteristics. The inclusion of polytethylene glycol affected the subcutaneous fat color, with higher content of hue angle and lower content of redness. The inclusion of the legume in the pasture resulted in a meat with less concentration SFA and greater PUFA concentration. The exclusive use of Aruana grass in lamb feed produced a meat with a higher resistance to lipid oxidation in six days of storage.

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## CONSIDERAÇÕES FINAIS

A qualidade do produto final (carne ou leite), depende principalmente do alimento oferecido ao animal. Os compostos secundários (taninos e tocoferoais) presentes nas plantas forrageiras, são antioxidantes naturais, que podem modificar o perfil lipídico da carne, cor e a oxidação lipídica, alterando o tempo de prateleira. A ingestão de carne com maior nível de antioxidante também pode interferir diretamente na saúde humana, pois constantemente o corpo humano fabrica radicais livres, que causam danos às células.

Nesta tese primeiramente realizamos uma meta-análise utilizando as palavras-chaves: tocoferol, carne e cordeiros. Após a busca e posterior compilação e análise dos dados obtidos dos artigos, realizamos duas obordagens principais. A primeira relacionada aos sistemas de terminação de cordeiros e a segunda referente aos níveis de tocoferol na dieta. Com base na meta-análise realizada, concluímos que os sistemas de alimentação (pasto ou concentrado) interferem nas características da carne. Os animais criados exclusivamente a pasto, apresentam menor relação ômega 6: ômega 3 e maior concentração de CLA na carne, em relação aos animais que receberam algum tipo de concentrado. Os níveis de tocoferol encontrados nas dietas, também interferiram na qualidade da carne, onde os animais que estavam recebendo os menores níveis de tocoferol, apresentaram consequentemente a menor concentração de alfa-tocoferol na carne e maior propensão à oxidação lipídica da carne.

Diversos trabalhos têm sido desenvolvidos pelo nosso grupo de pesquisa, no sentido de entender como os diferentes sistemas de alimentação de cordeiros influenciam no desenvolvimento dos animais, na sustentabilidade ambiental e econômica da cadeia produtiva. Pensando nisso realizamos um estudo onde se avaliou a produção de forragem e composição química da dieta dos cordeiros alimentados com pastagens tropicais e seus reflexos sobre a qualidade da carne. E verificamos que as pastagens tropicais apresentaram variação produtiva ao longo do tempo de utilização da pastagem. Que a inserção de leguminosas na dieta de cordeiros não altera a concentração de tocoferol, mas, ao longo dos períodos de avaliação da pastagem tropical, os níveis de taninos aumentaram na dieta contendo leguminosas. A participação da leguminosa na dieta de cordeiros aumentou o teor de omegas 3 e 6 disponíveis na dieta, essa proporção maior de omegas na dieta dos cordeiros, refletiu em mudança na proporção desses mesmos ácidos graxos na carne. A carne de cordeiros terminados exclusivamente em Capim Aruana apresentou menor relação n-6: n-3 e menor oxidação lipídica do que animais mantidos em feijão guandu ou em pastagem consorciada. A inclusão de PEG nas dietas dos cordeiros pode influenciar algumas características da carcaça, como o aumentar o valor do ângulo da tonalidade e diminuir a intensidade de vermelho da gordura subcutânea. Os dados da presente tese apontam que a produção de carnes, com bom teor de tocoferol, com perfil lipídico favorável à saúde humana pode ser obtido quando utilizamos pastagens tropicais. E quando utilizado exclusivamente gramíneas na alimentação, se obtém uma carne de cordeiro com menor oxidação lipídica e menor relação n6:n3. Espera-se que esses resultados tenham utilidade prática

no sentido de produzir cordeiros em pastagens tropicais, e de conseguir uma carne favorável à industria, à saúde do consumidor e que reverta em valorização do produtor, com maior renda aos produtores. Novos estudos com melhor controle das condições experimentais são necessários para se obter resultados mais acurados dos efeitos reais dos tipos de pastagem e o efeito dos antioxidantes das pastagens tropicais na qualidade da carcaça e da carne dos cordeiros.

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## VITA

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