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EFEITO DA INCLUSÃO DE NITRATO NA DIETA SOBRE A FERMENTAÇÃO RUMINAL, A EMISSÃO DE METANO ENTÉRICO E O DESEMPENHO DE OVINOS

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EFEITO DA INCLUSÃO DE NITRATO NA DIETA SOBRE A FERMENTAÇÃO RUMINAL, A EMISSÃO DE METANO ENTÉRICO E O DESEMPENHO DE OVINOS[1]

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RESUMO

Objetivou-se avaliar os efeitos da inclusão de nitrato encapsulado na dieta sobre a fermentação ruminal, a emissão de metano enterico e o desempenho de ovinos. Foram utilizados 18 cordeiros Santa Inês (peso vivo médio de 27 ± 5,55 kg) designados em blocos casualizados por peso, sendo oferecida uma dieta 40:60 feno/concentrado, como dieta basal e alocados a um dos seguintes tratamentos: dieta controle (dieta basal com 1,50% de uréia), Nitrato (dieta basal com 4,51% nitrato encapsulado) e Nitrato + CNSL (dieta basal com 4,51% nitrato encapsulado com liquido de casca de castanha de caju). Foi utilizado um período de adaptação de 28 dias e posteriormente dois períodos experimentais de 32 dias, sendo que nos últimos 6 dias de cada período foi feita a quantificação da emissão do metano. Cada 14 dias (dia 14, 28, 42 e 56) as amostras de sangue e liquido ruminal (6 e 3 horas após alimentação da manhã, respectivamente) foram coletadas. Após os 92 dias, o ensaio de metabolismo (6 dias) foi realizado. Em seguida, os animais foram abatidos e as carcaças foram avaliadas. A utilização de nitrato encapsulado diminuiu em 32% a emissão de metano em relação aos animais que receberam a dieta controle (28,57 vs 19,34 L/kg MS consumida) ($P<0,02$), sem que fossem detectadas diferenças de emissão de metano entre o tipo de encapsulamento ($P>0,05$). Os cordeiros que receberam Nitrato + CNSL apresentaram maior ($P<0.01$) produção total de AGCC quando comparados aos que receberam Nitrato ou dieta controle. A concentração de NH_3 ruminal foi reduzida ($P<0,01$) (24,33 vs 34,93 mg/100 ml) pelo nitrato encapsulado nas dietas, sem que fosse ($P>0,08$) detectado efeito sobre a % metahemoglobina (0,62 vs 1,0% MetHb). A inclusão de nitrato encapsulado aumentou ($P<0,01$) a concentração de acetato, propionato e butirato. A inclusão de nitrato encapsulado na dieta reduziu o número total de protozoários em 12% ($P<0,01$). O consumo de MS, GPD, digestibilidade aparente dos nutrientes, balanço-N e síntese de N-microbiano não foram afetados pelo adição de nitrato encapsulado ($P>0,05$). Não houve efeito sobre parâmetros plasmáticos (proteína total, albumina, AST, ALT e uréia), resíduos de nitrato e nitrito na carne fresca, cor de carne e as características de carcaça avaliadas. A inclusão de nitrato encapsulado na dieta permite mitigar a emissão de metano entérico, proporciona um ambiente adequado para a fermentação ruminal sem que sejam observados sinais clínicos de intoxicação em cordeiros em crescimento.

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[1]Tese de Doutorado em Zootecnia - Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (126 p.) Março, 2013.

EFFECT OF NITRATE INCLUSION IN THE DIET ON RUMEN FERMENTATION, ENTERIC METHANE EMISSION AND PERFORMANCE IN OVINE[1]

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ABSTRACT

The objective of this study was to evaluate the effect of encapsulated nitrate inclusion in the diet on rumen fermentation, enteric methane emission and performance in sheep. Eighteen Santa Inês male lambs (27 ± 5.55 kg of BW) were randomly blocked according to BW, with basal diet consisted of 40:60 roughage to concentrate and allocated to one of three treatments diets: 1) Basal diet supplemented with urea at 1.5 % of dietary DM (control), encapsulated nitrate at 4.51 % of dietary DM (Nitrate), and encapsulated nitrate with cashew nut shell liquid at 4.51 % of dietary DM (Nitrate + CNSL). Lambs were gradually adapted for 28 days and two experimental periods composed of 32 days in each. Within each period, the last 6 days were used for methane collection. Every 14 d (day 14, 28, 42 and 56) blood and ruminal fluid samples (6 and 3 h after morning feeding), respectively were collected. After 92 days the metabolic study was conducted for 6 days, subsequently all lambs were fasted, slaughtered and carcass traits were evaluated. The CH_4 emission was reduced ($P < 0.02$) by 32 % in encapsulated nitrate-fed lambs relative to control (28.57 vs 19.34 L/kg DMI). A greater ($P < 0.01$) total SCFA concentrations were observed with Nitrate + CNSL addition in the diet. The addition of encapsulated nitrate increased ($P < 0.01$) acetate, propionate and butyrate concentrations relative to control diet. Encapsulated nitrate diets was reduced ($P < 0.01$) ruminal NH_3-N (24.33 vs 34.93 mg/100 ml), with no effects ($P > 0.08$) on MetHb concentration (0.62 vs 1.0 % Hb). Total protozoa count was reduced ($P < 0.01$) by 12 % with encapsulated nitrate diet than does control diet. There were no differences among treatments in DMI, ADG and apparent nutrients digestibility, N-balance and microbial-N with encapsulated nitrate inclusion in the diet ($P > 0.05$). No differences ($P < 0.05$) were observed in plasma blood parameters (total protein, albumin, AST, ALT and urea), meat color objective, sodium nitrate and nitrite residual in fresh meat and carcass characteristics determined. In conclusion our data inferred that incorporation of encapsulated nitrate in growing lambs diet is an effective means for mitigating enteric methane emissions without compromising the performance or carcass characteristics with no clinical signs.

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[1]Doctoral thesis in Animal Science - Animal Production, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (126 p.) Março, 2013.

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CAPÍTULO 2.

LISTA DE ABREVIATURAS E SÍMBOLOS

µM	Micromolar
*a**	Redness
ADF	Acid detergent fiber
ADG	Average Daily Gain
AGCC	Acidos graxos de cadeia curta
ALT	Alanine amino-transferase
AST	Aspartato amino-transferase
AOAC	Association of Official Analytical Chemists
AST	Aspartate amino-transferase
*b**	Yellowness
BW	Body weight
C	Celsius
CCW	Cold carcass weight
CCY	Cold carcass yield
CENA	Center for nuclear energy in agriculture
CNSL	Cashew nut shell liquid
CP	Crude protein
CWL	Chilling weight loss
d	Day
dL	Deciliter
DMI	Dry matter intake
DOMI	Digestible organic matter intake
EE	Ether extract
EMN	Efficiency of microbial nitrogen
FBW	Final body weight
FEC	Fecal egg counts per gram
g	Gram
h	Hour
H	Hydrogen
Hb	Hemoglobin
HCW	Hot carcass weight
HCY	Hot carcass yield
IBW	Initial Body Weight
kg	Kilogram
L	Liter
*L**	Lightness
LANA	Laboratory of animal nutrition
MetHb	Methemoglobin
MFS	Methyl green-formalin-saline solution
mg	Milligram
mL	Milliliter
mM	Millimolar
MNS	Microbial nitrogen supply
N	Nitrogen
NAD	Nicotinamide adenine dinucleotide
NDF	Nuteral detergent fiber

$N\text{-}NH_3$	Nitrogênio amoniacal
NRC	National Research Council
OM	Organic matter
PD	Purine derivatives
pH	potencial Hidrogeniônico
RBCs	Red blood cells
SAS	Statistical analysis system
SCFA	Short chain fatty acid
SEM	Standard error of the mean.
SLBW	Slaughter live body weight
wk	Week

CAPÍTULO 1

1. INTRODUÇÃO

Nos países em desenvolvimento, o sistema de produção pecuário tem dificuldade em fornecer dietas que permitam atender as exigências nutricionais de animais ruminantes. Regiões situadas principalmente nos trópicos apresentam uma grande variação na disponibilidade e qualidade das forragens, principalmente durante a estação seca, que limitam a produtividade animal. A alimentação de baixa qualidade (forragens tropicais), devido à inadequada ou flutuação da oferta de nutrientes, conduz a baixos índices tanto reprodutivos como produtivos, bem como o aumento da susceptibilidade a doenças dos animais. Portanto, faz-se necessário buscar estratégias nutricionais alternativas, que maximizem a eficiência da utilização dos nutrientes disponíveis e otimizem o crescimento microbiano.

A otimização do processo fermentativo da matéria orgânica no rúmen, depende de um adequado fornecimento de carboidratos e nitrogênio degradáveis, assim como de macro e microminerais, além da manutenção de um baixo potencial redox (redução do NAD^+ para NADH). As bactérias metanogênicas (*Archaea*) usam H_2 e NADH para reduzir o CO_2 formando metano (CH_4), possibilitando que o NAD^+ seja regenerado e garantido a continuidade do metabolismo microbiano durante a fermentação das matérias primas. Um dos grandes desafios na nutrição de ruminantes é diminuir as emissões de CH_4 entérico em ruminantes. Neste sentido, tem sido claramente demonstrado que, as emissões de CH_4 podem ser diminuídas através de mecanismos dissipadores dos elétrons, provenientes da fermentação dos componentes da dieta e a conseqüente formação dos produtos intermediários no ambiente ruminal.

O CH_4 tem um papel importante na manutenção da fermentação no ecossistema ruminal, pois ajuda a garantir o baixo potencial redox, necessário em sistemas de fermentação anaeróbios. Nesse processo, o H_2 presente no fluido ruminal é utilizado pelas bacteriás metanogênicas (*Archaea*) para reduzir o CO_2 a CH_4, porém esta rota metabólica ocasiona perdas da energia bruta contida na dieta. Existem várias estratégias nutricionais que podem ajudar a reduzir as grandes quantidades de CH_4 produzidas durante a fermentação entérica em ruminantes consumindo volumosos de baixa qualidade. Uma das alternativas disponíveis é a incorporação de nitratos na dieta.

A presença de nitratos no rúmen redireciona o H_2 da metanogênese para redução dos mesmos, diminuindo assim a produção de CH_4. Além de captar o H_2 ruminal, os sais de nitratos podem substituir a uréia em dietas com baixos teores de proteína, fornecendo nitrogênio degradável e estimulando a fermentação ruminal. Na prática inclução de nitrato na alimentação de ruminantes não tem sido utilizada comercialmente, devido à possível toxicidade do nitrito que converte a hemoglobina em metahemoglobina (MetHb) e diminui o transporte de oxigênio pelo sangue.

A hipótese deste trabalho foi que adição de nitrato encapsulado na dieta de ovinos favorece a sincronia entre a redução de nitrato a nitrito e aumenta a capacidade de capturar o H_2 disponível, resultando em redução da emissão de CH_4 e excreção de nitrogênio, sem efeitos prejudiciais sobre a saúde e o desempenho animal.

Objetivo Geral

Avaliar o efeito da inclusão de nitrato encapsulado em dietas de ovinos sobre a emissão de CH_4 entérico *in vivo*, o desempenho e os parâmetros sanguíneos e ruminais.

Objetivos Específicos

Avaliar o efeito da inclusão de nitrato encapsulado sobre a emissão de CH_4 entérico *in vivo*, desempenho, fermentação ruminal e parâmetros sanguíneos.

Avaliar o efeito da inclusão de nitrato encapsulado sobre a digestibilidade aparente dos nutrientes, excreção dos derivados das purinas, parâmetros de sangue e características da carcaça e qualidade da carne dos animais.

2. REVISÃO DA LITERATURA

2.1. Emissão de metano mundial e no Brasil pela pecuária

A sustentabilidade dos sistemas de produção animal depende da manutenção do ambiente. Nas últimas décadas, tem crescido a preocupação ambiental pelo aumento da concentração de gases do efeito estufa (GEE) e seu efeito sobre o aumento da temperatura da terra (Steinfeld et al., 2006). A agricultura e a pecuária contribuem significativamente com as emissões de dióxido de carbono (CO_2), metano (CH_4) e óxido nitroso (N_2O) (IPCC, 2007). Estes gases têm um papel decisivo nas trocas energéticas entre o planeta e o meio ambiente. O CH_4 é o segundo GEE mais problemático após CO_2 (Moss et al. 2000), pois apresenta um potencial de aquecimento global 25 vezes maior que o CO_2 (Foster et al., 2007).

Estimativas realizadas nos países em desenvolvimento localizados na região tropical os caracterizam como importantes emissores de gases de efeito estufa, uma vez que as condições climáticas dessa região aumentam em muito o potencial de emissão de gases como o CH_4, que já contribui com 15 % da emissão global (Cotton e Pielke, 1995). As emissões de CH_4 a nível mundial são geradas por fontes antropogênicas (70 %) e naturais (30 %). A agricultura produz aproximadamente 10 a 12 % do total de emissões globais de GEEs antropogênicas (Smith et al., 2007). No mundo, os ruminantes são responsáveis por cerca de 32 % da emissão de CH_4 antropogênico (Perdok e Newbold, 2009), enquanto no Brasil esta contribuição chega a 70 %, principalmente em função do tamanho do rebanho nacional (MCT, 2006).

O'Mara (2011) citou de FAOSTAT (2010) que o rebanho bovino mundial é maior quando comparado com os rebanhos ovino e caprino, sendo responsáveis pelas maiores emissões de CH_4 entéricas. A Ásia detêm 28,6 % dos bovinos do mundo, encontrando-se as maiores concentrações destes na Índia e China. A América do Norte tem 8,2 %, Europa Ocidental 6,8 %, e Austrália / Nova Zelândia 2,8 %, dos bovinos do mundo. A maioria dos búfalos (175 milhões) e as cabras do mundo (822 milhões) encontram-se na Ásia, concentrando-se principalmente na Índia e China, enquanto os ovinos estão distribuídos globalmente (1,09 bilhões).

Segundo dados da EPA (EPA, 2006), as emissões globais de CH_4 entérico representaram 1929 milhões de toneladas de CO_2-equivalente, ou 91,9 milhões de toneladas de CH_4 em 2005. A Ásia foi o continente que mais emitiu CH_4 entérico, com cerca de 33 % das emissões globais. Outras regiões do mundo, com grande participação nas emissões de CH_4 entérico são a América Latina (23,9 %), África (14,5 %), Europa Ocidental (8,3 %) e América do Norte (7,1 %) (EPA, 2006 citado por O'Mara, 2011).

No ranking mundial de emissão de CH_4 entérico, a Índia e o Brasil são responsáveis com 14,5 e 10,3 Tg de CH_4/ano, respectivamente. O Brasil é apontado como o maior emissor de CH_4 (9,6 Tg de CH_4/ano), seguido da Índia (8,6 Tg de CH_4/ano) e dos Estados Unidos da América (5,1 Tg de CH_4/ano) quando é considerada apenas a emissão por bovinos (Thorpe, 2009).

Tem sido estabelecido que a intensidade da emissão do CH_4 na pecuária está relacionada com fatores dependentes do tipo de animal, consumo, alimento e grau de digestibilidade das dietas fornecidas (Johnson e

Johnson, 1995). As forrageiras tropicais apresentam menor valor nutritivo, quando comparadas com as forrageiras de clima temperado, conseqüentemente, a pecuária concentrada nas zonas tropicais, que tem sua dieta a base de forrageiras, emitem mais CH_4.

Existem alguns fatores que podem influenciar negativamente a digestibilidade da fibra, e conseqüentemente, a produção de CH_4, tais como a presença de compostos secundários (*ex*, taninos) em leguminosas e gramíneas C4 (Archimède et al., 2010). Portanto, é importante investir em pesquisas que melhorem a eficiência de utilização dos nutrientes contidos nos alimentos consumidos pelos animais e, ao mesmo tempo, reduzam a emissão de CH_4 para a atmosfera.

2.2. Fermentação entérica e produção de CH_4

Os ruminantes são animais que têm a capacidade de utilizar uma grande variedade de alimentos como fonte de nutrientes. Existe uma relação simbiótica entre o hospedeiro e os microrganismos ruminais, que possibilita o uso da parede celular de vegetais e nitrogênio não proteico (NNP) como fonte de nutrientes, que não são utilizados por outros animais. A relação simbiótica ocorre porque o animal fornece alimento e um ambiente adequado para o crescimento dos microrganismos, que por sua parte, suprem o animal com ácidos graxos de cadeia curta (AGCC) resultantes da fermentação e proteína microbiana.

No ambiente ruminal a atividade enzimática dos microrganismos hidrolisa a maior parte da matéria orgânica (MO) dietética para aminoácidos e açúcares simples (O'Mara, 2004). Esta digestão anaeróbia é possível devido ao sinergismo existente entre os diversos grupos de microrganismos existentes no rúmen. Os produtos finais de processo de fermentatção ruminal são os AGCC (Acético, Propiônico e Butírico), amônia (NH_3), células microbianas, H_2, CO_2 e CH_4. A qualidade e quantidade dos produtos finais do processo da fermentação ruminal são dependentes das espécies e atividade dos microrganismos presentes.

Os ruminantes, devido ao processo digestivo de fermentação entérica, são reconhecidos como importantes produtores de CH_4 (Lassey, 2008). A produção de CH_4 faz parte do processo de fermentação anaeróbia dos alimentos e sua reação de síntese constitui o principal dreno de H_2 no rúmen. A produção de CH_4 no rúmen envolve um conjunto de reações, nas quais participam diferentes espécies de microrganismos, sendo que a etapa final destas reações é realizado pelos microrganismos metanogênicos (Moss et al., 2000). O rúmen se caracteriza por ser um ambiente anaeróbio onde o carbono do CO_2 é o último aceptor de íons de H_2 gerando CH_4 (McDonald et al., 2002). Durante a oxidação de açúcares, o NAD^+ é reduzida a NADH que precisa ser re-oxidado a NAD^+ para permitir que a fermentação continue (Moss et al., 2000; McAllister e Newbold, 2008).

A estequiometria da conversão de um mol de glicose para AGCC e a proporção em que cada ácido é produzido, dependem da espécie bacteriana, que pode ser especializada em produzir um tipo ou outro e principalmente da concentração de H_2 dentro da célula (Kozloski, 2002). De acordo com o balanço estequiométrico da fermentação ruminal, os maiores produtores de H_2

e CH_4 são os microrganismos que produzem acetato e butirato (Hegarty e Gerdes, 1998). Embora o H_2 seja um dos principais subprodutos da fermentação das bactérias, protozoários e fungos, o excesso de H_2 não se acumula pela ação de microorganismos do grupo *Archeae*, principalmente metanogênicos (Boadi et al, 2004). Nesse grupo se encontraram os principais microorganismos metanogênicos responsáveis pela produção de CH_4, os quais se dividem em 4 gêneros: *Methanobrevibacter, Methanobacterium, Methanomicrobium e Methanosarcina* (McAllister et al., 1996). A produção de CH_4 é modulada principalmente pela presença de CO_2 e H_2 livres no ambiente ruminal.

Apesar do seu papel benéfico na manutenção de baixas pressões parciais de H_2 dentro do ecossistema ruminal, a produção de CH_4 é reconhecida como um processo de desperdício energético para o ruminante, na medida em que permite a conversão de substratos úteis em compostos não utilizáveis pelo hospedeiro, e que correspondem a uma perda de energia da dieta 4 a 12 % da energia bruta ingerida (Johnson e Johnson, 1995). Esse processo metabólico produz calor, o qual é dissipado como calor metabólico pela superfície corporal, em aumento CO_2 e CH_4, são eliminados durante a eructação (Lana et al., 1998).

A pesar da reação de formação de CH_4 ser considerado um processo consumidor de energia, no ambiente ruminal este processo drena o H_2 procedente de todas as reações químicas que ocorrem no rúmen, permitindo um maior rendimento total de adenosina-trifosfato (ATP) para manutenção da eficiência desse ecossistema. Isso indica que a produção de CH_4 é um fator primordial para otimização da eficiência de síntese de proteína microbiana e posterior disponibilização para o metabolismo do animal. O máximo rendimento energético na fermentação anaeróbia de carboidratos envolve a liberação de grande quantidade de H_2 que é utilizado para que ocorra a regeneração de NAD^+ sem interferir na disponibilidade de piruvato nem do acetil-CoA.

Nos processos fermentativos com alta produção de ácido propiônico ocorre um dreno de H_2 do meio, resultando numa relação inversa e altamente significativa entre a concentração de propiônato e CH_4 no líquido ruminal (Whitelaw et al., 1984). Outras reações consumidoras de H_2 incluem a conversão de NO_3 em NH_3, de SO_4 em H_2S e a saturação de ácidos graxos insaturados, contudo estas reações não apresentam grande importância quantitativa (Fahey e Berger, 1993). No rúmen, as *Archaea* são encontradas intimamente associadas com protozoários ciliados e em justaposição com bactérias, não sendo essa, no entanto, uma localização obrigatória (Finlay et al., 1994).

Os microorganismos metanogênicos podem ser encontrados tanto aderido na superfície celular dos protozoários, como na fase intracelular dos mesmos (Ushida e Jouany, 1996). Considerando que os protozoários ciliados têm um grande potencial de produção de H_2 no rúmen, essa associação das bacterias metanogênicas com protozoários indica uma relação simbiótica, em que as metanogênicas, por utilizarem o H_2 produzido pelos ciliados, favorecem a manutenção de um ambiente ruminal adequado ao desenvolvimento destes microrganismos (Van Soest, 1994).

A presença de outros aceptores de elétrons, além do CO_2, tem efeito sobre a presença e atividade dos produtores e utilizadores de H_2 (Morgavi et al., 2010). Entre os produtores de H_2, os protozoários têm um papel importante, que é reforçado pela sua estreita associação física com a metanogênese, o que favorece a transformação de H_2. Se a transferência entre produtores de H_2 e interespécies de H_2 é afetada, ocurre acumulação de H_2 no meio ruminal, que se não for removido, inibe os sistemas enzimáticos que envolvem o nicotinamida-adenina-dinucleotídio hidrogênio desidrogenase (NADH), enzima importante na fermentação dos carboidratos envolvidos em reações redox e inibe também a continuidade do metabolismo intracelular, resultando em a morte bacteriana.

2.3. Estratégias nutricionais para mitigação da emissão de CH_4 entérico

Pesquisa nas áreas de nutrição e microbiologia dos ruminantes vem sendo desenvolvidas para minimizar as perdas energéticas na forma de CH_4, direcionando esta energia para síntese de produtos como leite, carne e lã, e consequentemente aumentando a eficiência alimentar (Nagajara, 2003). Este aspecto, associado à redução da metanogênese, está relacionado à melhoria da qualidade de vida no planeta.

A utilização de suplementos, a incorporação na dieta de ingredientes tais como: nitratos, ionóforos, lipídios, compostos que favorecem o crescimento de fungos ruminais, leveduras, substâncias defaunantes (eliminação de protozoários) e/ou substâncias com propriedades anti-metanogênicas (saponinas e taninos condensados), são alternativas que podem diminuir as emissões por animal entre 20 e 40 %.

Qualquer estratégia nutricional que tenha como meta a mitigação de CH_4 entérico não pode prejudicar o desempenho animal e deve ter como foco um ou mais dos objetivos listados a continuação:

1. Inibir as reações que liberam H_2 no ambiente ruminal.
2. Capturar o H_2 por outras vias metabólicas que possam receber o H_2 durante a reoxidação de equivalentes redutores, resultando em produtos que possam ser utilizados pelo animal hospedeiro.
3. Eliminar uma parte das bactérias metanogênicas, pela diminuição da quantidade de H_2.

A manipulação da produção e utilização de H_2 é considerado a chave para controlar a emissão de CH_4 no rúmen (Joblin, 1999).

2.3.1. Redução da produção de H_2

2.3.1.1. Defaunação

A remoção de protozoários do rúmen ou defaunação, através de manipulação das dietas ou do uso de aditivos, pode estar associada com a diminuição da produção de CH_4 (Ushida et al., 1997; McAllister e Newbold, 2008). A relação simbiótica existente entre os protozoários e as bactérias metanogênicas, oferece hábitat para colonização de aproximadamente 20% das metanogênicas (Finlay et al., 1994). A defaunação ou a eliminação dos protozoários do rúmen, em dietas com alta energia e ricas em nitrogênio não

protéico resulta numa melhoria do desempenho do animal e uma redução da produção de CH_4 (Hegarty et al., 2008).

Por outro lado a presença de protozoários no rúmen tem um fator na manutenção de ambiente rúminal e processo fermentativo, através da ingestão de partículas alimentares e pelo armazenamento de amido, que pode trazer alguns benefícios sobe uniformizando a fermentação rúminal. Os protozoários podem servir uma fonte de nitrogênio após sua morte e degradação (Finlay et al., 1994).

2.3.1.2. Composição da dieta

A inclusão de concentrado na dieta gera alteração na fermentação ruminal, principalmente na diminuição da proporção de acetato: propionato. O processo de produção de propionato é provavelmente o maior aceptor de H_2, depois de CH_4. Conseqüentemente, a produção de CH_4 é menor quando os animais são alimentados com dietas ricas em concentrado (Beauchemin et al., 2008). Primavesi et al. (2004) relataram que a utilização de dietas com níveis de concentrado iguais ou menores do que 40 % do consumo de matéria seca, aumenta a emissão CH_4 por animal porém diminui a emissão por unidade de produção.

Quando ruminantes são alimentados com forragem, a fermentação ruminal produz mais CH_4 representando uma perda de 12 % de energia bruta na forma de CH_4. Quando estes animais recebem uma dieta alta em grãos produzem 2-3 % menos de CH_4 (Johnson e Johnson, 1995). Dietas à base de volumosos favorecem a formação de acetato, aumentando a produção de H_2 e conseqüentemente de CH_4 por unidade de matéria orgânica fermentada.

O tipo de alimento oferecido pode ter efeito importante sobre a produção de CH_4. Beauchemin e McGinn (2005) estudaram o efeito de grãos de milho ou cevada sobre a emissão de CH_4 em bovinos em confinamento. As emissões de CH_4 expressas por kg de matéria seca consumida (kg CH_4/CMS) e como percentagem do consumo de energia total (ET) foram menores ($P<0,05$) com a utilização do milho, do que com a cevada (9,2 vs 13,1 g / kg de CMS; 2,81 vs 4,03 % do ET). Os resultados indicam que o efeito da dieta sobre a produção de CH_4 entérico foi evidente em termos da proporção de grão ou concentrado na dieta. Entretanto, as emissões poderiam ser mais reduzidas usando uma dieta à base de milho.

2.3.2. Inibição das bactérias *Archeae* metanogênicas

2.3.2.1. Extratos de plantas (taninos, saponinas e óleos essenciais)

Os taninos são compostos polifenólicos que formam complexos principalmente com proteínas, e em menor grau com carboidratos estruturais e amido, reduzindo a digestibilidade e degradação destes (McSweeney, et al., 2001). Plantas que possuem compostos secundários como taninos e saponinas reduzem a emissão de CH_4 pelo efeito tóxico deste componente sobre os protozoários (Hart et al., 2008).

A inclusão de leguminosas com elevados teores de taninos (*Callinadra calothyrsus* e *Fleminga macrophylla*) provocou diminuição de 24 % na produção de CH_4 em carneiros, contudo esse efeito esteve associado à

redução na digestibilidade da matéria orgânica e da fibra (Tiemann et al., 2008). Em estudo *in vitro* utilizando a metodologia de produção de gás o aumento dos níves de taninos condensados (CT) na dieta (0, 10, 15, 20, 25 e 30 mg de extrato de *leucaena leucocephala*) gerou uma taxa decrescente na produção de CH_4 (mL/g MS) e número das bacterias metanogênicas (quadrática $P < 0,01$) com o aumento dos níveis de CT (Tan et al., 2011).

Os óleos essenciais são misturas complexas de metabólitos secundários (compostos fenólicos e terpenóides) associados com odor e cor de algumas plantas, que têm atividade antimicrobiana contra bactérias gram-negativas e gram-positivas (Wallace et al., 2002) e que podem ter atividade antimetanogênica pelo efeito inibitório sobre acúmulo de H_2 no rúmen. O efeito dos óleos essenciais sobre a fermentação ruminal está relacionado com a redução da degradação de proteínas, aminoácidos e amido (Hart et al., 2008). O líquido da casca da castanha de caju (cashew nut shell liquid, CNSL) é um co-produto da produção de castanha de caju em países tropicais. As propriedades antimicrobianas do extrato de CNSL estão relacionadas com os compostos fenólicos antibacterianos e o seu conteúdo de ácido anacárdico, cardanol e cardol, que são derivados do ácido salicílico com um C-15 alquilo grupo.

Watabane et al. (2010) avaliaram as propriedades potenciais antimicrobiana de CNSL, sobre a fermentação, produção de CH_4 e população bacteriana. Utilizaram o sistema de Rusitec durante um período de 7 dias. Foram testadas diferentes concentrações de CNSL em 0, 50, 100 ou 200 µg/mL, utilizando liquído rúminal dos bovinos alimentados com uma dieta contendo 30:70 feno: concentrado. Observaram inibição das bactérias gram-positivas e aumento da produção de propionato de uma forma dependente da dose com inibição máxima de CH_4 (70,1 %) e aumento de propionato (44,4 %) a dose de 200 µg/mL de suplementação, alem de aumento da concentração de AGCC totais e digestibilidade da matéria seca.

El-Zaiat et al. (2011) avaliaram a produção de CH_4, produção de gás, degradabilidade da matéria orgânica e parâmetros da fermentação, pela incorporação de doses crescentes (0, 15, 30 e 45 µL/75 mL) do óleo essencial de patchouli (*Pogostemon cablin*) em dietas com volumoso:concentrado (50:50), utilizando sistema semi-automático para produção de gás em incubação 24 h *in vitro*. Os resultados mostraram que os níveis moderados de incorporação do óleo essencial de patchouli não têm efeito significativo na produção de CH_4 ou pH, com tendencia para aumentar a degradabilidade verdadeira da matéria orgânica.

Os estudos sobre os efeitos de extratos de plantas sobre a fermentação ruminal, desempenho ou digestibilidade, mostram disaparidade grande entre número de estudos realizados *in vitro* contra os poucos realizados *in vivo*. O efeito de qualquer aditivo é depende os compostos intermediários e as bactérias ruminais. A diminuição da concentração das bactérias vivas em sistemas *in vitro*, os pesquisadores aumentaram os doses testadas *in vitro* para favorecer a interação entre os compostos intermediários e as bactérias ruminais. Assim, deve-se ter cautela na extrapolação dos resultados encontrados *in vitro* para situações *in vivo*.

Enquanto, os estudos realizados *in vitro* são um procedimento lógico para reduzir o custo da pesquisa durante as fases de testes exploratório de aditivos novos os quais podem ser posteriormente testados *in vivo*. As principais limitanções associadas com a utilização desse extratos de plantas, em termos da determinação das doses, são o custo alto da extração dos óleos essenciais ou compostos secundários, problemas de aceitabilidade, diminuição de consumo associativo e baixa digestibilidade dos nutrientes, faz com que os estudos *in vivo* é sejam importantes.

2.3.2.2. Vacinação

A vacinação estimula o sistema imunológico dos animais para a produção de anticorpos contra as bactérias produtoras de CH_4 (Wright et al., 2004). O CH_4 foi reduzido em 7,7 % utilizando duas vacinas desenvolvidas VF3 e VF7. Contudo a eficácia do uso de vacinas para controlar microrganismos metanogênicas pode variar pela dieta e localização geográfica do hospedeiro, dificultando uma abordagem única (Wright et al., 2007).

2.3.3. Utilização do H_2 em rotas alternativas benéficas

2.3.3.1. Inclusão de lipídeos

O uso de lipídeos não protegidos da digestão ruminal (ácidos graxos poli-insaturados) na dieta de ruminantes além de aumentar a densidade energética da dieta (Kumar et al., 2009), pode reduzir a produção de CH_4 entérico, fato atribuído à ação sobre microrganismos que direta ou indiretamente contribuem à formação de CH_4. Martin et al. (2008) sumarizaram dados de estudos *in vivo* avaliando os efeitos de diferentes fontes de lipídeos sobre a emissão de metano em bovinos e ovinos. O resultados mostraram uma redução média de 3,8 % na emissão de CH_4 (g/kg de MS ingerida) para cada 1% de gordura adicionada na dieta (% do CMS).

Grainger et al. (2010b), utilizando a técnica do gás traçador SF6, avaliaram os efeitos da suplementação de vacas leiteiras com caroço de algodão por 12 semanas sobre a metanogênese. Os autores observaram redução persistente na emissão de CH_4 (3,5 g de CH_4/kg de MS ingerida, em média) ao longo de 12 semanas com a adição de caroço de algodão (2,61 kg de MS/vaca/dia). Na primeira semana foi observada uma redução de 5,1 % na produção de CH_4 (g/kg de MS ingerida) e na 12 semana 14,5 %.

O uso de lipídeos em dietas de ruminantes tem efeitos negativos na fermentação da matéria orgânica ruminal por dois principais motivos: 1) efeito tóxico direto dos ácidos graxos aos microrganismos (Nagajara et al., 1997) e 2) efeito físico pelo recobrimento das partículas alimentares com gordura, e conseqüente a diminuição do contato destas partículas com enzimas, com diminuição da atividade das bactérias metanogênicas e no número de protozoários (Machmuller et al., 2003). Por outro lado, os lipídeos em estudos *in vivo* reduziu as emissões de CH_4, mas apresentaram efeitos indesejáveis, com a redução na digestibilidade de todos os nutrientes espicialmente da FDN e FDA e na relação acetato:propionato (Nagajara et al., 1997).

2.3.3.2. Leveduras

As leveduras são aditivos utilizados como probióticos (*Saccharomyces cerevisiae* e *Aspergillus oryzae*) pelos efeitos positivos que tem sobre a fermentação ruminal. Alguns destes efeitos estão relacionados com aumento da concentração de AGCC, menor concentração de amônia ruminal (Morais et al., 2006), aumento do crescimento e atividade bacteriana, aumentando da digestibilidade da matéria seca e da fibra (Ospina et al., 2009), aumento do consumo de matéria seca e melhor desempenho animal (Robinson, 2007).

A inclusão de leveduras em dietas de ruminantes pode estimular uma espécie de bactéria acetogênica "hidrogeniotrópica" que utiliza o H_2 para produção de acetato. Segundo Dehority (2003), as bactérias acetogênicas utilizam o H_2 ineficientemente, devido à baixa afinidade e capacidade "hidrogeniotrópica" de H_2 livre no rúmen (100 vezes menor do que observado para os microrganismos metanogênicos) nas condições normais do meio (Mackie e Bryant, 1994). Em culturas de bactérias acetogênicas e metanogênicas, sem adição de levedura, 19 % do H_2 foi utilizado para a síntese de acetato e 79 % para a produção de CH_4. Já na presença de levedura, 70 % do H_2 foi utilizado para a produção de acetato (Chaucheyras et al., 1995).

Rivera et al. (2010) estudaram os efeitos do uso de monensina, complexo de leveduras, ácidos graxos poliinsaturados e aminoácidos nos parâmetros de população de protozoários e na produção de CH_4. A relação acetato:propionato nos animais alimentados com a dieta com monensina foi menor que naqueles que receberam o complexo de leveduras e ácidos graxos poliinsaturados e aminoácidos, diminuindo a perda de energia na forma de CH_4. Foi evidenciado um aumentou significativo na população total de protozoários quando fornecido o complexo de leveduras, ácidos graxos poliinsaturados e aminoácidos em comparação à dieta com monênsina.

Possenti et al. (2008) avaliaram os efeitos do uso de leucena (*Leucaena leucocephala*) e levedura (*Saccharomyces cerevisiae*) em dietas para bovinos sobre a produção de CH_4, produção de AGCC e amônia. A produção de CH_4 foi mensurado com a técnica do traçador interno hexafluoreto de enxofre (SF6). Observaram redução da emissão de CH_4 em 12,3 % em relação à mesma dieta sem levedura e em 17,2 % quando os animais foram alimentados com 20 % de leucena com levedura. Verificou-se efeito associativo de leucena, quando fornecida em alto nível na dieta (50 % MS), e levedura na redução da emissão de CH_4 e na melhoria no padrão de fermentação no rúmen, o que pode reduzir as perdas de energia e melhorar eficiência energética do animal.

2.3.3.3. Ácidos orgânicos

Ácidos orgânicos (malato e fumarato) são precursores diretos de propionato, e reduzem da metanogênese (O´Mara, 2004). A incorporação dos ácidos orgânicos na dieta permite a utilização do H_2 livre no rúmen para formação de succinato, funcionando desta maneira, como um dreno alternativo para o H_2 e reduzindo a emissão de CH_4 (Lopez et al., 1999). A maioria dos estudos com suplementação de ácidos orgânicos utilizam o malato que

convertido em propionato via fumarato, aumenta a concentração ruminal de propionato e inibiu a produção de CH_4 (Martin et al, 1999). Espécies de bactérias tais como *Fibrobacter succinogenes, S. ruminantium* (*ruminantium*), *S. ruminantium* (*lactilytica*), *Veillonella parvula* e *Wolinella succinogenes* podem utilizar fumarato, competindo com as bacteriás metanogênicas pelo uso do H_2 (Castillo et al., 2004). Wallace et al. (2006) observaram uma redução de 75 % na produção de CH_4 com adição de 10 % de fumarato encapsulado na dieta de cordeiros, sem efeito negativo no crescimento dos animais. McCourt et al. (2008) não observaram efeito do fumarato encapsulado em vacas leiteiras.

O uso dos ácidos orgânicos na alimentação dos ruminantes deve atender à quantidade ideal a ser fornecida, já que doses muito grandes podem tornar o processo anti-econômico, devido à elevação do custo.

2.3.3.4. Ionóforos

Os ionóforos são antimicrobianos utilizados em sistemas da alimentação como aditivos visando maniplular o ambiente ruminal e modular a eficiência de produção de carne e leite (McGuffey et al., 2001). Apesar da restrição de seu uso nos países de União Européia, existem mais de 120 tipos comerciais de ionóforos disponíveis no mercado que utilizam como princípios ativos lasalocida, salinomicina, laidomicina e monensina, sendo esta última a mais estudada e utilizada. O efeito inibitório dos ionóforos sobre as bactérias (metanogênese) está associado com a capacidade dos ionóforos em ligar cátions, como sódio, e formar complexos lipofílicos para atravessar a membrana celular das bactérias e protozoários, alterando a permeabilidade da membrana celular, ocasionando um aumento da pressão osmótica e morte dos microrganismos (Morais et al., 2006).

Devido à existencia de parede celulare dupla, as bactérias gram-negativas são mais resistentes aos ionóforos (Morais et al ., 2006). Os efeitos anti-metanogênicos estão relacionados com a inibição da formação dos precursores do CH_4 - direto sobre a população de metanogênicas - ou com as bactérias que estão produzindo H_2. Estes dois mecanismos contribuem para alteração na fermentação de acetato para propionato, reduzindo a produção de CH_4 (Newbold et al., 1988). Também podem estar relacionados com a inibição do crescimento de protozoários ciliados (McAllister et al., 1996). Guan et al. (2006) verificaram uma redução de 30 % na emissão de CH_4 quando a dose de 33 ppm de monensina foi incluída em dietas com alto ou baixo teor de forragem.

Grainger et al. (2010a) avaliaram o uso de 471 mg/dia de monensina em vacas alimentadas com pasto de azevém suplementado com 4 kg/dia de grãos de cevada. Em ambas condições, a adição de monensina não aumentou a produção de leite e não promoveu efeito sobre a emissão de CH_4 entérico (g/dia, g/kg de leite e g/kg de MS ingerida). Provavelmente devido à adaptação de micróbios rúminais com a monensina, que não pode ser promovido como uma estratégia viável de mitigação da emissão de metano para vacas leiteiras em pastejo de azevém suplementadas com grão.

Na prática, o uso de ionóforos aumenta a produção de propionato, diminui a metanogênese, a proteólise e a desaminação das proteínas do alimento no rúmen e consequentemente, melhora o desempenho animal

(Tedeschi et al., 2003). Após fornecemento de ionóforos para os ruminantes em cerca de 8 semanas (com longo prazo) foi observado uma adaptação das bactérias ruminais. A resistência pode ocorrer de três maneiras: 1) síntese algunas enzimas pelos microrganismos ruminais que degradam os ionóforos e 2) mudança na permeabilidade da celula microbiana. Também a toxicidade dos ionóforos pode estar relacionada com o uso de doses excessivas ou inadequadas, fornecimento de doses erradas ou sem período de adaptação adequado (Russel e Strobel, 1989).

2.3.3.5. Inclusão de Nitrato

A inclusão de nitrato, em dietas de ruminantes tem efeito positivo na fermentação ruminal, e tem grande potencial para redução de CH_4, pois a molécula é um ótimo aceptor de elétrons (Van Zijderveld et al., 2010).

A redução de nitrato a amônia por organismos anaeróbicos é altamente competitiva com a produção de metano, pelo fato de consumir oito elétrons/mol de nitrato no processo, traduzindo-se em um potente sumidouro de H_2 no processo fermentativo (Leng e Preston, 2010).

2.4. Influência do nitrato na fermentação ruminal e produção de CH_4

No sistema biológico, o metabolismo energético é caracterizado por rações de oxiredução que libera energia pela transferência de elétrons. A fermentação ruminal e metabolismo microbiano seguem os mesmos fundamentos de transformações biológicas de diferentes formas da energia (química, térmica, elétrica,....etc.). Desse modo, a degradação da matéria orgânica é principalmente caracterizada por reações de oxiredução. O metabolismo de redução de nitrato a amônia no rúmen é predominante quando o conteúdo de matéria orgânica esta disponível (Leng, 2008).

A energia útil nesses sistemas deve ter capacidade de realizar por exemplo a síntese de macromoléculas ou transporte através da membrama. A direção das reações nessas sistemas é indicada pela variação de energia livre (função de Gibbs, ΔG). ΔG (kJ/mol) é uma função da atividade dos produtos e reagentes existem no sistema, quando as reações ocorrem na direção mais longe do seu equilíbrio, mais energia livre é liberada. Quando a reação atinge o equilíbrio, a ΔG é zero (nula). A direção desses reações pode ser positiva ou negativa (Kozloski, 2011).

A redução de nitrato a nitrito atinge ΔG = -130 kJ/mol de H_2 e subseqüente redução do nitrito a amônia atinge ΔG = -124 kJ/mol de H_2 liberando mais energia do que a redução do CO_2 a CH_4 a qual atinge ΔG = -16,9 kJ/mol de H_2 (Ungerfeld e Kohn, 2006). Parte dessa energia libertada pela fermentação dos substratos é utilizado para a formação de ATP microbiano, enquanto o resto dissipado na forma de produção de calor, que conduz o processo de fermentação mais para a frente (Kozloski, 2011).

O nitrato é considerado um potente inibidor da metanogênese em todos os sistemas anaeróbicos incluindo o rúmen, a partir da digestão fermentativa ruminal (Hungate, 1965; Akunna et al., 1994). A redução de nitrato a amônia, por exemplo, e composta por duas reações. Na face externa das bactérias que estão aderidas ao epitélio, as enzimas nitrato redutase e nitrito

redutase produzidas somente quando há presença de substrato (Cheng e Phillips, 1988). Primeiramente, o nitrato (NO_3^-) é reduzido a nitrito (NO_2^-) e posteriormente, o nitrito é reduzido a amônia (NH_3). Essa rota pode se tornar a principal rota de eliminação do H_2 se houver nitrato suficiente no ecossistema ruminal. (Kozloski, 2011).

$$\mathbf{NO_3^- + H_2 \rightarrow H_2O + NO_2^-}$$

$$\mathbf{NO_2^- + 3H_2 + 2H^+ \rightarrow 2H_2O + NH_4}$$

A amônia produzida estaria disponível ao anabolismo de aminoácidos e seria importante fonte de nitrogênio fermentável em dietas deficientes em proteína bruta, podendo assim, substituir parte do N da dieta (Van Zijderveld et al., 2010). A redução de nitratos em meio anaeróbico pode competir com o uso de H_2 para a propiogênese e metanogênese, pois é uma reação energeticamente mais favorável do que a redução de CO_2 e redireciona H_2 para redução de nitratos (Van Zijderveld et al., 2011).

A supressão da metanogênese por estes nitrocompostos pode não aumentar significativamente o acúmulo de H_2 ou alterar as proporções molares dos AGCC produzidos pelos microrganismos ruminais. Isto sugere que equivalentes redutores poupados da produção de CH_4 não foram redirecionados para produzir mais AGCC. A combinação de nitrato com sulfato (Van Zijderveld et al., 2011), o uso de bactérias nitrificantes, tais como *Escherichia coli* (Sar et al., 2005a,b) e o uso de nitrato em baixas concentrações (menores do que 12 µM) podem ser alternativas para mitigar as emissões de CH_4 em ruminantes.

O nitrato pode reduzir a produção do CH_4 persistentemente, sendo que a inibição da celulólise no longo prazo, pode ser contornada pela produção de amônia ruminal (Sar et al., 2005a, b) e síntese de proteína microbiana (Guo, 2009). Sar et al. (2005a,b) mostraram que o uso de nitrato reduziu a produção de CH_4 por culturas ruminais, também diminuiu AGCC em culturas *in vitro*, sugerindo um impacto potencialmente negativo sobre a nutrição ruminal.

Marais et al. (1988) relataram que, tanto o nitrato como nitrito, reduziram as atividades celulolíticas e xilanolítica *in vitro*, a contagem total de bacterias da microbiota ruminal, bem como o crescimento de culturas puras de *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens*, e *Succinogenes fibrobacter*. A metanogênese é inibida por uma baixa concentração de nitrito. O aumento de nitrato e nitrito reduz as bactérias metanogênicas, podendo reduzir a metanogênese ruminal, sem acúmulo de H_2 (Iwamoto, 2001b). A adição de 2 % de nitrato na dieta de vacas leiteiras aumentou a proporção de acetato, a custa de propionato e butirato (Farra e Satter, 1971).

Todavia, Nolan et al. (2010) não constataram esse fato, apesar de haver uma tendência de redução do propionato e butirato, e uma tendência de maior produção de acetato. Outra possível alteração devido à inclusão de nitrato na dieta de ruminantes é a redução da digestibilidade da fibra, e conseqüentemente da matéria seca, pois o nitrato tem efeito tóxico sobre os microorganismos celulolíticos (Leng e Preston, 2010).

Van Zijderveld et al. (2010) usaram 20 cordeiros para avaliar o efeito da adição de nitrato na dieta (2,6 % de matéria seca) e sulfato (2,6 % da matéria seca) sobre a emissão de CH_4 entérico e as concentrações de AGCC. A alimentação com nitrato ou sulfato não foi apresentaram efeitos sobre as concentrações de AGCC no fluido do rúmen após 24 h da alimentação. Os autores observaram que a redução das emissões de CH_4, devido à utilização de nitrato marcada imediatamente após o período de alimentação. Enquanto que a redução na metanogênese, devido ao sulfato, foi observado ao longo do dia. Os autores concluiram que, quando fornecidos com segurança, sais de nitrato e sulfato são agentes potentes para redução de CH_4 entérico.

O efeito tóxico aos microorganismos celulolíticos não é tão grande a ponto de afetar da digestibilidade da matéria seca. Glenn e Ely (1981) não observaram diferença na digestibilidade da MS e da fibra em detergente ácido (FDA) de festuca (*Festuca arundinacea*) em dietas de carneiros alimentados com inclusão de 0,8 % de nitrato de potássio. Esse resultado corrobora com as observações de Nolan et al. (2010), os quais não observaram alteração na digestibilidade total e na taxa de degradação da MS, em dietas contendo 4 % de nitrato de potássio. Van Zijderveld et al. (2011) também não constataram redução na digestibilidade da fibra em detergente neutro (FDN), do amido e nem do extrato etéreo em dietas de vacas em lactação, quando se incluiu 21 g de nitrato/kg MS de nitrato de cálcio.

2.5. Nitrato e formação de metahemoglobina (MetHb) no sangue

A utilização prática do nitrato como um aceptor de elétrons não é uma pratica comum devido à toxicidade associada com o seu intermediário reduzido, o nitrito (McAllister et al., 1996). Em animais não adaptados, a capacidade dos microrganismos ruminais de reduzirem nitrato a nitrito é maior que a capacidade de reduzir nitrito a amônia, ocorrendo um acumulo desse intermediário (nitrito) no rúmen. O nitrito acumulado no rúmen é absorvido, pelo pareder ruminal, levado à corrente sanguínea onde oxida a hemoglobina da forma ferrosa (Fe_2^+) para a férrica (Fe_3^+). A MetHb que é incapaz de transportar O_2 para os tecidos. A condição resultante é um estado geral de anoxia, que pode reduzir o desempenho animal e nos casos mais severos, ser fatal (Ozmen et al., 2005). Níveis de 30 a 40 % de MetHb provocam sinais clínicos e níveis de 80 a 90 % causam a morte.

A susceptibilidade das diferentes espécies animais depende da capacidade de transformar nitratos em nitritos. A espécie mais sensível é a suína, seguida da bovina, ovina e eqüina (Cheeke 1998, Radostits et al., 2000). Os bovinos e ovinos são duas espécies de ruminantes particularmente afetados pela toxicidade do nitrato. Ambas as espécies apresentam sintomas semelhantes em resposta a altos níveis de nitrato na forrageira. No entanto, os ovinos, têm uma maior tolerância ao nitrato, devido à capacidade de aumentar a concentração de glóbulos vermelhos no sangue.

Van Zijderveld et al. (2010) afirmaram que o valor mais elevado de MetHb no sangue dos cordeiros alimentados com nitrato foi de 7 %, quando o nitrato foi introduzido gradualmente por 28 dias na dieta de ovinos. Nolan et al. (2010) observaram um aumento significativo ($P<0,001$) na concentração de MetHb no sangue em ovinos alimentados com nitrato (0,36 % de MetHb no dia

14) em relação aos alimentados com uréia (0,76 % no dia 28), sem apresentados quadros de intoxicação de 4 semanas.

A formação de MetHb é um dos principais entraves da utilização do nitrato em dietas de ruminantes. Dentre as estratégias que podem ser utilizadas para evitar a formação de MetHb estão: a otimização do ambiente ruminal para síntese de proteína microbiana, a utilização de nitrato encapsulado e/ou a adaptação gradual dos animais ao produto.

2.6. Período de adaptação dos animais a dieta de nitratos

Os ruminantes alimentados com concentrações elevadas de nitrato na dieta, podem se adaptar rapidamente, pois as taxas de redução de nitrato a nitrito aumentam em cerca de 2,5 vezes maior que redução do nitrito, pelo aumento do número de bactérias redutoras de nitrato. Estes microrganismos são rapidamente produzir as enzimas nitrato redutase e nitrito redutase como resposta à presença de nitrato na dieta. Alaboudi e Jones (1985) relataram que, com ovelhas adaptadas gradualmente com nitrato na dieta, aumentaram as taxas de redução, tanto de nitrato como de nitrito até 3-10 vezes. O aumento de redução de nitrato no rúmen, está associado com a multiplicação rápida das bactérias aumentando a atividade de nitrato/nitrito redutases, tornando-os grupos predominantes nos animais adaptados (Allison e Reddy, 1984).

A introdução de nitrato pela primeira vez no rúmen, aumentou a taxa de redução de nitrato em líquido rúminal, em apenas 4 horas, até 15 vezes mais do que antes a introdução de nitrato. Assim, acumulo de tanto nitratos e nitritos no rúmen depende do tempo que o animal tenha sido adaptado ao nitrato na dieta (Allison e Reddy, 1984; Alaboudi e Jones, 1985).

Uma outra maneira para minimizar a sua velocidade de intoxicação de nitrito pode ser feita pela modulação de sua aparecimento e progressivamente reduzir nos primeiros sinais clinícos. Mais recentemente, as propriedades de liberação lenta têm sido conseguido através da utilização de revestimentos com óleos ou polímeros. Um método alternativo é a utilização de compostos de nitratos encapsulados. Por ser uma tecnologia nova para diminuir a liberação de nitrito e suas concentrações no ambiente ruminal do que nitrato convencional.

CAPÍTULO 2[1]

[1]According the form and style required by Journal of Animal Science for publication.

Enteric methane emission, performance and rumen fermentation in lambs fed encapsulated calcium nitrate

ABSTRACT

Two encapsulated nitrate products were used in this study to evaluate the effects on *in vivo* methane emission, rumen fermentation parameters and growth in growing lambs. Eighteen Santa Inês male lambs (mean BW 27 ± 5.55 kg, 4 to 5 mo old) were assigned to a randomized complete block design experiment with 6 blocks and 3 diets. Animals were assigned to 1 of 3 experimental diets and fed a basal diet containing a roughage:concentrate ratio of 40:60 for *ad libitum* intake. Treatments were: 1) basal diet plus 1.5% as urea (DM basis; control), 2) basal diet plus 4.51% encapsulated nitrate (DM basis; Nitrate) or 3) basal diet plus 4.51% encapsulated nitrate containing cashew nut shell liquid (DM basis; Nitrate + CNSL). All diets were iso-nitrogenous (average CP = 13.47%). Lambs were fed experimental diets for 92 d (28 d for adaptation, and two experimental periods composed of 32 d of data collections). Within each period, the last 6 days were used for methane collection. Every 14 d lambs BW were recorded (after a 16 h fast). Blood and ruminal fluid samples (day 14, 28, 42 and 56) were collected 6 and 3 h after morning feeding, respectively. Data were analyzed using the Mixed procedure of SAS. DMI, ADG and gain: feed (G:F) were not affected by encapsulated nitrate. Enteric methane production was inhibited by 32.3% with encapsulated nitrate supplementation ($P < 0.05$). Encapsulated nitrate increased ($P < 0.01$) total ruminal SCFA, acetate, propionate and butyrate concentrations. Nitrate + CNSL diet decreased methane inhibition (by 32.3 %), increased propionate (by 20.98 %) and butyrate (by 39.59 %) concentrations. Ruminal pH, acetate:propionate ratio

were unaffected ($P > 0.05$) by encapsulated nitrate addition. Ruminal ammonia concentrations declined in lambs fed Nitrate (25%) and Nitrate + CNSL (36%) compared with control ($P < 0.05$), whereas ruminal and blood nitrite concentrations were increased by encapsulated nitrate inclusion. The blood methemoglobin concentration (MetHb %) was not influenced by treatment, but tended to increase ($P = 0.08$) in lambs supplemented with Nitrate compared to controls (1% relative to 0.62). Encapsulated nitrate enhanced red blood cell number ($P < 0.01$), indicating a compensatory mechanism for decreased blood oxygen in lambs fed dietary nitrate. All encapsulated nitrate treatments decreased ($P < 0.01$) total rumen protozoa numbers (11.84%) compared with control. Our data indicate that encapsulated nitrate modified the rumen fermentation pattern which resulted in decreased methane production without negatively impacting growth performance in growing lambs.

Key words: cashew nut shell liquid, methane, methemoglobin, sheep

INTRODUCTION

Ruminant enteric methane (CH_4) production is predicted to increase by 30% by 2020 (O'Mara, 2010) and is recognized as an energy loss from animals. Fermentation of ingested feed in the rumen results in H_2 formation which is used by methanogenic *Archaea* during methanogenesis as a substrate to produce methane (McAllister and Newbold, 2008). A strategy to reduce methane production is to increase the activity of an alternative pathway for H_2 utilization (López et al., 1999). Recently, nitrocompounds such as nitrate salts have been considered as a potential feed source to replace urea in ruminant diets, and may reduce ruminal CH_4 formation because it may be an alternative H_2 sink to CO_2 via the reduction routes to ammonia (Leng, 2008).

In fact, several studies concluded that there is a potential effect for using nitrate as a potent inhibitor of rumen methanogenesis (Van Zijderveld et al., 2011; Hulshof et al., 2012; Li et al., 2012). In addition, cashew nut shell liquid (CNSL) is a co-product of cashew nut production in tropical countries such as Brazil. The CNSL has antimicrobial activity through their phenolic compounds that inhibit specific species (gram-positive bacteria) of rumen bacteria (Kubo et al., 1993). Therefore, CNSL has been considered to be a propionate enhancer (Watanabe et al., 2010), which contributed to decreased ruminal CH_4 formation (Shinkai et al., 2012). As a consequence of nitrite poisoning risk, the practical use of nitrate in ruminant diets is diminished (Zhou et al., 2012). We hypothesized that this adverse effect can be reduced by feeding encapsulated nitrate or encapsulated nitrate plus CNSL, which could decrease CH_4 production without negative impact on animal health or lamb performance. Accordingly, our objectives were to investigate the effects of inclusion of encapsulated nitrate and

encapsulated nitrate plus CNSL on enteric CH_4 emission, rumen fermentation and growth performance of growing lambs.

MATERIALS AND METHODS

This experiment was carried out at Laboratory of Animal Nutrition, Center for Nuclear Energy in Agriculture (LANA-CENA/USP), University of São Paulo (USP), located in Piracicaba, state of São Paulo, Brazil. All experimental protocol and animal use procedures followed guidelines recommended by the Internal Commission for Environmental Ethics and Experimentation with Animals of the CENA/USP.

Animals and Housing

Eighteen Santa Inês ram lambs (mean BW 27 ± 5.55 kg, 4 to 5 mo old at the beginning of the study) were blocked according to initial BW and randomly allotted to 18 individual pens (150×110×120 cm). At the onset of the experiment lambs were dewormed with 2 mL of Ripercoll as anthelmintic (Fort Dodge Saúde Animal® LTDA, Campinas, Brazil) and 3 mL of Valbendazole (PHIBRO Saúde Animal®, internacional® LTDA, Guarulhos, São Paulo, Brazil) according to fecal egg counts per gram (FEC) analyses and average BW and injected with 2.5-5 mL of a vitamin A D3, and E (Fabricante®, Bayer S.A, São Paulo, Brazil).

Experimental Design and Diets

The experiment lasted for 92 d consisting of 28 d of adaptation (to feeding diets and treatments) and two experimental periods of consisted of 32 d for sampling and data collection. Lambs were assigned to treatment in a randomized complete block design with 6 blocks and 3 diets. Each block (3 pens with 1 lamb each) was defined by BW and age at the beginning of the experiment.

Treatments were: 1) basal diet plus 1.5% as urea (15 g/kg DM basis; control), 2) basal diet plus 4.51% encapsulated nitrate (45.1 g/kg DM basis; Nitrate) or 3) basal diet plus 4.51% encapsulated nitrate containing cashew nut shell liquid (45.1 g/kg DM basis; Nitrate + CNSL). The basal diet used was isonitrogenous and consisted of 40% Tifton-85 (*Cynodon sp*) hay (chopped approximately 5 mm length) and 60% concentrate which was based on ground corn, soybean meal and a mineral mixture (DM basis). Diets were designed to meet NRC (2006) recommendations for growing lambs.

The feed ingredients and chemical composition of the experimental diets are presented in Table 1. The experimental encapsulated nitrate products were manufactured by GRASP® Ind. e Com. LTDA (Curitiba, Paraná, Brazil). Both products are protected by an international patent application and were in a granule form as a double salt of ammonium and calcium nitrate decahydrate with magnesium sulfate monohydrate coated with a slow release matrix. The encapsulated Nitrate contained (DM basis): DM 86.17%; Total-N 14.98 %; Total-NO_3 61.15%; Total SO_4 7.3%; Ca 17.84%; the encapsulated Nitrate plus cashew nut shell liquid (CNSL) contained (DM basis): DM 86.17%; Total-N 14.98%; Total-NO_3 61.15%; Total SO_4 7.3%; Ca 17.84%; CNSL 2.96 %.

Feeding Management and Data Collection

Total mixed diets were offered twice a day in two equal portions at 0900 and 1600 h. Feed and fresh water were offered of *ad libitum* access. An adaptation period (28-d) served to introduce the treatment concentrates gradually into the diets and the experiment conditions. Every 7 d an incremental portion of 33% of the total amount of concentrate treatment was replaced by 1 of the 3 experimental concentrates (control,

Nitrate and Nitrate + CNSL) for each treatment until the lambs were fed the total amount of experimental concentrate after 21 d.

The lambs continued to be fed their respective concentrates for 7 d more to give the rumen microbes a sufficient time to increase activity of nitrate and nitrite reductases. To ascertain the *ad libitum* intake, feed intake was measured during the adaptation period for all lambs. The amount of feed offered was adjusted daily on the basis of the feed intake of the previous day, allowing refusal amount of approximately 10% of daily feed intake. Body weights were recorded every 2-wk (after a 16 h fast) before the morning feeding to determine the average daily gain (ADG) and gain:feed (G:F). Feeds were sampled weekly for each animal and frozen at -20°C for later analyses.

Laboratory Analyses

Feed samples were thawed, dried in a forced-air oven at 55°C for 48 h and ground to pass a 1-mm screen. In ground samples, DM (oven drying at 105°C for 24 h) OM (determined by difference after heating at 550°C for 4 h), CP (6.25×N) and EE (using the ANKOM extraction system, model XT10I, New York, USA) were determined according to AOAC (2006) methods. Sequentially in the same sample, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined in filter bags with a heat stable α-amylase and expressed exclusive of residual ash as described by Van Soest (1991) and adapted by Mertens (2002).

Rumen Fermentation Constituents Determination

Rumen fluid samples (30 ml) were taken 3 h after the morning feeding every 2-wk (day 14, 28, 42 and 56) by using an oesophageal tube.

Rumen pH was measured immediately using a portable digitaled pH meter (Digimed model DM21, Tecnal, São Paulo, Brazil). The rumen sample was divided into separate subsample for ammonia (NH_3-N) and short-chain fatty acids (SCFA) analyses, which were stored at -20°C for future analyses.

The ruminal NH_3-N concentration was analyzed using micro-kjeldahl by steam distillation with sodium tetraborate solution (5%), collected in boric acid solution (20%) and determined by titration with solution of H_2SO_4 (0.05N) as described by Preston (1995). Ruminal fluid samples were centrifuged at 15,000 *x g* for 20 min at 4°C.

The SCFA concentrations were determined according to manufacture's conditions with some modifications according to Palmiquist and Conrad (1971) using a gas chromatograph (GC HP 7890A, Automatic Injector HP 7683B, Agilent Technologies, Palo Alto, CA, USA) equipped with a capillary column HP-FFAP (19091F-112; 0.320 mm OD, 0.50 µm ID, 25 m length, J&W Agilent Technologies Inc., Palo Alto, CA, EUA). A 1 µL aliquot were injected using a 20:1 split ratio with 31.35 mL/min of H_2 flux (9.20 psi). Injector and FID temperatures were kept at 260°C . Oven heating slope was: 80°C (1 min), 120°C (20°C /min; 3 min), 205°C (10°C /min; 2 min), with 16.5 min of total analytical time. Hydrogen at 1.35 mL/min was used as carrier gas. Detector hydrogen, synthetic air, and nitrogen fluxes (make up) were kept at 40, 400, and 40 mL/min, respectively.

Another 2 mL rumen fluid subsample was mixed with 4 mL of methyl green-formalin-saline (MFS) solution and stored in glass bottle at room temperature for later total protozoa counts. Total protozoa numbers were counted using Neubauer Improved Bright-Line counting chamber according to the procedure described by Dehority et al. (1983).

Methaemoglobin (MetHb), Hemoglobin (Hb) and Red blood cells (RBCs) Determination

Blood samples (5 ml) were taken 6 h after the morning feeding every 14 d (day 14, 28, 42 and 56) from the jugular vein using vacutainer tubes containing K2E (K2 EDTA, BD, Franklin Lakes, NJ, USA). Blood Hb and RBCs counted by light microscopy using a hemocytomer chamber were assayed within 30 min of collection time using following the conventional method described by Helper (1966).

The MetHb concentration was assayed within 30 min of blood collection, via modified spectrophotometric procedure based on a phenomenon that the absorbance maximum of weakly acidic MetHb at 630 nm disappears by addition of cyanide. MetHb concentration determined as the ratios of the absorbance changes induced by cyanide before and after addition of potassium ferricyanide following to the methods described by Sato (2005).

Nitrate and Nitrite Determination

Ruminal fluid and blood samples were centrifuged at 2,000 x g for 15 min at 4°C to obtain plasma, which was immediately frozen at -20°C until required for analyses.

Nitrate concentration was determined according to the methods of "Arrow Straight Nitric Oxide Measurement System" (Lazar Research Laboratories, INC. Los Angeles, USA), using micro ion selective electrodes (Model No. ISM-146NOXM) in conjunction with an ion analyzer coupled with computer program to conveniently measure, tabulate and records the values.

Nitrite concentration was determined colorimetrically using spectrophotometer at 525 nm, using test of Merck Merckoquant® nitrite (Merck KGaA, Darmstadt, Germany) kit.

Methane Production Determination

Total methane production (L/d at standard temperature and pressure) was measured for 2 d in each measurement period for each lamb as developed by Abdalla *et al*. (2012), during the last six days of the adaptation period, when the animals were moved to the respiration chambers. Moreover, the 62 d for experimental period, methane was measured two times for each treatment within 25-d intervals between measurements. Six simple individual (length × width × height: 157×71×167 cm with 1.9 m^3 in volume) respiration chambers were available for quantifying the methane emission and two animals per treatment according to blocks were allocated to the chambers in a staggered manner. The lambs were transferred back into the individual pens after finished the gas exchange assay.

The daily experimental diets were offered at 0900 and 1600 h for each animal in the chamber. Temperature (23 ± 2°C) and humidity (87 ± 4%) were measured at regular intervals. Methane concentration was quantified by using a gas chromatograph (Shimadzu GC-2014, SINC Brazil, São Paulo, Brazil). A pure methane dose was injected into each chamber to determine the recovery rate and the measured methane production data was adjusted for 100% recovery.

Statistical Analyses

Body weight, feed intake, average daily gain, feed efficiency, methane production, as well as ruminal and blood constituents data were analyzed as a completely randomized block design with 3 treatments and 6 blocks with repeated

measures over time by using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC), The block and sheep were included as random effects. The best fitted covariance structure was autoregressive 1 (AR1). The LSMEANS option (SAS Inst. Inc., Cary, NC) was used to generate individual treatment means. Effects of treatment, time, and the interaction of treatment × time were defined by the F-test of ANOVA. Comparison´s among treatments were performed by Tukey's test. Effects were declared significant at $P < 0.05$. The model used was: $Y = \mu + B_i + T_j + BT_{ij} + D_k + DT_{jk} + E_{ijk}$, where μ is the overall mean, Bi is the block effect (i = 1-6), Tj is the treatment effect (j = 1-3), BT_{ij} is the residual error associated with the sheep effect (block × treatment); D_k is the effect of date of measure (k = 1-4); DT_{jk} is the interaction of treatment × time, and E_{ijk} is the residual error.

The interaction between treatment × periods was analyzed using linear regression model, where the dependent variables were DMI and Blood Hb, and the independent variable was the measured time. The comparisons between regression equations were analyzed using dummy variables test according the model proposed by Kleinbaum et al. (1998).

RESULTS AND DISCUSSION

Performance and Methane Production

In this study, we demonstrated that is the first research using encapsulated nitrate in sheep diet. The chemical composition of the control fed diet was similar to encapsulated nitrate fed diets (Table 2). No differences ($P > 0.05$) in DMI (expressed as g/d, g/kg of BW and g/kg of $BW^{0.75}$) were observed between treatments. Neither control nor encapsulated nitrate diets affected ADG and final BW (Table 2). The DMI

(expressed as g/d, g/kg of BW and g/kg of $BW^{0.75}$) were affected by treatment × time interaction (P = 0.04, Table 2). On control diet DMI increased by 6.38 g per kg metabolic body weight.

The interaction between treatment × periods was significantly ($P < 0.05$) for DMI. However, comparisons between regression equations using dummy variables test, showing that the intercepts and regressors of three treatment equations (Control, Nitrate and Nitrate + CNSL) were not statistical different ($P > 0.05$) ($Y = 930 + 2.65X$, $R^2 = 0.09$; $Y = 815.5 + 5.9156X$, $R^2 = 0.37$ and $Y = 872.17 + 6.3822X$, $R^2 = 0.50$), respectively, Figure 1).

Feed efficiency was not different (P = 0.84) among treatments for the overall feeding period (Table 2). In terms of DMI and ADG, our results are in agreement with the results reported by Nolan et al. (2010), Van Zijderveld et al. (2010) and Li et al. (2012) when with nitrate inclusion in the diet relative to urea. In the latter study Hulshof et al. (2012) reported that DMI tended to be lower in beef cattle fed sugarcane based diet with 22 g nitrate/kg DM compared with a urea control diet. Changes in DMI could be due to some negative effects of nitrite on ruminal fiber digestibility (Marais et al., 1988), even though in the third chapter using the same experimental diets, NDF digestibility was unaffected by encapsulated nitrate inclusion. These results could suggest that feeding the encapsulated nitrate could be less effective than conventional nitrate on DMI and ADG.

Methane production for the control diet and encapsulated nitrate diets expressed as L/d, L/kg DMI or L/kg $BW^{0.75}$ are presented in Table 2. Lambs fed encapsulated nitrate diets had reduced (P = 0.02) CH_4 production (L/kg DMI). There was no difference ($P > 0.05$) observed in CH_4 production between the two encapsulated

nitrate types. Additionally, the encapsulated nitrate diets decreased (P = 0.05) CH_4 expressed as metabolic body weight ($BW^{0.75}$). Methane production was decreased by 32.3% when 1.5% urea was replaced by 4.51 % encapsulated nitrate in the diet. No differences were observed when CNSL was added to encapsulate nitrate (Nitrate + CNSL) compared with encapsulated Nitrate (P > 0.05). These results are in contrast to findings by Watanabe et al. (2010) and Shinkai et al., (2012) for CNSL as a methane-inhibiting agent. Results from our study provided further evidence that the encapsulated nitrate inhibited CH_4 production in lambs. The nitrate addition in the diets can effectively in reduce CH_4 formation by ruminal microbes (Bozic et al., 2009; Zhou et al., 2011).

Our results are agreement with recently published studies with sheep, which reported that inhibition of CH_4 production was 23% (after 18 d adaptation) when nitrate replaced urea by 4 % in the diet (Nolan et al., 2010). Similarly, Van Zijderveld et al. (2010) found that CH_4 emission was decreased by 32 % in sheep fed nitrate with 4 wk adaptation period. Apparently, several mechanisms might contribute to the inhibition of CH_4 production from lambs fed nitrate. First, nitrate reduction to nitrite is a means of diverting H_2 away from CH_4 formation and can serve as alternative electron acceptors which captured the H_2 produced during fermentation (Ungerfeld and Kohn 2006). Second, inhibitory effects of nitrate on H_2-producing bacteria (*R. albus and R. flavefaciens*), could reduce rumen H_2 availability, leading to decreased methanogenesis in the rumen (Zhou et al., 2012). The direct toxicity of nitrite to methanogens could result in decreased CH_4 production (Zhou et al., 2012).

The stoichiometry of the complete reduction of one mole nitrate reduced CH_4 production by one mole (25.8 g of CH_4 reduction/100 g of nitrate fed) has been

described by Van Zijderveld et al. (2010). In our experiment lambs consumed diets with encapsulated Nitrate and encapsulated Nitrate + CNSL on average 28.23 and 28.45 g per day, this would theoretically reduce CH_4 by 6.59 and 4.97 g CH_4/d. Thus, the CH_4 effective reduction was 90.48% and 67.71% of the expected inhibition, respectively. Our data showed that the theoretical efficiency of our encapsulated nitrate was higher than reported by Van Zijderveld et al. (2011) and was closely related to 78% observed by Nolan et al. (2010) and 89% and 97.5% of Van Zijderveld et al. (2010) and Li et al. (2012) when nitrate included in the diet, respectively.

In this sense, we can probably assume an explanation for the lower use of H_2 for CH_4 formation, redirecting of H_2 from methanogenesis toward nitrate reduction, rumen ammonia and more acetate and propionate (McAllister and Newbold, 2008). Thus, inclusion of encapsulated nitrate in sheep diets could have an important impact on reducing rumen CH_4 foramtion.

Blood Nitrate, Nitrite and Methemoglobin (MetHb) Concentrations

The possible toxicity of rumen nitrite accumulation is due to increased blood MetHb formation (Iwamoto et al., 2001), which impairs the ability of hemoglobin (Hb) to transport oxygen to tissues (Ozmen et al., 2005). Neither type of encapsulated nitrate induced any change in blood Hb and nitrate concentrations compared with control on average 11.97 g/100 mL and 36.33 mM, respectively (Table 3).

The interaction between treatment × periods for blood Hb (g/dL) was significantly ($P > 0.05$). However, data from dummy variables test, comparing the three linear regression equations of treatment diets showed that the intercepts and regressor of the three equations (control, Nitrate and Nitrate + CNSL) were not statistical different

($P > 0.05$) ($Y = 12.122 – 0.0083X$, $R^2 = 0.011$, $Y = 11.316 + 0.026X$, $R^2 = 0.18$ and $Y = 10.828 + 0.0219X$, $R^2 = 0.074$, respectively, Figure 2)

Lamb health could be favorably affected by increasing blood Hb, when consumed nitrate in their diets and which could be due to compensate the decreased oxygen-carrying capacity of their blood (Winter and Hokanson, 1964). Normally, MetHb % represents only 1-2 % of total Hb (Langlois and Calabrese, 1992). Regarding the blood MetHb, no differences in MetHb % were observed ($P > 0.05$) when lambs were fed Nitrate and Nitrate + CNSL diets relative to control diet (1.08, 0.92 and 0.62%, respectively, Table 3). MetHb % did not reach 2 % of Hb and tended ($P = 0.08$) to increase with encapsulated nitrate when compared to the urea diet. Additionally, MetHb levels were less than that considered potentially toxic (30 to 40% of Hb) for sheep (Bruning-Fann and Kaneene, 1993). In our experiment, the inhibitory effect of high blood MetHb % on oxygen transfer to the body tissues could be disappear when encapsulated nitrate is included in the diet (El-Zaiat et al. 2011).

Differences among treatments in blood nitrite concentration were detected. Lambs fed Nitrate increased ($P = 0.05$) blood nitrite concentration compared to the control diet (2.40 vs. 2.04 µM, Table 3). The high ruminal nitrite concentration (more than 3 mM) is very toxic for rumen bacteria and could result in acute animal intoxication (Kozloski, 2011). The exceptionally higher ruminal nitrite concentration (Table 4) and blood elevated nitrite concentrations could be due to their ruminal metabolism and the absorption of nitrite accumulated across the rumen wall to the blood stream (Morris et al., 1958).

However, amounts of nitrite detected could be much below to the toxic levels evidenced by no clinical signs of toxicity. The gradual adaptation for nitrate diets

could allow to increase rumen nitrite-reducing bacteria which reduce the accumulation and toxic effect of nitrite (Allison and Reddy, 1984; Van Zijderveld et al., 2010). Although the MetHb % and increased blood nitrite, all lambs appeared to be healthy throughout the experiment without any signs of toxicity.

It is noteworthy that lambs fed encapsulated nitrate diets increased ($P < 0.01$) the RBC number compared with the control diet (12.38 vs. 10.36, $x10^6$/mL, respectively, Table 3). However, no differences in RBC number ($P > 0.05$) were observed for the overall feeding period among the two encapsulated nitrate inclusion treatments. These differences could be related to the main function of RBC, which maintain aerobic metabolism by transporting O_2 and CO_2 to and from body tissues. The results obtained in our study is agreement with the data reported by Jainudeen et al. (1964) that Holstein heifers showed elevated circulating erythrocytes and Hb concentration when fed nitrate (440 and 660 mg/kg of BW) for a long period of time. Our results showed that lambs fed encapsulated nitrate diets attained more MetHb levels, which could be a response to stimulate more RBC production. This also could be caused by a compensatory mechanism trying to carry sufficient and nearly normal amounts of O_2. Retarded growth, disturbed feed intake and feed efficiency did not occur.

Ruminal Fermentation Constituents

The inclusion of encapsulated nitrate had no ($P > 0.05$) effect on ruminal pH values (Table 4). These data are consistent with Nolan et al. (2010) who reported that inclusion of nitrate in the diet did not affect rumen pH in sheep. The present results are in contrast with the findings of Li et al. (2012) who recently observed a significant decline in ruminal pH when nitrate diets were fed. Clearly, rumen conditions in term of

pH are not constant, but are influenced by diet. When the qualitative nature of the diet was altered, about 10 d were needed to complete the major adjustments in the rumen microbial population (Warner, 1962).

As shown in Table 4, inclusion of encapsulated nitrate to the diet decreased ($P < 0.01$) ruminal ammonia concentration compared with control diet. Lambs fed Nitrate + CNSL decreased ($P < 0.01$) NH_3-N concentrations by 36% compared with lambs fed Nitrate (25 %) or control diet (22.29 vs. 26.37 and 34.93 mg/100 ml, respectively). These results could be due to the CNSL antimicrobial inhibitory effect on proteolytic or deaminating bacteria or stimulate of ruminal ammonia assimilation (Watanabe et al., 2010). On the other hand, recent results reported by Hulshof et al. (2012) observed that ruminal ammonia concentration was greater for nitrate diet fed to cattle than those fed urea diet, as a result of nitrate reduction process with nitrate feeding (Lewis, 1951).

In contrast, Nolan et al. (2010) observed no effect on rumen ammonia concentration of sheep fed nitrate diet. Additionally, the sufficient concentrations of ruminal ammonia ensure persistent nitrogen availability for rumen microbes, which may allow for maximal microbial protein synthesis. The optimal ammonia concentration for efficient microbial growth and maximum rumen fermentation activity are between 19 and 23 mg/dL (Mehrez et al., 1977) which may confirm that our results are within the optimal range.

In this study, although the ruminal ammonia concentration of both encapsulated nitrate diets were significantly lower than control, in the third chapter the same microbial nitrogen supply to the small intestine was observed. Thus, efficient rumen function process was indicated by the optimal ruminal pH and ammonia

concentration, which synchronize with other available nutrients for microbial growth that reflected the redirection of H_2 for acetate and total SCFA production. Consequently, it might be also due to increases in the rumen microbe population as adequate levels of ammonia could promote rumen microbial growth, improving carbohydrate fermentation and subsequently elevating total SCFA production (Jackson, 1971).

The total SCFA concentration was ($P < 0.01$) increased by 9.96 % and 21.67 % for Nitrate and Nitrate + CNSL respectively, compared with control (Table 4). Nitrate inclusion may influence ruminal total SCFA concentrations, these alterations in the proportions of SCFA might be indicative of greater reducing equivalent release due to nitrate reduction (Russell and Wallace, 1997). Acetate concentration was increased ($P < 0.01$) in lambs fed Nitrate + CNSL (by 31 %) compared to Nitrate (by 13 %) or control diet (64.04 and 55.21 vs. 48.88 mM) respectively. Nolan et al. (2010) reported that there was a tendency towards a higher molar percentage of acetate with nitrate-inclusion in sheep diet. However, Hulshof et al. (2012) found that the concentration of total SCFA was not affected, whilst the proportion of acetate tended to be greater for the nitrate diet.

The propionate and butyrate concentrations were not affected ($P > 0.05$) by addition of Nitrate compared with control (Table 4). Conversely, Nitrate + CNSL enhanced ($P = 0.04$) propionate and butyrate ($P < 0.01$) concentrations compared with control. In agreement with results of the present study, Shinkai et al. (2012) reported that feeding fistulated Holstein cows fed with a CNSL pellets blended with silica or with several other ingredients (4 g/100 kg BW/d) decreased CH_4 production (38.3 % and 19.3 %, respectively) and increased propionate proportion and caused a decrease in

acetate and total SCFA levels with both pellets type. These results could also suggest that electrons spared from CH_4 production were redirected to the production of more reduced fermentation acids (propionate and butyrate). This is contradictory to the finding of a previous study when sheep receiving 2.6 % of dietary DM had no differences in molar proportions of SCFA and a shift in SCFA proportions from butyrate to acetate (Van Zijderveld et al., 2010).

This is the first *in vivo* experiment to show that encapsulated Nitrate + CNSL which might be effective in directing rumen fermentation to greater propionate production which is associated with the decrease in CH_4 emission and increased total SCFA concentration, acetate and butyrate proportions compared to control diet. These fermentation changes likely are caused by a modification in rumen microbes due to the presence of CNSL antimicrobial phenolic compounds (anacardic acid with alkyl chains) which have been suggested to enhance propionate production (Watanabe et al., 2010). The CNSL addition to encapsulated nitrate could induce the growth inhibition of specific species of gram-positive bacteria, which could enhance the growth of gram-negative bacteria (because of their tolerance of CNSL phenolic compound) resulting in a redirection of hydrogen for greater propionate production.

Our results are in accordance with earlier studies of Li et al. (2012) and inconsistent with previous observation of Farra and Satter (1971) who stated that the competition for electrons between acetyl-CoA and nitrate when electrons are diverted to nitrate and butyrate synthesis is decreased, resulting in a higher production of acetate which occurred with nitrate compared to control. Consequently, the increase in acetate production would be due to increases a CO_2-reducing process to produce acetate, which is a way to dispose of metabolic H_2 during microbial metabolism (McAllister and

Newbold, 2008). In supporting of this hypothesis, Czerkawsik (1972) demonstrated that H_2 can be utilized to produce more highly reduced acids in the rumen such as propionate and butyrate. As an alternative, the increase in propionate concentration is consistent with decreased methane production due to a shift in ruminal fermentation patterns to propionate (Lovett et al., 2003).

Additionally, the difference between our study and the experiment of Van Zijderveld et al. (2010) is that rumen fluid samples in our study were collected 3 h after morning feeding, whereas in the other study samples were collected 24 h after feeding. No differences were observed between the incorporation of Nitrate or Nitrate + CNSL in the diet on propionate and butyrate concentrations (averaged 15.97, 11.25 mM and 17.87, 13.01 mM, respectively).

Similarly, the addition of encapsulated nitrate to the diet did not alter isobutyrate, valerate, isovalerate concentrations. The acetate:propionate ratio was unaffected ($P > 0.05$) by encapsulated nitrate addition; which could be due to the similar change pattern of acetate and propionate. In our experiment, 60% concentrate in the ration certainly enhances rapid reduction of nitrate and might be related to the higher digestible OM intake, which was reflected in greater SCFA concentrations that could be a consequence of greater rumen fermentation and passage rate from the rumen, which affected by the optimal pH (López et al., 1994).

Total protozoa number was decreased ($P < 0.01$) by 11.89 % with encapsulated nitrate compared with the control diet (Table 4). No significant ($P > 0.05$) differences in total protozoa counts were observed among the two encapsulated nitrate diets. These results suggest that compared with nitrate treatment, the addition of CNSL did not modify the total protozoa number. The corresponding induction of fermentation

changes with nitrate diets on ruminal protozoa number are conflicting, while some studies reported that there is no effect of nitrate on protozoal number, other studies confirmed the reduction in protozoa count by inclusion of nitrate in the diet. Our results are in contradiction with the earlier results reported by Nolan et al. (2010) and Li et al. (2012) that the total protozoa population were not affected by nitrate inclusion in the diet. Also, Van Zijderveld et al. (2010) found that the protozoa population was unaffected by inclusion of nitrate and sulfate in the diet.

Abundance of ruminal protozoa might be susceptible to the possible toxic effects of nitrite (Lewis, 1951) depending on their reliance on the electron transfer for energy production (Marias, 1988). Nevertheless, the methanogenic Archaea are symbiotically and metabolically associated with protozoa, so a reduction in methanogens would probably affect the protozoa populations (Morgavi et al., 2010) must be taken into account. Additionally, in our study the inhibition of methane with the reduction in total protozoa number, support the hypothesis that reduced protozoa activities are associated with lower methanogenesis, which could be due to a reduction in the H_2 availability from protozoa to methanogenic bacteria (Bhatt et al., 2009, McAllister and Newbold, 2008). Protozoa numbers are strongly affected by nitrate diets confirming decreases in protozoal numbers, which may be associated with a reduction of ruminal proteolysis and deamination that appeared clearly in lowered ammonia concentration and could improve the capture of dietary-N and cycling of microbial-N in the rumen (Hristov and Jouany, 2005).

The addition of both Nitrate and Nitrate + CNSL increased ($P < 0.05$) ruminal nitrite concentration compared with the control diet (5.00, 4.76 and 4.02 µM, Table 4) respectively. These results are probably due to increases in reducing

nitrate/nitrite bacteria numbers (Alaboudi and Jones, 1985), along with decreased H_2 concentrations associated with rumen protozoa depression (Eryavuz et al., 2003), which could stimulate rumen bacteria activity. The present results are in accordance with findings reported by Lewis (1951) who reported that nitrite concentration was elevated as a consequence of nitrate feeding, due to the reduction of nitrate and nitrite in the rumen. Our results suggest little differences between Nitrate or Nitrate + CNSL in regard to the effect on ruminal fermentation, suggesting that these encapsulated nitrate types could induce sufficient N to improve rumen fermentation without negative effects on intermediate metabolites on rumen pH and DMI.

CONCLUSIONS

The use of encapsulated nitrate as compared to urea as a N source in lamb diets could serve as an alternate electron acceptor with further reductions in rumen CH_4 production. The addition of encapsulated nitrate improved ruminal fermentation profiles in terms of SCFA and ammonia concentration without any adverse effects on animal performance. Dietary addition of CNSL with encapsulated nitrate has positive effect on total SCFA and acetate concentration associated with lower ruminal NH_3-N concentrations without any effect on ruminal methane production. All lambs appeared to be healthy and showed negligible blood MetHb % with no clinical signs associated with feeding these products throughout the experimental period. This study demonstrates that encapsulated nitrate could be used as a feed additive for ruminant feeding. Future research is needed and may focus on microbial diversity in order to better describe biological potential of these products on rumen microorganisms.

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Table 1. Feed ingredients and chemical composition of experimental diets (%, DM) fed to lambs

Items	Experimental diets		
	Control	Nitrate	Nitrate + CNSL
Ingredients (% DM)			
Chopped Tifton-85 hay	40.00	40.00	40.00
Ground corn	50.90	48.99	48.99
Soybean meal	5.00	5.00	5.00
Urea	1.50	---	---
Encapsulated nitrate	---	4.51	---
Encapsulated nitrate + CNSL	---	---	4.51
Mineral mixtures[1]	1.50	1.50	1.50
Limestone	1.10	---	---
Chemical composition (g/kg of DM)			
DM	893.47	900.25	895.82
OM	953.60	940.27	937.97
CP	135.07	134.30	134.80
EE	44.72	49.73	46.83
NDF	678.75	673.20	678.10
ADF	240.28	236.47	239.30
Lignin	49.73	44.72	46.83

[1] Composition: 85 g of P, 165 g of Ca, 10 g of Mg, 25 g of S, 120 g of Na, 350 mg/kg of F, 756 mg/kg of Cu, 2800 mg/kg of Zn, 2180 mg/kg of Mn, 66 mg/kg of I, 44 mg/kg of Co, and 14 mg/kg of Se.

Table 2. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on growth performance and methane production of growing lambs

Variable	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		Treat.
Initial BW, kg	26.75	27.25	27.17	---	---
Final BW, kg	37.80	36.83	37.03	0.882	0.80
DMI, g/d	1112	1029	1037	44.7	0.39
DMI, g/kg BW	34.91	32.76	33.21	0.978	0.30
DMI, g/kg $BW^{0.75}$	82.75	77.39	78.24	2.52	0.31
ADG, g	173	156	153	18.3	0.71
G:F, %	15.9	15.0	14.7	0.0144	0.84
CH_4, L/d	27.50^{a}	18.27^{b}	20.54^{ab}	2.363	0.05
CH_4, L/kg DMI	28.57^{a}	19.14^{b}	19.53^{b}	2.178	0.02
CH_4, L/kg $BW^{0.75}$	2.04^{a}	1.37^{b}	1.53^{ab}	0.176	0.05

[a,b] Within a row, means without a common superscript differ, $P < 0.05$,
DMI: dry matter intake, ADG: average daily gain, G:F: Feed efficiency.

Table 3. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on blood hematological constituents of growing lambs

Variable	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		Treat.
Hb, g/100 mL	11.81	12.29	11.65	0.416	0.54
MetHb, %	0.62	1.08	0.92	0.131	0.08
RBCs, x 10^6/mL	10.36^a	12.75^b	12.01^b	0.246	<0.01
Nitrate, mM	30.50	36.43	36.22	3.69	0.46
Nitrite, µM	2.04^b	2.40^a	2.19^{ab}	0.093	0.05

[a,b] Within a row, means without a common superscript differ, $P < 0.05$
RBCs: red blood cells, Hb: hemoglobin, MetHb: metahemoglobin.

Table 4. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on rumen fermentation constituents of growing lambs

Variable	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		Treat.
pH	6.76	6.78	6.74	0.063	0.92
NH_3, mg/100 ml	34.93^a	26.37^b	22.29^c	0.281	<0.01
SCFA, m*M*					
Total	90.46^c	99.47^b	110.07^a	2.091	<0.01
Acetate	48.88^c	55.21^b	64.04^a	1.31	<0.01
Propionate	14.77^b	15.97^{ab}	17.87^a	0.81	0.04
Butyrate	9.32^b	11.25^{ab}	13.01^a	0.60	<0.01
Isobutyrate	6.68	7.46	6.26	0.43	0.16
Valerate	6.04	5.74	5.56	0.59	0.85
Isovalerate	4.76	3.85	3.33	0.54	0.19
A:P	3.57	3.61	3.67	0.20	0.94
Protozoa, x10^5/mL	22.55^a	19.90^b	19.87^b	0.41	<0.01
Nitrite, µM	4.02^b	5.00^a	4.76^a	0.133	<0.01

[a,b,c] Within a row, means without a common superscript differ, $P < 0.05$
SCFA: short chain fatty acid, A:P: Acetate :Propionate ratio.

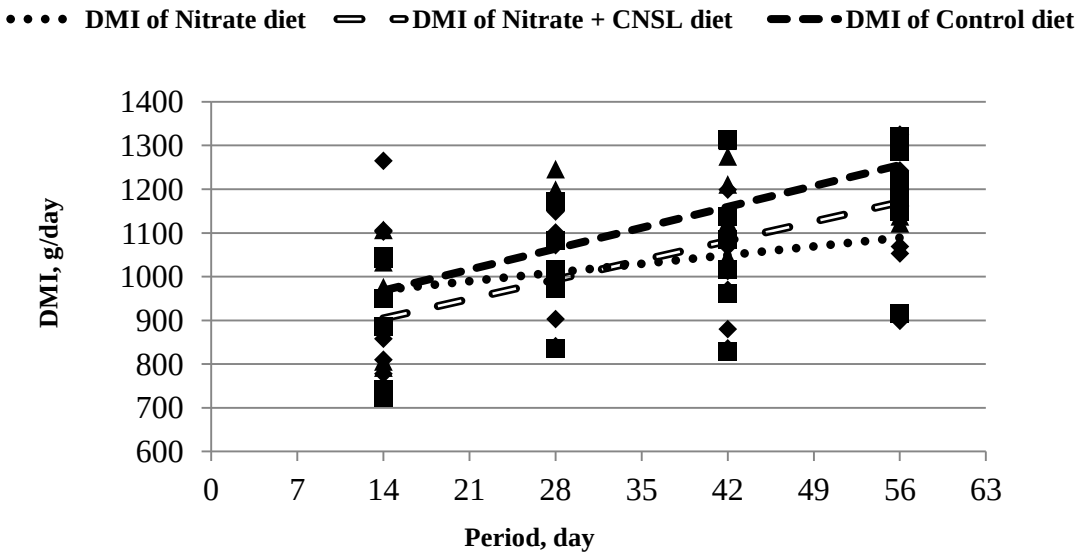

Figure 1. The effect of period × treatment interaction on dry matter intake (DMI, g/d).

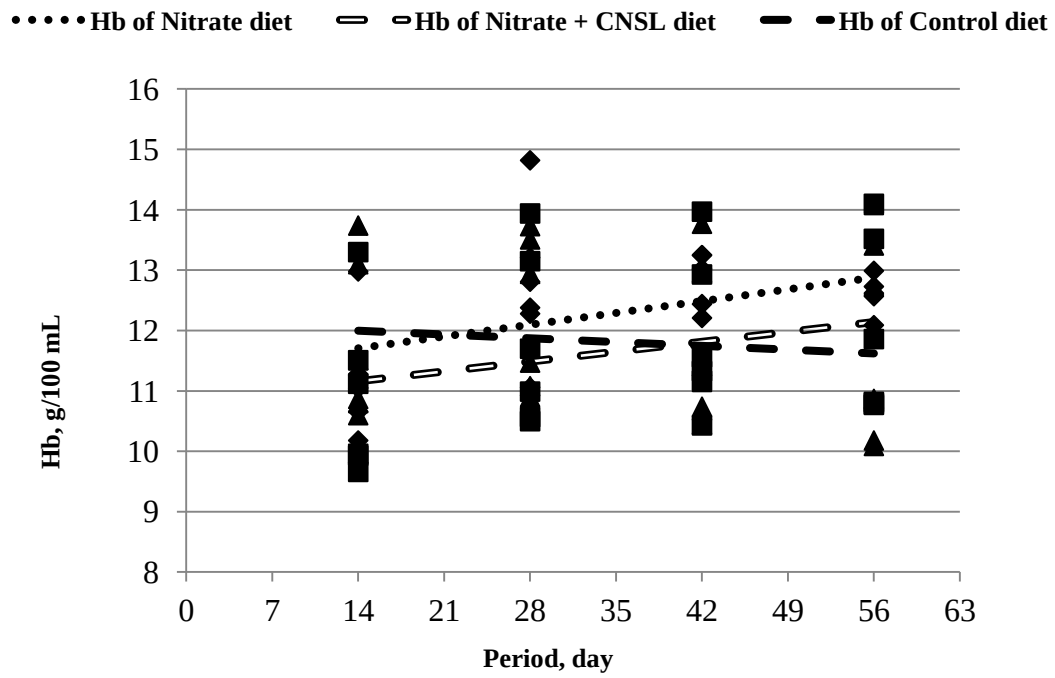

Figure 2. The effect of period × treatment interaction on blood hemoglobin (Hb, g/100 mL).

CAPÍTULO 3[1]

[1]According the form and style required by Journal of Animal Science for publication.

Apparent nutrients digestibility, nitrogen balance and carcass traits in lambs fed encapsulated calcium nitrate

ABSTRACT

The objective of this experiment was to determine the effect of encapsulated nitrate on apparent nutrients digestibility, nitrogen balance, carcass characteristics and some blood constituents in growing lambs. Eighteen Santa Inês male lambs (mean BW 36.2 ± 5 kg, 6 to 7 mo old) lambs were housed in metabolism pens. A randomized complete block design experiment was used with 6 blocks and 3 diets. Animals were assigned to 1 of 3 experimental diets and fed *ad libitum* the same basal diet described in the second chapter. Treatments were basal diet plus urea 1.5% in the dietary DM (control), encapsulated nitrate replaced urea by 4.51% in the dietary DM (Nitrate) or encapsulated nitrate plus cashew nut shell liquid (CNSL) replaced urea by 4.51 % in the dietary DM (Nitrate + CNSL). Experimental period consisted of 6 d for data and samples collection. Apparent digestibility of DM, OM, CP, EE, NDF, and ADF and nitrogen balance were determined. Blood samples were drawn every 2-wk (day 14, 28, 42 and 56) during the feeding trial to determine blood biochemical parameters. After completion of metabolism trial lambs were fasted, slaughtered and carcass traits were evaluated. Data were analyzed by the SAS Mixed procedure. The DMI an nitrogen balance were unaffected, and all lambs were in positive nitrogen balance. Apparent nutrients digestibility were not affected by encapsulated nitrate diets. Urinary urea excretion declined (P = 0.01) in lambs fed encapsulated nitrate diets compared with fed control diet, whereas plasma urea concentrations were not (P = 0.16) modified. Lambs fed encapsulated nitrate diets had greater (P < 0.01) urinary nitrite

excreted. The total urinary purine derivatives excretion and creatinine were not influenced in response to encapsulated nitrate inclusion. Microbial nitrogen supply was similar (P = 0.73) for all treatment diets. The plasma concentrations of total protein, albumin, AST and ALT were not modified by dietary encapsulated nitrate. Meat and carcass characteristics were unaffected ($P > 0.05$) with encapsulated nitrate inclusion. Residues of sodium nitrate in fresh meat ($P < 0.05$) were not affected by treatments. The residues of sodium nitrite were undetected. Meat color (L^*, a^* and b^*) was not changed ($P < 0.05$) by either Nitrate or Nitrate + CNSL. Replacement of urea by encapsulated nitrate for growing lambs did not influence the nutrients digestibility and nitrogen balance with no clinical signs or deleterious impacts on hematological variables and carcass characteristics.

Key words: nitrate, carcass characteristics, digestibility, purine derivatives

INTRODUCTION

Dietary urea has been used as inexpensive fermentable nitrogen source in ruminant diets to increase feed intake, DM digestibility and microbial protein synthesis (Taylor-Edwards et al., 2009). Nitrate salts could potentially replace urea to provide a nitrogen source for microbial growth and increasing animal productivity (Leng, 2008). The progress in using nitrate in ruminant diet has been discouraged due to nitrite accumulation and poisoning syndrome (McAllister et al., 1996; Sar et al., 2005). An alternative approach is to use slow-release characteristics using coatings based on polymers, which could be a potential way to minimize the excess of nitrite and subsequently rumen nitrite toxicity. Cashew nut shell liquid (CNSL) has an inhibitory effect on proteolytic or deaminating bacteria without detrimental effects on the digestibility (Watanabe et al., 2010).

In second chapter, rumen methane production was potentially decreased and DMI, feed efficiency, ADG and MetHb% were not affected when urea replaced by encapsulated nitrate or encapsulated nitrate plus CNSL in lamb diets. Little information on nutrients digestibility, purine derivatives and carcass and meat characteristics of lambs fed encapsulated nitrate is substantially documented. The hypothesis was that encapsulated nitrate would favor synchrony between the reduction rate of nitrate to nitrite and the ability to capture available hydrogen would alter nitrogen utilization in lambs without any adverse effects on DMI, apparent nutrients digestibility, nitrogen balance, carcass quality and without negatively affecting animal health. The objectives of our study were to investigate the apparent nutrients digestibility, nitrogen retention, carcass characteristics responses with blood metabolites of growing Santa Inês lambs using the experimental treatments of encapsulated nitrate.

MATERIALS AND METHODS

Regrading to the second chapter, animals and the experiment protocol were approved by the same Environmental Ethics and Animal Care Committee of the CENA/USP.

Animal and Housing

Upon complication the feeding trial, all lambs (18 Santa Inês male lambs with mean BW of 36.2 ± 5 kg, 6 to 7 mo old) were placed in metabolism cages (1.35 × 0.57 × 1.05 m) equipped with feeders and water troughs and designed to allow the separation and collection of feces and urine.

Experimental Design and Diets

The experiment lasted of 6 d of sample and data collection (after 28 d as adaptation period and 64 d of feeding trial). Lambs were designed using the same experimental design in the second chapter within 6 blocks and 3 diets by live body weight.

The same three experimental treatments in the second chapter were used as the following: 1.5% urea of dietary DM (control), encapsulated nitrate replaced urea by 4.51% in the dietary DM (Nitrate) or encapsulated nitrate plus cashew nut shell liquid (CNSL) replaced urea by 4.51% in the dietary DM (Nitrate + CNSL). The basal diet used was designed to meet NRC (2006) recommendations for growing lambs and consisted of 40% forage based on Tifton-85 (*Cynodon sp*) hay (chopped approximately 5 mm length) and 60% concentrate based on ground corn, soybean meal and mineral mixture (DM basis). The feed ingredients and chemical composition of the experimental diets are presented in Table 1 in second chapter. Regarding to the second chapter, the

experimental encapsulated nitrate products and its composition were manufactured by GRASP® Ind. e Com. LTDA (Curitiba, Paraná, Brazil).

Feeding Managements and Data Collection

Animals were fed a mixed basal diet with their treatments twice a day in two equal portions at 0900 and 1600 h, with allowed *ad libitum* access to feed and fresh water. Feed offered and orts quantities were recorded and weighted daily. Treatments started in adaptation period by 33% of the total amount of concentrates and increased every 7 d for each treatment until the lambs fed the total amount of experimental concentrates after 21 d. The lambs continued feeding their respective treatments for 7 d more to give a sufficient adaptation for rumen microbes. Adaptation period served to introduce the treatment concentrates gradually into the diets and the experiment conditions

The amounts of feed intake offered was adjusted daily on the basis of the feed intake on the previous day, calculated by weighting-back refusals daily, allowing refusal amount of approximately 10% of daily feed intake. Orts were recorded every day to determine daily DMI and stored in the freezer at -20 °C until analyses. Total feces and urine samples were collected for all lambs at 0800 h before the morning feeding. At the end of the collection period, total fecal samples from individual lamb were mixed, dried at 65°C for 48 h in a forced-air oven and ground through a 1-mm mesh sieve. Total feces were collected, weighed and stored in the freezer at -20°C until analyses.

The total urine volume was recorded daily and collected into containers with approximately 100 mL of 10% H_2SO_4 to bring down the pH to below 3 to prevent purine derivatives destruction by bacteria and ammonia volatilization. Total volume of

urine a 10 % daily aliquot was pooled over the 6 day per lamb stored at -20°C for total N and purine derivatives analyses. Blood samples (5 ml) were collected by jugular vein 6 h after the morning feeding every 2-wk from the jugular vein using vacutainer tubes containing K2E (K2 EDTA, BD, Franklin Lakes, NJ, USA) for fresh hematological blood analyses.

Laboratory Analyses

Feed, orts, and feces samples for each animal were thawed, dried in a forced-air oven at 55°C for 48 h and ground to pass a 1-mm screen for future analyses. In ground samples DM was determined by oven drying at 105°C for 24 h, and organic matter (OM) was determined by difference after heating at 550°C for 4 h, crude protein (CP as 6.25×N) and ether extract (EE) using the extraction system (ANKON brand, model XT10I, New York, USA) were determined according to AOAC (2006) methods. After thawing, composited urine samples were analyzed for total N according to AOAC (2006). Urinary N concentration was corrected for the addition of acid. Apparent nutriets digestibility and N retention were calculated by difference. Sequentially in the same sample, neutral detergent fiber (NDF), acid detergent fiber (ADF) were determined in filter bags with a heat stable amylase and expressed exclusive of residual ash as described by Van Soest (1991) and adapted to Mertens (2002).

Blood Parameters Determinations

Blood samples were taken 6 h after the morning feeding every 14 d (day 14, 28, 42 and 56 during the feeding trial according to the second chapter) from the jugular vein using vacutainer tubes containing K2E (K2 EDTA, BD, Franklin Lakes, NJ, USA).

The blood samples were centrifuged (2,000 x g for 15 min at 4°C) and the plasma was immediately frozen -20°C for future analyses. Biochemical blood

parameters were assayed colorimetrically using spectrophotometer methods for urea nitrogen, total protein, albumin and activity of alanine (ALT) and aspartate (AST) aminotransferase, using a commercial Labtest (Diagnóstica S.A.1 Lagoa Santa, MG, Brazil) kit.

Urine Urea, Nitrate and Nitrite Determinations

Urine samples were centrifuged (5,000 x g for 20 min at 4°C) and immediately frozen at -20°C until future analyses.

Urinary urea concentration was determined colorimetrically using spectrophotometer methods, using a commercial Labtest (Diagnóstica S.A.1 Lagoa Santa, MG, Brazil) kit.

Urine nitrate concentrations were determined according to the methods of Arrow straight Nitric Oxide Measurement System (Lazar Research Laboratories, INC. Los Angeles, USA), using micro ion selective electrodes (Model No. ISM-146NOXM) in conjunction with an ion analyzer which coupled with computer program to conveniently measure, tabulate and record the values.

Urine nitrite concentration was determined colorimetrically using spectrophotometer at 525 nm, using test of Merck Merckoquant® nitrite (Merck KGaA, Darmstadt, Germany) kit.

Urine Purine Derivatives (PD) Determinations

Urinary excretion of PD (uric acid, allantoin, xanthine and hypoxanthine) and creatinine were determined using HPLC analyses following the procedure of Chen and Gomes (1992). Urinary PD is widely used to estimate microbial nitrogen (MN) flow to the small intestine (Shingfield and Offer, 1998). The PD concentrations were

corrected for the addition of acid. Total urinary PD excreted (mmol/day) were estimated as the sum of all four PD compounds.

The relationship between the excretion of PD in urine and absorption of microbial purine was estimated using the flowing equation:

$PD_{ex} = 0.84 \times PD_{abs} + (0.150W^{0.75}e^{-0.25X})$ where PD_{ex} (mmol/d) is the excretion of PD in urine; PD_{abs} (mmol/d) is the absorption of microbial purine, $(0.150W^{0.75}e^{-0.25X})$ represents excretion of the endogenous PD, $W^{0.75}$ represents the metabolic BW (kg) of the animal.

$MN_{PD} = (PD_{abs} \times 70)/(0.116 \times 0.83 \times 1000) = PD_{abs} \times 0.727$

Slaughter and Carcass Assessment

Upon completion of the metabolism trial, all lambs attained on average 37 kg of BW (16-h fast) were slaughtered at a commercial abattoir. Lambs slaughter live BW (SLBW) was recorded after 16 h of fasting (Table 6). Carcasses were refrigerated for 24 h at 4°C . Carcass characteristics evaluated were hot and cold carcass yield (HCY and CCY, respectively), cold carcass weight (CCW) and chilling weight loss (CWL), fat thickness over the 12th rib, and rib eye area (obtained after chilling for 24 h at 4°C) were recorded. The hot carcass yield was calculated as (HCW/SLBW) × 100 (obtained at the slaughter time). The cold carcass yield was calculated as (CCW/SLBW) × 100. Chilling weight loss (CWL) was calculated as ((HCW-CCW)/HCW) × 100. Carcass traits were evaluated using the methods proposed by Osório et al. (1998).

After weighing chilled carcasses were split along the vertebral column into 2 symmetrical sections and ribbed between the 12th and 13th ribs to expose the rib eye area. Samples comprising the 11th, 12th and 13th ribs from the left side was separated from the carcass and frozen at -20°C for further measurements. The rib eye area was

measured (obtained after chilling for 24 h at 4°C) from a tracing using an appropriate pen on a transparent plastic film in cm^2 using a standard transparent checked base (1 cm^2/cell). The dorsal 12th-rib fat thickness (mm) was measured using an outside caliper graduated in millimeters.

Meat Objective Color Measurements

The objective meat color readings of the surface 11th rib muscle were taken on fresh cuts soon after slaughter the exposed surface of the muscle was removed to avoid any effect of oxidation. Triplicate readings were made on separated zones in each sample and average values were calculated.

Meat objective color was determined with colorimeter (Konica Minolta, Chroma Meter, CR-400, Mahwah, New Jersey, USA) using the following parameters: L^* (lightness), a^* (redness) and b^* (yellowness) (CIELAB). The parameters were calibrated in a standard white porcelain with Y=93.7, x=0.3160 and y=0.3323 and with a measurement area of 8 mm in diameter, with an observation angle of 10° and an illuminant D65 with the specular component included.

Meat Sodium Nitrate and Nitrite Residual Determinations

A sample comprising the 12th and 13th ribs of each lamb was vacuum-packaged and frozen at -20°C for nitrate and nitrite residual determinations. Sodium nitrate and nitrite residues in fresh meat were determined spectrophotometrically at 540 nm according to the methods described by IAL (2005) (Instituto Adolfo Lutz, métodos químicos e físicos para análise de alimentos, São Paulo, Brasil).

Residual sodium Nitrite in fresh meat is based on the reactions of nitrite diazotization with sulfanilic acid and copulation with alpha-naphthylamine hydrochloride in acidic medium, forming the α-naphthylamino-p-azobenzene-p-sulfonic

(pink colored). Nitrate is reduced to nitrite column metallic cadmium in an alkaline medium. Residual sodium Nitrate in fresh meat based on the reaction of diazotizing sulfanilic acid with nitrite and copulation with α-naphthylamine hydrochloride in acidic medium, forming the α-naphthylamino-p-azobenzene-p-sulfonic.

Statistical Analyses

Feed intake, apparent nutrients digestibility, N-balance, blood and urine constituents, purine derivatives and carcass characteristics data were analyzed using the Mixed procedure (SAS Inst. Inc., Cary, NC) as completely randomized block design with 3 treatments and 6 blocks. The block was included as a random effect. The LSMEANS option was used to generate individual treatment means. Comparison´s among treatments were performed by Turkey's test. Effects were declared significant at $P < 0.05$. The model was: $Y = \mu + B_i + Tj + Eij$, where μ is the overall mean, B_i is the block effect (i = 1-6), T_j is the treatment effect (j = 1-3), and E_{ij} is the residual error.

RESULTS AND DISCUSSION

Dry Matter Intake

Data of feed intake, apparent nutrients digestibility are presented in Table 1. There was no effect ($P = 0.48$) of encapsulated nitrate on total DMI (average 1062 g/d). The lambs DMI averaged by 1.06 kg DM/animal/d was similar to the mean recommended values by NRC (2006) for sheep, which is between 1.0 and 1.3 kg DM/animal/d. our results are agreed with the reported results by Li et al. (2012) who found that 1.37 kg DMI /animal/d for sheep fed diet with inclusion of 3% calcium nitrate. The current results are consistent with recent studies with sheep by Van Zijderveld et al. (2010) and with goats by Trinh et al. (2009).

Recently, Hulshof et al. (2012) teported that no effect on feed intake was observed in beef cattle fed sugarcane based diet supplemented with 22 g nitrate/kg DM. Additionally, Van Zijderveld et al. (2011) testing a total mixed ration containing dietary nitrate source 21 g nitrate/kg DM fed to lactating Holstein-Friesian cows, did not find any effect on DMI by nitrate inclusion. The present study showed no adverse effects of encapsulated nitrate on DMI and the lambs appeared healthy throughout the experiment. In the second chapter, growing lambs feeding the same experimental treatment diets also showed unaffected DMI (1112 and 1033 g/animal/d, respectively). The unchanged DMI observed might be explained by the short main retention time in sheep as a consequence of greater ruminal fermentation rate, supporting faster rumen flow rates and disappearance (López et al., 1994).

Apparent Nutrients Digestibility

In the present study, the apparent digestibilities of DM, OM, CP, NDF, ADF and EE were not influenced ($P > 0.05$) by encapsulated nitrate inclusion (Table 1). Feeding encapsulated nitrate did not influence ($P = 0.50$) the digestible organic matter intake (DOMI). This result is in accordance with the observation reported by Nolan et al. (2010) who observed no effect of 4% calcium nitrate when fed to cannulated sheep on whole tract or ruminal DM digestibility. Similarly, Li et al. (2012) reported that apparent DM and N digestibility was not affected by 3% calcium nitrate fed to sheep. Although CP digestibility pattern is directly reflect the increase of dietary degradable nitrogen compounds, the CP digestibility not affected by both encapsulated nitrate relative to control ($P = 0.50$). These results could suggest that the effect of encapsulated nitrate diets on these nutrients digestibility is similar to those with urea diet.

The present results are in contrast to those reported by Marais et al. (1988) who found that addition of both nitrate and nitrite lowered the fiber digestibility corroborated by increase ruminal fill which reflect the DMI reduction. In this response, the encapsulated nitrate probably attributed to a slower release rate of nitrate/nitrite in the rumen, resulting lower nitrite toxicity which did not disrupt or influened DMI and over all nutrients digestibility.

Nitrogen Utilization

The mean values of N-intake, fecal-N, urinary excretion of urea, nitrate, nitrite and N balance are given in Table 2. All lambs were in positive N balance. Nitrogen intake was not influenced (P = 0.37) by encapsulated nitrate inclusion, which reflected by the comparable DMI observed (Table 1). Fecal, urinary and total N excretion was similar (P > 0.05) in lambs fed encapsulated nitrate compared with control diet. Lambs retained nitrogen and retained-N expressed as a percentage of N-intake and N-absorbed was also unaltered by the diets (Table 2). The present result is supported by the results reported by Trinh et al. (2009) in which goats had no difference in N-retention when adapted over 14 d when nitrate and urea were incorporated in the diets.

The amount of N-intake of encapsulated nitrate diets could be able to support a comparable N loss (feces and urine) similar to control diet (Table 2). Additionally, sulfur incorporated with the encapsulated nitrate could altered the nitrogen balance by increasing the utilization of nitrate nitrogen. Sokolowski et al. (1969) reported that lambs were able to retain more nitrogen when sulfur was added to nitrate diet.

Lambs fed Nitrate and Nitrate + CNSL diets reduced (P = 0.01) urinary urea excretion relative to control (3.31, 3.80 and 5.87 g/day respectively, Table 2). Greater urinary urea excretion observed with control diet might be due to increased liver urea removal capacity, corresponding a greater ruminal ammonia concentrations which diffused into blood stream, and converted to urea by the liver and excreted in urine (Firkins and Reynolds, 2005), which also supported by ruminal ammonia concentration (26.37 and 22.29 vs. 34.93 mg/100 ml, respectively) presented in the second chapter. No significant differences (P > 0.05) were found in urinary excretion of nitrate and nitrite (g/d) values of all tratments.

The urinary nitrate concentration tended (P = 0.06) to increase with encapsulated nitrate inclusion in the diets compared with control diet (Table 2). Unaccounted nitrate for ruminal reduction process could be absorbed throughout the rumen wall to blood stream and excreted in the urine (Alaboudi and Jones, 1985). Additionally, urinary nitrate excretion could be a consequence to the higher intestinal nitrite absorption which oxidized to nitrate almost completely during the first passage throughout the liver then excreted in the urine (Granger et al. 1991).

Compared with the control diet, the inclusion of Nitrate and Nitrate + CNSL diets significantly (P < 0.05) increased urinary nitrite concentration (1.57, 2.32 and 2.14 µM, respectively, Table 2). These results associated with greater rumen nitrite concentrations (4.88 and 4.02 µM), resulting in more blood nitrite concentrations (2.04 vs. 2.29 µM) presented in the second chapter. Rumen nitrite could be absorbed cross the rumen wall into the blood stream and subsequently lost in urine (Takahashi et al., 1998). At the same time, increased urine nitrite excretion was a consequence of increased plasma nitrite concentrations with encapsulated nitrate inclusion.

This rise response in urinary nitrite excretion for encapsulated nitrate treatments could be substantial enough to decrease the nitrite intoxication impact. In contrast to our findings Hüsler and Blüm, (2001) reported that urinary nitrite excretion was not enhanced even when plasma nitrite concentrations increased, because the transient rise in blood plasma was too small and nitrite conversion to nitrate.

Purine derivative excretion (PD_{ex}) and Microbial Nitrogen Supply (MNS)

Mean values for daily urinary PD_{ex}, creatinine, PD_{ab} and MNS of lambs are shown in Table 3. No changes ($P > 0.05$) in urinary excretion of allantoin precursors (uric acid, hypoxanthine, and xanthine) were observed either with Nitrate or with Nitrate + CNSL compared with the control diet. Moreover, here were no significant differences in total PD_{ab} in the urine of lambs fed encapsulated nitrate diets or control diet and ranged from 5.42 to 5.71 mmol/d/kg $BW^{0.75}$, respectively. There were no differences ($P > 0.05$) of treatments on creatinine excretion averaged by 7.01 mmol/day. Our data suggest the same urinary PD and creatinine excretion in lambs fed encapsulated nitrate or control diet.

A strong positive correlation between ruminant excretions of urinary PD and microbial N flow to the duodenum has been observed (Moorby et al., 2006). Most of the PD excreted in urine comes from partial metabolism of microbial nucleic acid, which efficiently absorbed in small intestine and the majority of their metabolites is excreted in the urine throughout the kidney, with some specific urinary recovery in each animal species (Belenguer et al., 2002). Moreover, urinary PD_{ex} variations are mainly related to the supply of microbial nucleic acid, which relying on purine metabolizing enzymes (Verbic, et al., 1990).

The PD proportions as percentage of the sum are about 60-80 % for allantoin, 10-30 % for uric acid and 5-10 % for hypoxanthine plus xanthine for sheep as described by Chen and Gomes (1992). Results obtained from lambs in this experiment (Table 4) confirmed about 75.1% for allantoin, 18.9% for uric acid and 5.94% for xanthine plus hypoxanthine which are within the range to those observed in sheep (Chen and Gomes, 1992). The present results further confirmed that allantoin was quantitatively the major purine derivative excreted in the urine. This observation is consistent with the results observed by Chen et al. (1992) and Galo et al. (2003).

Therefore, it indicated that the encapsulated nitrate inclusion in lamb diet did not change the urinary PD proportions and creatinine, but this could require further study. Neither type of encapsulated nitrate induced any notable ($P > 0.05$) change in MNS flow from the rumen compared with control diet (Table 4). The MNS when expressed as g microbial-N/kg DOMI ($P = 0.50$) were not affected by supplementation with encapsulated nitrate (Table 3). Thus, it could be due to the enhanced synchronization of essential substrates released in the rumen (the available amount of carbohydrates, Sulfur, branched-chain fatty acids, passage rate and ruminal pH), which could contribute to increase microbial energy efficiency (g of microbial-N/kg of digestible organic matter intake).

The present study provides the first data obtained from inclusion of encapsulated nitrate in sheep diet concerning the urinary excretion of PD and suggest a similar sensitivity of sheep to diets containing encapsulated nitrate compared with control diet with urea. Although no differences were observed across diets, the inclusion of encapsulated nitrate may have reduced nitrite toxicity to the ruminal microbes, which could improve the nutrients digestibility, improvement in N-balance and MNS. The

rumen nitrate/nitrite reduction to ammonia and the reductive process to detoxify nitrite could provide more rumen microbial growth and microbial-N flow (Cheng et al., 1988).

Blood plasma constituents

Blood plasma biochemical constituents measured 6 h after the morning feeding are presented in Table 4. Inclusion of encapsulated nitrate had no effect on (P = 0.16) blood urea concentration compared with the control. The urinary-N and urea excretion are indicative to blood urea levels, which was positively correlated with ruminal ammonia levels.

Unmodified ($P > 0.05$) plasma urea concentrations occurred, may be related to increased hepatic ammonia removal capacity in lambs fed control diet, to compensate the greater portal ammonia absorption, so that net urinary urea excretion by the kidneys was still substantially greater compared with encapsulated nitrate diets. One interesting observation was the encapsulated nitrate could slowly converted to ruminal ammonia, resulting lower ruminal ammonia as demonstrated in rumen fermentation results in the second chapter, then transformed into urea in the liver accompanied by little amount excreted in the urine.

The urinary urea excretion depression occurred with encapsulated nitrate diets, could be due to the reduction in renal plasma flow and glomerular filtration rate, which increased urea reabsorption from tubules in ruminants (Leng et al., 1985), leading to exert the same blood urea concentration and therefore helped to maintain urea recycling to the rumen (Tebot et al., 2002).

In addition, the liver enzymes studied (aspartate (AST) and alanine (ALT) amino-transferase) showed no differences (P = 0.10) with encapsulated nitrate inclusion (Table 4). There were no significant (P = 0.72) differences detected for plasma albumin

and total protein concentrations among the treatments (Table 4). It is established that high nitrate intake could increase AST and ALT concentrations, which might be related with hepatotoxic effect, which could impair and cause pathological changes in liver functions (Ogur et al., 2005).

Consequently, the blood plasma albumin and total protein levels are considered to be associated with hepatic and renal damage resulting in protein loss (Roussel et al., 1997). Nevertheless, the examined liver enzymes, total protein, albumin and urea were not affected by treatments; the values observed in this study are within the normal reference range (Kaneko et al., 1997), indicating normal hepatic and renal capacity functions during the experiment. So, these results suggest that Nitrate and Nitrate + CNSL could provide a safety margin to avoid the toxic concentrations potential of nitrate without any associated toxicity symptoms.

Carcass Characteristics

Literature and information are very Little on the impact of nitrate on carcass characteristics and its residues in meat. In this study, no carcass characteristics studied were affected by the inclusion of encapsulated nitrate in the diet (Table 5). The observed carcass characteristics values were within the range reported by Urano et al. (2006) for Santa Inês breed lambs with 37.4 kg of live slaughter weight: 49.6% hot carcass yield, 48.4% cold carcass yield, 2.5% chilling weight loss, 15.5 cm^2 of rib eye area, and 1.5 mm of fat thickness.

So, the results obtained for chilling weight loss was (0.55 %) was below the expected and thickness was a little above (2.41 mm) (Table 5). Moisture evaporation during chilling is responsible for carcass weight losses, so reducing chilling losses play an important role to obtain higher carcass yield (Rodríguez et al., 2011).

In this study, the isonitrogenous diet supplied in all treatments could be justified by the absence of significant influences of encapsulated nitrate supplementation on productive performance resulting in similar quantitative carcass characteristics. This result was very close to the results found by Sokolowski et al. (1969) who found that no significant differences were observed in dressing percentage for fattening lambs receiving 3.2% potassium nitrate incorporated in a complete diet.

The lightness measured in fresh muscle was not influenced (P = 0.83) by encapsulated nitrate (Table 5). None of the meat color instrumental studied (L^*, lightness), (a^*, redness) and (b^*, yellowness) were affected by the inclusion of encapsulated nitrate diets (Table 5).

Regarding to nitrate residues, no significant differences in sodium nitrate residue (average, 0.330 mg/kg fresh meat) were found by the inclusion of encapsulated nitrate as a replacement for urea (Table 5), contrasting the humans nitrate ingestion is approximately 40 to 100 mg/day (USEPA, 1987). The results for all meat samples revealed that sodium nitrite residue were undetectable. The changes in a^* and b^* values could describe the meat color deterioration from red to brown which reflected the redox state of meat pigment (Mancini and Hunt, 2005).

It bears noting that the inclusion of encapsulated nitrate in lamb diets negatively affected meat color, knowing that is the most important aspect of consumer acceptability (Kerry et al., 2000), which positively associated with meat freshness and quality (Morrissey et al., 1994). Thus, unaffected blood MetHb level response (presented in the second chapter, Table 3) and increased urinary nitrite excretion, could closely mirrored a similar meat color with encapsulated nitrate diets compared to control. Regarding the undetectable residual nitrite amounts in meat samples could refer

to nitrite and meat pigment reactions, which described the normal meat color. As was described previously, the relationship between meat color, blood MetHb concentration, urinary nitrite excreted, nitrate and nitrite residues in fresh muscles were consistent.

CONCLUSION

Results of this study indicate that encapsulated nitrate or inclusion of CNSL with encapsulated nitrate can replace of urea in growing lambs diets wtihout detrimental effects on DMI, apparent nutrients digestibility, N-balance and carcass characteristics. However, the addition of encapsulated nitrate had no negative effects on objective color, nitrates and nitrites residues in fresh meat. Dietary encapsulated nitrate could be possible to have an effective role in nitrate-N utilization, which could have a beneficial environmental impact. Our results pointed out a new technology with a potential use of encapsulated nitrate in the practical feeding. Moreover, lambs health and meat characteristics seem to be normal which indicates a greater suitability for feeding this product to growing lambs.

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Table 1. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on dry matter intake, apparent nutrients digestibility of growing lambs

Items	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		
DM Intake, g/d	1068	999	1119	67.1	0.48
Digestibility, %					
DM	62.26	61.49	62.65	1.63	0.88
OM	64.06	63.33	63.99	1.53	0.93
CP	64.43	66.84	68.04	2.11	0.50
NDF	58.44	57.72	58.26	1.54	0.94
ADF	29.53	32.69	29.93	1.48	0.31
EE	74.23	66.55	67.22	2.77	0.15
DOMI, kg/d	0.655	0.599	0.673	0.04	0.50

DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; DOMI: Digestible Organic Matter Intake.

Table 2. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on nitrogen metabolism of growing lambs

	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		
N-intake, g/d	24.67	21.96	24.41	1.42	0.37
Fecal-N, g/d	8.45	7.31	7.60	0.45	0.24
Urinary excretion					
Urine-N, g/d	9.82	7.66	7.51	1.75	0.60
Urea, g/d	5.87^{a}	3.31^{b}	3.80^{b}	0.49	0.01
Nitrate, g/d	2.07	2.16	1.77	0.30	0.64
Nitrite, µg/d	78.38	70.56	84.64	16.42	0.83
Nitrate, mM	32.36	58.41	37.65	6.97	0.06
Nitrite, µM	1.57^{b}	2.32^{a}	2.14^{a}	0.07	<0.01
Retained-N, g/d	6.41	6.98	9.31	1.78	0.50
g/kg N-intake	260.22	310.19	386.58	70.67	0.47
g/kg N-absorbed	405.03	467.71	558.72	103.90	0.59

^{a,b} Within a row, means without a common superscript letter differ, $P < 0.05$

Table 3. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on urinary excretion of purine derivatives (PD), Microbial-N supply (MNS) and efficiency microbial-N (EMN) of growing lambs

Items	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		
Purine derivatives, mmol/day					
Uric acid	3.35	3.09	2.84	0.24	0.37
Allantoin	10.80	13.18	12.79	1.64	0.57
Xanthine and Hypoxanthine	1.06	1.02	0.85	0.07	0.13
Total PD_{ex}, mmol/day	15.21	17.29	16.48	1.85	0.73
PD_{ab}, mmol/d/kg $BW^{0.75}$, mmol/day	5.42	5.71	5.56	0.25	0.72
Creatinine, mmol/day	7.19	6.92	6.93	0.53	0.92
MNS, g/d	3.94	4.15	4.04	0.18	0.73
MNS, g/kg DOMI	10.22	9.34	10.50	0.69	0.50

PD_{ex}: Purine derivatives excreted, PD_{ab}: Purine derivatives absorbed, DOMI: Digestibible Organic Matter Intake.

Table 4. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on blood biochemical parameters of growing lambs

Items	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		
Urea, mg/100 mL	36.74	33.09	30.58	2.09	0.16
Total protein, g/100 mL	7.00	7.31	7.27	0.15	0.35
Albumin, g/100 mL	3.19	3.20	3.12	0.07	0.72
AST, IU/L	51.44	46.51	45.27	1.93	0.10
ALT, IU/L	6.15	8.51	6.82	0.71	0.10

AST: Aspartate amino-transferase, ALT: Alanine amino-transferase.

Table 5. Effects of encapsulated nitrate or encapsulated nitrate plus cashew nut shell liquid as a replacement of urea on carcass characteristics of growing lambs

	Treatments			SEM	*P*-value
	Control	Nitrate	Nitrate + CNSL		
Carcass characteristics					
SLBW, kg	37.09	37.83	37.77	1.43	0.93
HCW, kg	17.69	17.61	18.13	0.49	0.81
CCW, kg	17.61	17.51	18.02	0.64	0.83
HCY, %	47.70	46.39	48.08	0.57	0.15
CCY, %	47.49	46.16	47.80	0.57	0.17
CWL, %	0.44	0.58	0.65	0.087	0.27
Rib eye area, cm^2	13.81	14.85	14.61	1.05	0.79
Fat thickness, mm	2.70	2.37	2.17	0.21	0.23
Lightness (*L**)	39.01	38.78	38.24	0.91	0.83
Redness (*a**)	16.62	16.79	16.15	0.35	0.41
Yellowness (*b**)	4.42	4.88	4.71	0.55	0.85
Residues in meat					
Sodium nitrate, mg/kg fresh meat	0.283	0.494	0.215	0.18	0.63
Sodium nitrite, mg/kg fresh meat	0	0	0	-	-

SLBW: Slaughter live BW, HCW: Hot carcass weight, CCW: cold carcass weight, HCY: hot carcass yield, CCY: cold carcass yield, CWL: chilling weight loss.

3. CONSIDERAÇÔES GERAIS

A inclusão de nitrato encapsulado em substituição a uréia pode propiciar maior eficiência do processo de redução de nitrato à NH_3, evitando o acúmulo de nitrito no sangue com um período de adaptação suficiente. Existem trabalhos que identificam o uso de nitrato e o que fazer, mas não se preocupam como fazer, contudo alguns fatores devem ser levados em considerações na incorporação na dieta. Esses trabalhos procuram os métodos para diminuir emissão de CH_4 do que os outros efeitos relacionados com essa diminuição.

A incorporação de nitrato encapsulado na dieta pode reduzir em 32 % a emissão de CH_4 em ovinos da raça Santa Inês, funcionando como um dissipador alternativo de hidrogênio que captura o H_2 produzido durante a fermentação. O incremento gradual da inclusão de nitrato encapsulado na dieta pode ter permitido a rápida multiplicação de microrganismos que utilizam nitrato e nitrito, o que minimiza os riscos de uma possível intoxicação dos animais. Consequentemete, não foram observados sintoma clínicos da toxicidade do nitrito pela formação MetHb % .

A inclusão de nitrato encapsulado na dieta dos cordeiros, apresenta maior número de células vermelhas do sangue em relação ao tratamento controle (uréia), o que pode explicar o efeito compensatório para o equilíbrio de O_2 e CO_2.

Neste estudo, a incorporação de nitrato encapsulado na dieta dos ovinos não gerou diferenças significativas sobre o desempenho dos animais (CMS, GMD, eficiência alimentar e peso vivo final). Os cordeiros alimentados com dieta contendo nitrato encapsulado não apresentaram alteração na digestibilidade aparentes dos nutrientes. Com isso pode-se inferir que os microorganismos celulolíticos não foram influenciados pela inclusão de nitrato encapsulado. A substituição de uréia por nitrato encapsulado na dieta dos cordeiros não alterou a excreção urinária de derivados de purinas e consequentemente o nitrogenio microbiano, além de estarem com balanço nitrogenado positivo, apresentaram menor resposta na excreção urinária da uréia.

A incorporação de nitrato encapsulado na dieta dos ovinos não teve efeito sobre os valores de pH do líquido ruminal e relação Acetato:Propionato. Contudo, foi observada uma diminuição entre 25 e 36% na concentração de NH_3-N ruminal e 12% no número total de protozoários. Entretanto, observou-se que a inclusão de nitrato encapsulado na dieta aumentou significativamente as concentrações de AGCC totais, acetato e propionato no rúmen.

Embora, outros trabalhos se preocuparam somente em afirmar a necessidade de utilização de nitrato sobre a mitigação de CH_4 entríco, não se perocupando com os efeitos resíduais na carcaça. Neste estudo, todos os cordeiros alimentados com nitrato encapsulado não apresentaram diferenças nas características de carcaça, parâmetros de cor de carne L* (luminosidade), a* (vermelhidão) e b* (amarelo), resíduos de nitrato de sódio na carne fresca. Além disso, o resíduo de nitrito de sódio em todas as amostras de carne foram zero. Isso demonstra que não há impacto dos resíduos de nitratos e nitritos na carne não constituindo um risco para a saúde pública.

Os resultados sugerem que o uso de nitrato encapsulado pode ser considerado como uma tecnologia inovadora e com potencial de uso para alimentação de ovinos em fase de crescimento sem afetar a saúde, o desempenho e a qualidade da carne. No entanto, mais pesquisas são necessárias para melhor a compreensão e elucidar os efeitos destes nitratos encapsulados na resposta das populações microbianas ruminais individuais a longo prazo.

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5. APÊNDICES

APÊNDICE 1 – NORMAS PARA PUBLICAÇÃO DA REVISTA:

Journal of Animal Science
(REVISED 2012)

The Instructions for Authors to the Journal of Animal Science (JAS) is divided into 2 sections:

I. Manuscript Preparation, which gives the Style and Form to be used by authors in the preparation of manuscripts; and

II. Policies and Procedures of JAS, which provides details concerning the mission of JAS, contact information, care and use of animals, the types of articles accepted by JAS, submitting manuscripts to JAS (including copyright policies), the review procedures and policies, and papers in press, author proofs, and publication charges.

I. MANUSCRIPT PREPARATION (STYLE AND FORM)

The most important thing you can do as you prepare your manuscript is to consult a recent issue of JAS in terms of the acceptable format for headings, title page, Abstract, Key words, Introduction, Materials and Methods, Results, Discussion (or combined Results and Discussion), Literature Cited, and tables and figures (including figure captions), which are described in more detail below. **Failure to adhere to the style and form will result in immediate rejection of the manuscript.**

General. Papers must be written in English and must use the American spelling and usage as well as standard scientific usage, as given in the following online resources: For general style and form, authors should follow that recommended in Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers. 7th ed. Council of Science Editors, Reston, VA.

• For American English spelling and usage: Merriam-Webster Online (http://www.m-w.com/).

• For numbers usage, consult the Policies Regarding Number Usage later in this document.

• For SI units, the following site (National Institute of Standards and Technology) provides a comprehensive guide: http://physics.nist.gov/cuu/Units/index.html For capitalization and spelling of plants, consult the USDA Plants website (http://plants.usda.gov). For anatomical nomenclature, consult the current Nomina Anatomica Veterinaria (http://www.wavaamav.org/Downloads/nav_2005.pdf). Manuscripts should be prepared double-spaced in Microsoft Word, with lines and pages numbered consecutively, using Times New Roman font at 12 points. Special characters (e.g., Greek and symbols) should be inserted using the symbols palette available in this font. Complex equations should be entered using Math-Type. Tables and figures should be placed in separate sections at the end of the manuscript (not placed in the text). Authors should prepare their manuscript in Microsoft Word and upload the manuscripts using the fewest files possible to facilitate the review and editing processes. Manuscripts should contain the following sections (Appendices or Online-Only Data Supplements, described below, are optional), in this order:

Title Page. The title page includes a running head (the first word only and any proper nouns capitalized and no more than 45 characters plus spaces); the title (only the first word and any proper nouns capitalized, as brief as possible, and including the species involved); names of authors (e.g., T. E. Smith; no title, positions, or degrees) and institutions, including the department, city, state or country (all with first letters capitalized), and ZIP or postal code. Affiliations are footnoted using the symbols *, †, ‡, §, #, ‖, ¶ and are placed below the author names. Footnotes on the first page (present ad-dress, and e-mail address of the corresponding author) are referenced by superscript numbers. Acknowledgments, including acknowledgements of grants, experiment station, or journal series number, are given as a footnote to the title. Authors who hold patents related to the research presented in the manuscript should include a statement in a footnote.

Abstract. The abstract consists of no more than 2,500 keystrokes (characters plus spaces) in one paragraph and summarizes the pertinent results (with statistical evidence; i.e., P-values) in a brief but understandable form, beginning with a clear statement of the objective and ending with the conclusions, with no references cited. Abbreviations in the abstract that are not Standard JAS abbreviations must be defined at first use.

Key Words. List up to 6 key words or phrases including the species, variables tested, and the major response criteria. The first letter of each key word is lowercase (unless a proper noun); key words are separated by commas and presented in alphabetical order; and no abbreviations should be used. Because major words in the title are not used for the subject index, which is published in the last issue of each volume of JAS, appropriate words from the title (or synonyms) should be listed as key words.

Introduction. The Introduction must not exceed 2,000 keystrokes (characters plus spaces) and briefly justifies the research, specifies the hypotheses to be tested, and gives the objective(s). Extensive discussion of relevant literature should be included in the Discussion.

Materials and Methods. A clear description or specific original reference is required for all biological, analytical, and statistical procedures. All modifications of procedures must be explained. Diets, dates of experimental activities if appropriate, animals [breed, sex, age, body weight, and weighing conditions (i.e., with or without restriction of feed and water)], surgical techniques, measurements, and statistical models should be described clearly and fully. Appropriate statistical methods should be used, although the biology should be emphasized. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of the statistical analysis should justify the interpretations and conclusions. The experimental unit is the smallest unit to which an individual treatment is imposed. Measurements on the same experimental unit over time also are not independent and should not be considered as independent experimental units.

Provide a validation for assays [e.g., mean and CV for repeated analysis of a sample (both between and within-assay if available) and the sensitivity (minimum amount or concentration detectable)]. Also, provide a publication reference for the methodology used in kits. Centrifugal force should be provided in × g, not rpm, and duration and temperature of centrifugation must be included. Include volume of blood collected, container used, and amount of preservative or anticoagulant (e.g., heparin).

Results. The results are presented in the form of tables or figures when feasible. If data are discussed in the text but not presented in the tables or figures, specify "data not shown" in the text. The text should explain or elaborate on the tabular data, but numbers should not be repeated within the text. Sufficient data, all with some index of variation attached (including significance level; i.e., P-value), should be presented to allow the reader to interpret the results of the experiment. Reporting the actual P-value is preferred to the use of the terms significant and highly significant. Thus, the observed significance level (e.g., P = 0.027) should be presented, thereby allowing the reader to decide what to reject. Other probability (alpha) levels may be discussed if properly qualified so that the reader is not misled (e.g., trends in the data).

Discussion. The discussion should interpret the results clearly and concisely in terms of biological mechanisms and significance and also should integrate the research findings with the body of previously published literature to provide the reader with abroad base on which to accept or reject the hypotheses tested. A stand-alone Discussion section should not refer to any tables or figures, nor should it include P-values (unless citing a P-value from another work).

Results and Discussion. In JAS, authors have the option of combining the results and discussion into one section.

Literature Cited. To be listed in the Literature Cited section, papers must be published or accepted for publication ("in press"). Personal communications and unpublished data must not be included in the Literature Cited section. See the Literature Cited Guide-lines later in this document.

Tables and Figures. Tables and figures must be prepared so they stand alone. Author-defined abbreviations must be defined (or redefined) in each table and figure. Manufacturer name and location should be provided for any proprietary product appearing in a table or figure. Tables must be created using the table feature in MS Word (for instructions, see Guidelines for Creating Tables in Microsoft Word (http://jas.fass.org/misc/ifora.shtml). Refer to a recent issue of JAS for examples of table construction. When possible, tables should be organized to fit across the page without running broadside. Each column must have a head-ing (e.g., Item, Ingredient, Trait, Fatty acid). Units should be separated from headings by a comma. Limit the data field to the minimum needed for meaningful comparison within the accuracy of the methods. In the body of the table, references to footnotes should be numerals. Each footnote should begin on a new line. To indicate significant differences among means within a row or column, superscript lowercase letters are used; the preferred statement in the footnotes is: "Within a row (or column), means without a common super-script differ (P < 0.05)." Figures should be placed at the end of

the manuscript and should follow the Quality Guidelines for JAS Figures (http://jas.fass.org/misc/ifora.shtml). Each figure should be placed on a separate page (separated by section breaks) and identified by the figure number. Figure captions should be typed double spaced on a separate page. The use of color in figures should be avoided unless it is essential to understanding the figure. There is an additional fee for color figures that are printed in the journal (see Manuscript Central for more information).

Appendices. To provide readers with numerical examples or give extensive detail of analytical procedures, an appendix or appendices can be included. However, if the supplemental material is of interest only to a limited number of JAS readers, it should not be included as an appendix. Instead, mention that supplemental information is available on request from the author; addresses for websites with appropriate supplemental information are acceptable. If extensive, the data may be included as an e-supplement to the manuscript (see Online-Only Data Supplements). Appendices should follow the Literature Cited section and be introduced by a major heading.

Online-Only Data Supplements. Authors can present material online that cannot physically be dis-played in the print journal (e.g., Excel files, video), that might be cost-prohibitive (e.g., color figures), or that provides data sets too detailed for publication in print. A note will appear in the print version that more material can be found online. Material posted online only must go through the review process, and consequently should be in an format easily accessible by most reviewers and readers.

Additional Usage Notes

Numbers. See JAS Policies Regarding Number Usage later in this document.

Abbreviations. Abbreviations in the text that are not standard JAS abbreviations must be defined at first use. Authors should not use standard JAS abbre -viations (e.g., t = metric ton and cannot be used as an abbreviation for temperature). In addition, authors should not use abbreviations accepted by JAS , such as abbreviations for elements (e.g., S = sulfur and C = carbon and cannot be used as author-defined abbrevi-ations). Once defined, author-identified abbreviations should always be used, except to begin a sentence. Author-identified abbreviations need to be redefined in the abstract, at first use in the body of the paper, in each table, and in each figure. Authors should avoid excessive use of author-defined abbreviations. See Standard JAS abbreviations later in this document, which includes standard abbreviations for physical units, units of time, statistical symbols and abbreviations, and others. Standard JAS abbreviations should always be used except to begin a sentence or unless otherwise contraindicated (e.g., units of time should only be abbreviated when used with a number).

Gene and Protein Names. Because there is no universally accepted style for gene and protein names that applies to all species, the Journal of Animal Science asks the authors to assume the responsibility of using the convention appropriate for the particular species. Some general guidelines can be found in the CSE Manual for Authors, Editors, and Publishers (7th ed., 2006). For example, the gene that codes for the protein p53 is TP53 in humans

and Trp53 in mice (note that, by convention, gene names are italicized; also note that protein names are generally not italicized).

Quantitative Trait Loci and DNA Markers and Microarray Data. Papers that publish quantitative trait loci (QTL) or DNA marker association results for livestock are strongly encouraged to make their data available in an electronic form to one of the publicly available livestock QTL databases after the manuscript appears in publication [the date on which the paper is posted to the JAS -Papers in Press website (http://jas.fass.org/papbyrecent.dtl) repre-sents the official publication date]. Current QTL da -tabases for livestock include, but may not be limited to, the Animal QTL database (http://www.animal -genome.org/QTLdb) and the Bovine QTL database (http://bovineqtl.tamu.edu /). Similarly, for microarray data we request that all authors using microarray data analysis in their research submit a complete data set to 1 of 3 databases before submission of a manuscript: the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/projects/geo), the EMBL-EBI ArrayExpress repository (http://www.ebi.ac.uk/arrayexpress), or the Center for Information Biology Gene Expression (CIBEX) database (http://cibex.nig.ac.jp/index.jsp).

Commercial Products. The use of names of commercial products should be minimized. When a commercial product is used as part of an experiment, the manufacturer name, and location (city and state if in the United States; city and country otherwise) or a website address should be given parenthetically at first mention in text, tables, and figures. The generic name should be used subsequently. No ™ or ® sym -bols should be used.

General Usage.

• Note that “and/or” is not permitted; choose the more appropriate meaning or use “x or y or both.”

• Report time using the 24-h system (e.g., 1410 h rather than 2:10 p.m.).

• Use italics to designate genus and species (*Bos taurus*) and botanical varieties (*Medicago sativa* var. Potomac). Designations for botanical cultivars should be preceded by “cv.” or enclosed in single quotes (e.g., *Festuca arundinacea* cv. Kentucky 31 or *Festuca arundinacea* ‘Kentucky 31’).

• Specify the basis (as-fed or dry matter) for dietary ingredient and chemical composition data listed in text or in tables. Similarly, specify the basis for tissue composition data (e.g., wet or dry basis).

• Calculations of efficiency should be expressed as output divided by input (i.e., gain:feed, not feed:gain). This avoids the spurious positive and negative infinity values when body weight gain is zero or negative. It also avoids the con-fusion associated with discussing an improvement as being a decrease.

• A diet is a feedstuff or a mixture of feedstuffs; a ration is the daily allotment of the diet.

• Restrict the use of “while” and “since” to meanings related to time. Appropriate substitutes include “and,” “but,” or “whereas” for “while” and “because” or “although” for “since.”

• The words “Table” and “Figure” are capitalized and not abbreviated when used in the text to refer to a specific table or figure. Experiment and equation should be abbreviated to Exp. and Eq., respectively, when preceding a numeral.

• Avoid jargon unfamiliar to scientists from other disciplines. Do not use the term "head" to refer to an animal or group of animals. Instead, use animal, sow, ewe, steer, heifer, cattle, etc.
• Avoid bi- as a prefix because of its ambiguity; biweekly means twice per week and once every 2 weeks.
• Breed and variety names should be capitalized (Landrace, Hereford). Trademarked or registered names should be capitalized, but no ™ or ® symbols should be used.

II. POLICIES AND PROCEDURES OF JAS (return to Style and Form)

The mission of the American Society of Animal Science (ASAS) is to foster communication and collaboration among individuals and organizations associated with animal science research, education, industry, or administration "To discover, disseminate, and apply knowledge for sustainable use of animals for food and other human needs." The Journal of Animal Science (JAS), which is published monthly by ASAS, accepts manuscripts presenting information for publication with this mission in mind. The editorial policies of JAS are established by the editor-in-chief, managing editor, division and associate editors, and editorial board, subject to review by the publications commit-tee, board of directors, and the membership of ASAS. The views expressed in papers published in JAS represent the opinions of the author(s) and do not necessarily reflect the official policy of the institution with which the author is affiliated, the ASAS, or the editor-in-chief. It is the responsibility of the authors to ensure the accuracy of collection, analysis, and interpretation of data in manuscripts and ultimately to guarantee the veracity of the contents of articles published in JAS. The JAS is one of the most frequently cited, peer-reviewed, agriculturally oriented research journals in the world, based on statistics published by ISI Inc. (Philadelphia, PA). Its high ranking in several ISI categories, including impact factor, attests to the quality standards maintained by the JAS editorial board, editors, and staff and by authors who submit manuscripts for publication.

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For information on the scientific content of the jour-nal, contact the Editor-in-Chief, Dr. Steven A. Zinn, Department of Animal Science, University of Con-necticut, Storrs, CT 06269-4040; telephone 860-486-0861; fax 860-486-4375; email: steven.zinn@uconn.edu.

For questions on submitting a paper and Manu-script Central, contact Brett Holte, Submission Services Manager, telephone 608-268-3970; email:bholte@sciencesocieties.org. For assistance with author proofs, contact Emily Mueller, Managing Editor; email: emueller@science-societies.org

Care and Use of Animals

All authors submitting to JAS must complete the Care and Use of Animals form certifying that any re-search that involves animals has followed established standards for the humane care and use of animals and must specify which standards were used. Only investigations that have followed high standards for the humane care and use of animals in research will be reported in JAS. The manuscript must include a statement of institutional animal care and use committee (IACUC) (or equivalent) approval of all animal procedures.

The IA-CUC statement should appear as the first item in the Materials and Methods. The manuscript should discuss anesthetics, analgesics, tranquilizers, and care taken to minimize pain and discomfort during preoperative, operative, and postoperative procedures. If research requires discomfort to the animals or stressful conditions, justification for these conditions must be evident in papers published in JAS.

Types of Articles

Articles published in JAS encompass a broad range of research topics in animal production and fundamental aspects of genetics, nutrition, physiology, and preparation and utilization of animal products. Articles typically report research with beef cattle, companion animals, goats, horses, pigs, and sheep; however, studies involving other farm animals, aquatic and wildlife species, and laboratory animal species that address fundamental questions related to the biology of live -stock, companion animals, and other managed animals will be considered for publication. Manuscripts that report research on production issues in animals other than those constituting the main focus of the journal should be submitted to other journals. The preceding paragraph is not meant to exclude manuscripts but, rather, is a clarification of the focus of the journal. If there are any questions concerning the appropriateness of a manuscript for the journal, please contact the editor-in-chief. Research Articles. Results of work contained in manuscripts submitted to JAS must not have been published or submitted previously in a refereed scientific journal. Previous presentation at a scientific meeting or the use of data in field day reports or similar documents, including press publications or postings to personal or departmental websites, does not preclude the publication of such data in JAS. Articles simultaneously posted to websites and submitted to JAS should carry a disclaimer on the website that this version of the paper has not undergone JAS peer-review and is not to be considered the final published form of the article. If the article is published in JAS, the author should post the PDF (reprint) version of the article to the website so proper credit can be given to JAS as the publisher of the article. Because JAS holds the copyright to articles it publishes, posting altered JAS articles that are represented as exact duplicates of the published version constitutes copyright violation.

Review Articles. The journal publishes board-invited review articles each year; these reviews are identified by the editor-in-chief in consultation with the editors. Occasionally proposals for review articles to be published in JAS may be solicited by division editors, after consultation with the editor-in-chief; the authors will be responsible for publication charges for these articles. Unsolicited review articles will not be considered.

Special Topics. Papers will be considered for publication in this division that present Biographical or Historical Sketches, or that present viewpoints dealing with Contemporary Issues or Teaching in the animal sciences, or Perspectives that put a particular current topic into context in terms of its relationship or important to an entire area. Biographies and Histories are part of the Special Topics Division but will be published on the ASAS web-site (http://www.asas.org/pub_biohist.asp) as well as in the Association News section of the journal. The frequency of publication depends on the availability of the prepared sketches. See:

http://jas.fass.org/misc/JAS_Bigraphical_Sketch_Instruct.pdf for more information.

Contemporary Issues include topics such as environ-mental concerns, legislative proposals, systems analysis, and others. Teaching papers may discuss innovative pedagogical methods, philosophy of education, or solutions to teaching problems in animal science. Al-though Contemporary Issues or Teaching papers do not have to include original data, whenever appropriate the stated assertions should be substantiated by references to established information from credible published sources. Special Topics papers will be subject to peer review in a manner similar to other submissions. Because of the nature of these papers, their format may vary from that of standard scientific articles.

Technical Notes. A technical note is a vehicle to report a new method, technique, or procedure of interest to JAS readers. When possible, a technical note should include a comparison of results from the new method with those from previous methods, using appropriate statistical tests. The advantages and disadvantages of the new procedure should be discussed. When typeset, a technical note shall not exceed 6 pages (9 typed manuscript pages), including tables and figures. The words “Technical note” shall be the first words of the title of such manuscripts. The review process for a technical note will be the same as that for other manuscripts.

Letters to the Editor. Letters judged suitable for publication will be printed in a “Letters to the Editor” section of JAS. The purpose of this section is to provide a forum for scientific exchange relating to matters published in JAS. To be acceptable for publication, letters must adhere to the following guidelines: 1) only letters that address matters of science and relate to information published in JAS will be considered. In general, letters should not exceed 5,000 characters plus spaces and should contain no more than 5 citations; 2) Let-ters should provide supporting evidence based on published data for the points made or must develop logical scientific hypotheses; letters based on conjecture or on unsubstantiated claims will not normally be published. No new data may be presented in the letters; 3) Letters will be considered by the editor-in-chief and if deemed appropriate for publication, the author(s) of original paper(s) will be invited to write a letter of response. Normally both letters will be published together; and 4) All letters will be subject to acceptance and editing by the editor-in-chief and editing by the technical editor.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be submitted electronically at http://mc.manuscriptcentral.com/jas. Authors who have questions about using the electronic manuscript submission system should contact Brett Holte, Submission Services Manager at bholte@sciencesocieties.org. Authors who are unable to submit electronically should contact Brett Holte (bholte@sciencesocieties.org) for assistance; include your manuscript as an attachment (saved as a Microsoft Word file). Staff at ASAS head -quarters will post manuscripts by proxy, but authors should be aware that delays might occur in the review process.

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REVIEW OF MANUSCRIPTS

General Procedures. The suitability of all manuscripts for publication in JAS is judged by the re-viewers and associate editors, division editors, and the editor-in-chief. All communications regarding a submitted manuscript should maintain confidentiality. Associate editors handle correspondence with the author and promptly advise the division editor whether a manuscript should be rejected or accepted. The division editor's decision to reject or accept is based on the associate editor's recommendation and his or her own review of the manuscript. The division editor forwards document files for accepted manuscripts to the editor-in-chief for further review and editing, after which the editor-in-chief forwards the document file(s) to the technical editors. Note that most manuscripts that are eventually published are first returned by the associate editor to the author for re-vision, and in addition, the division editor may ask for changes before acceptance. The editor-in-chief is the final arbiter regarding acceptance or rejection of manuscripts submitted for publication.

Rejections. There are 3 main grounds for rejection of manuscripts. First, manuscripts that are not written clearly, concisely, and coherently or that do not conform to JAS style and form guidelines will be rejected without review. Authors whose first language is not English are urged to have their paper reviewed by an editing service. Second, the substance of the manuscript may not meet JAS standards: the work may be incomplete, the evidence may not support the conclusions, the experimental approach may be poorly conceived, or the work may repeat established fact or represent no advance of existing knowledge. Third, al-though the work may be sound and the results valid, the paper may be better suited for publication else-where.

Appeals. If a manuscript is rejected, as a first course of action the author may discuss the matter with the associate editor or division editor responsible for the manuscript. Decisions must be appealed to the editor-in-chief if the author(s) believe(s) that the judgment was erroneous or unfair. A letter presenting the reasons for the appeal should be sent to the editor-in-chief. The editor-in-chief will review the author's reasons, as well as all materials related to the manuscript and, after consulting with the editors who reviewed the manuscript, will render a decision whether to accept or deny the appeal. A rejected manuscript may be resubmitted for publication in another division of JAS only if this course of action has been specifically recommended by the

associate editor or division editor originally assigned to the manuscript and the transfer has been approved by the editor-in-chief.

Revisions. Most manuscripts that are eventually published are returned to the author(s) for revision. Normally, the revised manuscript must be returned to the associate editor via JAS Manuscript Central within 6 weeks from the date of receipt by the author or the manuscript will be withdrawn. Extenuating circumstances must be communicated to the technical editing staff, who will consult with the editor-in-chief before granting an extension. A Revision Checklist (http://jas.fass.org/misc/ifora.shtml) is sent with re-quests for revision to assist the authors.

PAPERS IN PRESS, AUTHOR PROOFS, AND PUBLICATION CHARGES

Papers in Press. To facilitate earlier dissemination, accepted manuscripts will be assigned a digital object identifier (DOI) and posted to the JAS Papers in Press site (http://jas.fass.org/papbyrecent.dtl) in the form in which they are accepted; because this does not represent the final, published form of the manuscript, the authors bear the primary responsibility for the content of manuscripts posted to the publish-ahead-of-print site.

Author Proofs. Accepted manuscripts are forwarded by the editor-in-chief to the editorial office for technical editing and typesetting. At this point, the technical editor may contact the authors for missing information or figure revisions. The manuscript is then type-set, figures reproduced, and author proofs prepared. Correspondence concerning the accepted manuscript should be directed to the technical editor. Proofs of all manuscripts will be provided to the corresponding author and should be read carefully and checked against the typed manuscript; accuracy of the galley proof is the author's responsibility. Corrections may be returned by fax, mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive correction is required, changes should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Editor queries should be answered on the galley proofs; failure to do so may delay publication. Excessive author changes made at the proof stage may result in a $250 surcharge.

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STANDARD JAS ABBREVIATIONS (return to Style and Form)

The following abbreviations should be used with-out definition in JAS; plural abbreviations do not require a final “s”. Use of 3-letter abbreviations for amino acids (e.g., Ala) and use of standard abbreviations for elements (e.g., S) are acceptable in JAS. For chemical units and abbreviations, refer to the ACS Style Guide (published by the American Chemical Society, Washington, DC).

Physical units

Item	Unit
Bq	becquerel
°C	degree Celsius
cal	calorie
Ci	curie
cM	centimorgan (spell out morgan if used with-out a prefix)
Da	dalton
Eq	equivalent (only can be used with a prefix)
g	gram
ha	hectare
Hz	hertz
IU	international unit
J	joule
L	liter
lx	lux
m	meter
M	molar (concentration; preferred over mol/L)
mol	mole
N	Normal (concentration)
Pa	pascal
rpm	revolutions/minute (not to be used to indicate centrifugal force)
t	metric ton (1,000 kg)
V	volt
W	watt

Units of time

Item	Unit
s	second(s)
min	minute(s)
h	hour(s)
d	day(s)
wk	week(s)
mo	month(s)
yr	year(s)

Statistical symbols and abbreviations

Item	Unit
ANOVA	analysis of variance
CV	coefficient of variation
df	degree(s) of freedom (spell out if used without units)
F	F-distribution (variance ratio)
LSD	least significant difference
n	sample size (used arenthetically or in footnotes)
P	probability
r	simple correlation coefficient
r^2	simple coefficient of determination
R	multiple correlation coefficient
R^2	multiple coefficient of determination
s^2	variance (sample)
SD	standard deviation (sample)
SE	standard error
SED	standard error of the differences of means
SEM	standard error of the mean
t t -	(or Student) distribution
α	probability of Type I error
β	probability of Type II error
μ	mean (population)
σ	standard deviation (population)
σ^2	variance (population)
χ^2	chi-squared distribution

Others

Item	Unit
AA	amino acid(s)
ACTH	adrenocorticotropic hormone
ADF	unless designated otherwise)
ADFI	average daily feed intake (not to be con-fused with DMI)
ADG	average daily gain
ADIN	acid detergent insoluble nitrogen
ADL	acid detergent lignin
ADP	adenosine diphosphate
AI	artificial insemination
AIA	acid insoluble ash
ARS	Agricultural Research Service
ATP	adenosine triphosphate
avg	average (use only in tables, not in the text)
BCS	body condition score

BLUE	best linear unbiased estimate
BLUP	best linear unbiased prediction
bp	base pair
BSA	bovine serum albumin
BTA	Bos taurus chromosome
BW	body weight (used for live weight)
cDNA	complementary deoxyribonucleic acid
C/EBP	CAAT-enhancer binding protein
cfu	colony-forming unit
CIE	International Commission on Illumination (Commission Internationale d'Eclairage)
CLA	conjugated linoleic acid
CoA	coenzyme A
Co-	cobalt
EDTA	ethylenediaminetetraacetate
CP	crude protein (N × 6.25)
D	dextro-diam. diameter
DE	digestible energy
DEAE	(dimethylamino)ethyl (as in DEAE-cellu -lose)
DFD	dark, firm, and dry (meat)
DM	dry matter
DMI	dry matter intake
DNA	deoxyribonucleic acid
EBV	estimated breeding value(s)
eCG	equine chorionic gonadotropin
EDTA	ethylenediaminetetraacetic acid
EFA	essential fatty acid
EIA	enzymeimmunoassay
ELISA	enzyme-linked immunosorbent assay
EPD	expected progeny difference(s)
Eq.	Equation(s)
Exp.	experiment (always followed by a numeral)
FFA	free fatty acid(s)
FSH	follicle-stimulating hormone
g	gravity
GE	gross energy
G:F	gain-to-feed ratio
GLC	gas-liquid chromatography
GLM	general linear model
GnRH	gonadotropin-releasing hormone
GH	growth hormone
GHRH	growth hormone-releasing hormone
hCG	human chorionic gonadotropin
HCW	hot carcass weight
HEPES	N -(2-hydroxyethyl)piperazine-N ′-2-ethanesulfonic acid
HPLC	high-performance (pressure) liquid chromatography
i.d.	inside diameter
Ig	immunoglobulin (when used to identify a specific immunoglobulin)
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor-binding protein(s)
IL	interleukin
IVDMD	in vitro dry matter disappearance
kb	kilobase(s)
KPH	kidney, pelvic, heart fat
l	levo-
LD50	lethal dose 50%
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
LM	longissimus muscle
ME	metabolizable energy
MP	metabolizable protein
mRNA	messenger ribonucleic acid
MUFA	monounsaturated fatty acid
NAD	nicotinamide adenine dinucleotide
NADH	reduced form of NAD
NDF	neutral detergent fiber
NDIN	neutral detergent insoluble nitrogen
NE	net energy
NEg	net energy for gain
NEl	net energy for lactation
NEm	net energy for maintenance
NEFA	nonesterified fatty acid
No.	number (use only in tables, not in the text)
NPN	nonprotein nitrogen
NRC	National Research Council
o.d.	outside diameter
OM	organic matter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PG	prostaglandin
PMSG	pregnant mare's serum gonadotropin
PPAR	peroxisome proliferator-activated receptor
PSE	pale, soft, and exudative (meat)
PUFA	polyunsaturated fatty acid(s)
QTL	quantitative trait locus (loci)
RDP	ruminally degradable protein
REML	restricted maximum likelihood
RFLP	restriction fragment length polymor -phism
RIA	radioimmunoassay
RNA	ribonucleic acid
RQ	respiratory quotient
RUP	ruminally undegradable protein
rRNA	ribosomal ribonucleic acid
SAS	Statistical Analysis System
SDS	sodium dodecyl sulfate

SFA	saturated fatty acid
SNP	single nucleotide polymorphism
spp.	species
ssp.	subspecies
SSC	Sus scrofa chromosome
ST	somatotropin
TDN	total digestible nutrients
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)aminomethan e
tRNA	transfer ribonucleic acid
TSAA	total sulfur amino acids
USDA	US Department of Agriculture
UV	ultraviolet
VFA	volatile fatty acid(s)
vol	volume
vol/vol	volume/volume (used only in parenthe-ses)
vs.	versus
wt	weight (use only in tables, not in the text)
wt/vol	weight/volume (used only in parenthe-ses)
wt/wt	weight/weight (used only in parenthe-ses)

LITERATURE CITED GUIDELINES FOR JOURNAL OF ANIMAL SCIENCE

Citations in the Text.

In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence struc-ture requires that the authors' names be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993). When there are more than 2 authors of an article, the first author's name is followed by the abbreviation et al. More than 1 article listed in the same sentence or parentheses must be in chronological order first and alphabetical order for 2 publications in the same year. Published
articles, and not abstracts, should be cited whenever possible; if the work was originally described in an ab-stract, the author(s) should use a literature search to determine if the work has been published as a peer-reviewed article.
Work that has not been accepted for publication shall be listed in the text as "J. E. Jones (institution, city, and state or country, personal communication)." The author's own unpublished work should be listed in the text as "(J. Smith, unpublished data)." Person-al communications and unpublished data must not be included in the Literature Cited section.

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To be listed in the Litera-ture Cited section, papers must be published or ac-cepted for publication ("in press"). In the Literature Cited section, references are listed alphabetically by the author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, 2 or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters after the date. All authors' names must appear in the Lit -erature Cited section. Journals shall be abbreviated according to the conventional ISO abbreviations used by PubMed (http://www.ncbi.nlm.nih.gov/entrez/que-ry.fcgi ; then, under "More Resources," find "Journals in NCBI Databases," and enter the journal title in the search box. A list of standard abbreviations for frequently cited journals and abbreviations used in citations is available at http://www.asas.org/jas/jour -nal_abbrevs.pdf. One-word titles must be spelled out.
Inclusive page numbers must be provided. Sample references are as follows:

1. Books and articles within edited books:

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

NRC. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Robinson, P. H., E. K. Okine, and J. J. Kennelly. 1992. Measurement of protein digestion in ruminants. Page 121 in Modern Methods in Protein Nutri - tion and Metabolism. S. Nissen, ed. Academic Press, San Diego, CA.

2. Handbooks, technical bulletins, theses, and dissertations

Goering, H. K., and P. J. Van Soest. 1970. Forage Fi-ber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Sigma. 1984. Total hemoglobin: Quantitative, colori-metric determination in whole blood at 530–550 nm. Tech. Bull. No. 525. rev. ed. Sigma Chemi - cal, St. Louis, MO.

Ward, J. D. 1995. Effects of copper deficiency on per-formance and immune function of cattle. PhD Diss. North Carolina State Univ., Raleigh.

3. Journal articles and abstracts

Cleale, R. M., IV, R. A. Britton, T. J. Klopfenstein, M. L. Bauer, D. L. Harmon, and L. D. Satterlee. 1987a. Induced non-enzymatic browning of soy - bean meal. II. Ruminal escape and net portal ab -sorption of soybean protein treated with xylose. J. Anim. Sci. 65:1319–1326.

Hall, J. B., R. B. Staigmiller, R. E. Short, R. A. Bel-lows, S. E. Bartlett, and D. A. Phelps. 1993. Body composition at puberty in beef heifers as influenced by nutrition and breed. J. Anim. Sci. 71(Suppl. 1):205. (Abstr.)

4. Conference proceedings

NMC. 1995. Summary of peer-reviewed publications on efficacy of premilking and postmilking teat disinfections published since 1980. Pages 82–92 in Natl. Mastitis Counc. Reg. Meet. Proc., Har -risburg, PA. Natl. Mastitis Counc., Arlington, VA.

Talmant, A., X. Fernandez, P. Sellier, and G. Monin. 1989. Glycolytic potential in longissimus dorsi muscle of Large White pigs as measured after *in vivo* sampling. Page 1129 in Proc. 35th Int. Con -gr. Meat Sci. Technol., Copenhagen, Denmark.

Van der Werf, J. H. J. 1990. A note on the use of conditional models to estimate additive genetic variance in selected populations. Proc. 4th World Congr. Genet. Appl. Livest. Prod., Edinburgh, Scotland XIII:476–479.

5. Electronic Publications

FDA. 2001. Effect of the use of antimicrobials in food-producing animals on pathogen load: Systematic review of the published literature. Accessed Dec**.** 14, 2001.http://www.fda.gov/cvm/antimicrobial/PathRpt.PDF.

Huntington, G. B., D. L. Harmon, N. B. Kristensen, K. C. Hanson, and J. W. Spears. 2006. Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. Anim. Feed Sci. Technol. 130:225–241. doi: 10.1016/j.anifeedsci.2006.01.012

Le Neindre, P., C. Terlouw, X. Boivin, A. Boissy, and J. Lensink. 2001. Behavioral research and its application to livestock transport and policy: A European perspective. J. Anim. Sci. 79(E-Suppl.) Accessed Oct. 7, 2001. http://www.asas.org/jas/jas0905.pdf .

Policies regarding number usage for journal of animal science

In 2006, JAS adopted the proposed changes for number style by the Council of Science Editors for the seventh edition of their Scientific Style and Format. The greatest change is more widespread use of numerals for single-digit numbers. A full description of the new number style is available in Scientific Style and Format. A summary of the CSE number style policies is as follows:

- All cardinal numbers are written as numerals except when they begin a sentence or appear in a title, when 2 numerals are adjacent in a sentence (spell out the number most easily expressed in words; e.g., two 10-kg samples), or when a number is used as a figure of speech.
- Numbers less than 1 are written with a preceding (leading) zero (e.g., 0.75).
- A comma separator is used in numbers greater than 999.
- Numerals should be used to designate ratios and multiplication factors (e.g., 2:1, 3-fold increase).
- If a number is spelled out at the beginning of a sentence, its associated unit is also spelled out (e.g., Ten milliliters of fluid . . ., not Ten mL of fluid . . .).
- Units of measurement not associated with a number should be spelled out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically.
- Single-digit ordinals are spelled out (i.e., first through ninth); larger ordinals are expressed in numeric form. Single-digit ordinals may be expressed numerically when they form part of a series (e.g., 1st, 3rd, 10th, 20th, not first, third, 10th, 20th).

General number usage policies of JAS are as follows:

- Measures must be presented in the metric sys-tem (SI or Système International d'Unités; see: http://physics.nist.gov/cuu/Units/introduction. html, or http://physics.nist.gov/Pubs/SP330/sp330.pdf .
- When a term must be expressed in nonmetric units for clarity (e.g., bushel weight), give such values in parentheses after the metric value.
- Use "to" instead of a hyphen to indicate a nu-merical range in text.
- Avoid the use of multiplying factors (e.g., × 106) in table columns or rows, or in figure axis labels because of the uncertainty whether the data are to be, or already have been, converted by the factor. • Avoid ambiguity by stating units (e.g., num-bers of spermatozoa, millions/mL).
- Do not use more than one slant line (for "per") in a single expression (e.g., use 5 mg/(g · d) or 5 mg·g–1 · d–1 instead of 5 mg/g/d). Mathemati -cally, "per" implies division; when 2 "per" occur consecutively, it is unclear precisely what is being divided by what.
- Dietary energy may be expressed in calories or in joules; the standard SI unit for energy is the joule.
- Hyphenate units of measure used as preceding adjectives (e.g., 5-kg sample). Hyphens are not used with percent or degree signs.
- Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <, etc.) when these signs occur between 2 values.
- Convert "mg %" to other units, such as mg/L or mg/mL; use "mol/100 mol" rather than "molar percent."

APENDICE 2 – Análise de SAS Proc. Mixed referentes ao capítulo 2:

Dry Matter Intake (DMI, g/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.97	1.95	0.1727
Treat	2	10.2	1.03	0.3925
Measure	3	28.9	23.07	<.0001
Treat*Measure	6	28.9	2.48	0.0465

Dry Matter Intake in g/ kg Body Weight (DMI, g/kg BW)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.87	3.63	0.0401
Treat	2	9.93	1.36	0.3014
Measure	3	29.5	4.63	0.0091
Treat*Measure	6	29.5	2.56	0.0409

Dry Matter Intake g/kg Metabolic Body Weight (DMI, g/kg $BW^{0.75}$)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.92	1.43	0.2948
Treat	2	10	1.30	0.3137
Measure	3	29.4	3.72	0.0222
Treat*Measure	6	29.4	2.62	0.0369

Average Daily Gain (ADG, g)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.9	1.31	0.3338
Treat	2	10.1	0.36	0.7072

Feed Efficiency

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.7	2.26	0.1296
Treat	2	9.91	0.18	0.8386

Final Body Weight (FBW, kg)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8.58	0.48	0.7835
Treat	2	8.82	0.23	0.8005

Methane emission Liter/Day (CH_4, L/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.85	0.16	0.9702
Treat	2	9.9	4.14	0.0493

Methane emission Liter/kg Dry Matter Intake (CH_4, L/kg DMI)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.67	0.38	0.8536
Treat	2	9.65	6.01	0.0202

Methane emission Liter/kg Metabolic Body Weight (CH_4, L/kg $BW^{0.75}$)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.85	0.56	0.7319
Treat	2	9.92	3.93	0.0552

Blood Hamoglobin concentration (Hb, g/100 mL)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.98	2.05	0.1570
Treat	2	10.1	0.65	0.5443
Measure	3	29.2	12.35	<.0001
Treat*Measure	6	29.2	2.44	0.0492

Red blood cells concentration (RBCs, 10^6/mL)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.61	2.02	0.1644
Treat	2	9.87	24.60	0.0001

Blood Methamoglobin concentration (MetHb, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.14	0.9797
Treat	2	10	3.20	0.0840

Blood Nitrate concentration (mM)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.95	1.12	0.4074
Treat	2	10	0.83	0.4632

Blood Nitrite concentration (µM)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.96	0.47	0.7929
Treat	2	10.8	3.89	0.0534

Ruminal pH

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.93	0.36	0.8656
Treat	2	9.97	0.09	0.9185

Ruminal ammonia concentration (NH_3, mg/100 ml)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.95	0.74	0.6105
Treat	2	9.96	526.19	<.0001

Total rumen Protozoa ($X10^5$/mL)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.61	1.27	0.3498
Treat	2	9.63	14.22	0.0013

Ruminal Total volatile fatty acids (Total-VFA, m*M*)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.21	1.39	0.3128
Treat	2	9.14	22.04	0.0003

Rumen acetate concentration (C2, m*M*)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.5	0.50	0.7722
Treat	2	9.61	33.44	<.0001

Rumen propionate concentration (C3, m*M*)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	19	2.79	0.0471
Treat	2	18.3	3.72	0.0442

Acetate:propionate ratio (C2:C3)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	13.3	2.61	0.0749
Treat	2	12.5	0.06	0.9405

Rumen butyrate concentration (C4, m*M*)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	19.1	1.34	0.2914
Treat	2	19.6	9.43	0.0014

Rumen valerate concentration (C5, m*M*)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.9	2.46	0.1068
Treat	2	10.1	0.17	0.8478

Rumen iso-butyrate concentration (iso-C4, m*M*)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	23.7	0.50	0.7742
Treat	2	26.2	1.94	0.1639

Rumen iso-valerate concentration (iso-C5, m*M*)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	21.7	2.01	0.1172
Treat	2	23	1.81	0.1860

Ruminal Nitrite concentration (µM)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	19.6	0.67	0.6519
Treat	2	19.3	14.69	0.0001

APENDICE 3 – Análise de SAS Proc. Mixed referentes ao capítulo 3:

Dry matter Digestibility (DMD, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.98	0.4775
Treat	2	10	0.13	0.8814

Organic matter Digestibility (OMD, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.97	0.4814
Treat	2	10	0.07	0.9326

Crude protein Digestibility (CPD, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	1.05	0.4422
Treat	2	10	0.75	0.4970

Neutral Detergent Fiber Digestibility (FDND, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	1.65	0.2349
Treat	2	10	0.05	0.9552

Acid Detergent Fiber Digestibility (ADFD, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	9.50	0.0015
Treat	2	10	1.34	0.3061

Ether Extract Digestibility (EED)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	1.65	0.2344
Treat	2	10	2.35	0.1453

Digestible Organic Matter Intake (DOMI, kg/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.99	0.4706
Treat	2	10	0.75	0.4984

Nitrogen intake (N-intake, g/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.94	0.4945
Treat	2	10	1.10	0.3696

Nitrogen in feaces (Feces-N, g/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	1.63	0.2395
Treat	2	10	1.66	0.2389

Urinary Nitrogen (Urine-N, g/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.32	0.8875
Treat	2	10	0.54	0.5965

Urinary urea Nitrogen (g/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	2.42	0.1100
Treat	2	10	7.41	0.0106

Urinary Nitrate concentration (mM)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	1.80	0.2000
Treat	2	10	3.90	0.0559

Urinary Nitrite concentration (µM)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	3.88	0.0325
Treat	2	10	25.08	0.0001

Retained Nitrogen (N-balance, g/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.24	0.9371
Treat	2	10	0.74	0.5022

Retained-N g/kg N-intake

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.19	0.9601
Treat	2	10	0.81	0.4717

Retained-N g/kg N-absorbed

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.18	0.9647
Treat	2	10	0.55	0.5917

Urinary allantoin concentration (mmol/day)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.16	0.9719
Treat	2	10	0.60	0.5653

Urinary uric acid concentration (mmol/day)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	1.11	0.4132
Treat	2	10	1.09	0.3743

Urinary Xanthine and Hypothanthine concentration (mmol/day)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	1.48	0.2780
Treat	2	10	2.54	0.1278

Total Purine derivatives excreted (mmol/day)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.19	0.9599
Treat	2	10	0.32	0.7326

Urinary Creatinine concentration (mmol/day)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	2.15	0.1423
Treat	2	10	0.08	0.9238

Microbial Purine Derivatives Absorbed (mmol/day)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.34	0.8792
Treat	2	10	0.34	0.7221

Microbial Nitrogen supply (MNS, g/d)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.34	0.8767
Treat	2	10	0.33	0.7277

Microbial Nitrogen supply (MNS, g/kg DOMI)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	10	0.99	0.4705
Treat	2	10	0.75	0.4984

Total Protein (g/100 mL)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.95	0.41	0.8300
Treat	2	9.94	1.16	0.3521

Albumin (g/100 mL)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	14	0.86	0.5313
Treat	2	13.5	0.34	0.7171

Blood Urea (mg/100 mL)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.98	0.89	0.5251
Treat	2	10.1	2.20	0.1607

Aspartate amino-transferase (AST, IU/L)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.97	7.06	0.0046
Treat	2	10.4	2.85	0.1029

Alanine amino-transferase (ALT, IU/L)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9.28	1.89	0.1890
Treat	2	9.65	2.91	0.1022

Slaughter live Body Weight (SLBW, kg)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8	4.99	0.0228
Treat	2	8	0.08	0.9256

Hot carcass weight (HCW, kg)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8	6.39	0.0112
Treat	2	8	0.22	0.8110

Cold carcass weight (CCW, kg)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8	6.36	0.0113
Treat	2	8	0.19	0.8270

Hot carcass yield (HCY, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8	3.37	0.0620
Treat	2	8	2.42	0.1506

Cold carcass yield (CCY, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8	3.09	0.0760
Treat	2	8	2.27	0.1654

Chilling weight loss (CWL, %)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8	1.11	0.4244
Treat	2	8	1.56	0.2687

Rib eye area, (cm^2)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	0.14	0.9773
Treat	2	9	0.25	0.7850

Fat thickness, (mm)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	8	1.71	0.2372
Treat	2	8	1.75	0.2346

Meat pH at slaughter

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	1.18	0.3892
Treat	2	9	0.52	0.6114

Meat pH after chilling

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	3.11	0.0664
Treat	2	9	2.05	0.1841

Muscles instrumental color (Lightness, *L**)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	1.87	0.1946
Treat	2	9	0.19	0.8319

Muscles instrumental color (Redness, *a**)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	7.59	0.0047
Treat	2	9	0.98	0.4134

Muscles instrumental color (Yellowness, *b**)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	1.83	0.2036
Treat	2	9	0.17	0.8464

Meat Residual-Sodium nitrate (mg/kg fresh meat)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	0.44	0.8136
Treat	2	9	0.48	0.6314

Rib weight (g)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	1.60	0.2552
Treat	2	9	0.06	0.9398

Muscle weight (g)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	1.18	0.3908
Treat	2	9	0.08	0.9231

Fat weight (g)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	0.98	0.4783
Treat	2	9	0.03	0.9735

Bone weight (g)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	1.50	0.2815
Treat	2	9	0.37	0.7025

Muscle (%)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	0.43	0.8159
Treat	2	9	0.12	0.8853

Fat (%)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	0.31	0.8930
Treat	2	9	0.43	0.6658

Bones (%)

Effect	Num DF	Den DF	F Value	Pr > F
Block	5	9	0.37	0.8541
Treat	2	9	1.32	0.3152

6. VITA

Hani Mohammed Mohammed El-Zaiat, filho de Mohammed Mohammed El-zaiat e Mariam Mahmoud Mohammed Younis, nascido em 09 de Janeiro de 1983, em Behera – Egito.

Estudou no colégio Mahalet Al-amir, onde completou o 1° grau em 1997, no mesmo ano ingressou o 2° grau no colégio Rashid finalizando em 2000. No ano de 2000 ingressou no curso de Agronomia da Universidade de Alexandria, onde em 2004 se formou em Ciência Agrícola na Produção Animal. Em 2005 começou a trabalhar no departamento de Produção Animal e ingressou no curso de Mestrado em Produção Animal sob a orientação do professor Abd El-Aziz M. Nour, onde em 2009 se formou.

Em Março 2009 começou a trabalhar como Assistant lecturer no departamento de Produção Animal, Faculdade da Agronomia na Universidade de Alexandria, Egito. Ingressou no curso de Doutorado em Zootecnia, Março de 2010, sob a orientação do professor Harold Ospina Patino no Programa de Pós-Graduação em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul.