UNIVERSIDADE FEDERAL DE GOIÁS
ESCOLA DE VETERINÁRIA E ZOOTECNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

NITRATO ENCAPSULADO EM SUBSTITUIÇÃO AO FARELO DE SOJA NA TERMINAÇÃO DE BOVINOS DE CORTE EM CONFINAMENTO

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Título: NITRATO ENCAPSULADO EM SUBSTITUIÇÃO AO FARELO DE SOJA NA TERMINAÇÃO DE BOVINOS DE CORTE EM CONFINAMENTO Palavras-chave: **Desempenho animal, metahemoglobinemia, nelore, proteína**

Título em outra língua: **Encapsulated nitrate replacing soybean meal on growth, eating behavior, and blood metabolites of feedlot finishing young bulls**

Palavras-chave em outra língua: **feedlot performance, methemoglobin, nitrite, non-protein nitrogen, slow-release nitrate**

Área de concentração: **Produção Animal** Data defesa: (dd/mm/aaaa) **09/06/2015**

Programa de Pós-Graduação:**Ciência Animal**

Orientador(a):**Juliano José de Resende Fernandes** E-mail:

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NITRATO ENCAPSULADO EM SUBSTITUIÇÃO AO FARELO DE SOJA NA TERMINAÇÃO DE BOVINOS DE CORTE EM CONFINAMENTO

Tese apresentada para obtenção do grau de Doutor em Ciência Animal junto à Escola de Veterinária e Zootecnia da Universidade Federal de Goiás

Área de Concentração:
Produção Animal

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GOIÂNIA
2015

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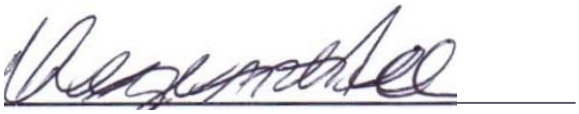
Pereira, Marcela Luzia Rodrigues Pereira
NITRATO ENCAPSULADO EM SUBSTITUIÇÃO AO FARELO DE SOJA NA TERMINAÇÃO DE BOVINOS DE CORTE EM CONFINAMENTO [manuscrito] / Marcela Luzia Rodrigues Pereira Pereira. - 2015.
XCI, 91 f.

Orientador: Prof. Dr. Juliano José de Resende Fernandes Fernandes; co-orientador Dr. Victor Rezende Moreira Couto Couto; co-orientador Dr. Flávio Augusto Portela Santos Santos.
Tese (Doutorado) - Universidade Federal de Goiás, Escola de Veterinária e Zootecnia (EVZ) , Programa de Pós-Graduação em Ciência Animal, Goiânia, 2015.
Bibliografia.
Inclui siglas, abreviaturas, gráfico, tabelas, lista de figuras, lista de tabelas.

1. Desempenho animal. 2. metahemoglobinemia. 3. nelore. 4. proteína. I. Fernandes, Juliano José de Resende Fernandes , orient. II. Couto, Victor Rezende Moreira Couto , co-orient. III. Título.

MARCELA LUZIA RODRIGUES PEREIRA

Tese defendida e aprovada em 09 de Junho de 2015, pela seguinte Banca Examinadora:



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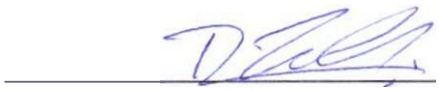

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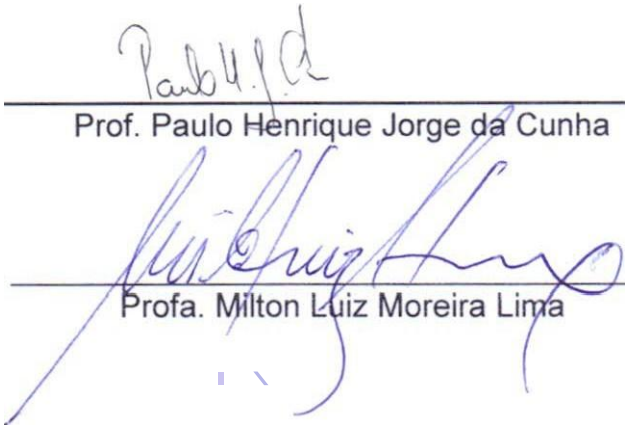

Prof. Paulo Henrique Jorge da Cunha

Profa. Milton Luiz Moreira Lima

Dedico:

Aos meus pais: Marcos e Valdete

Minhas irmãs: Kelem, Juliana e Arligeny

Meus sobrinhos: Beatriz, Pabline, Luana, Marcos Emilio, Kerolayne, Amanda e Ketly.

AGRADECIMENTOS

Agradeço a Deus, por ter me dado saúde, força e coragem para enfrentar os desafios encontrados, possibilitando a realização deste trabalho.

À minha mãe, Valdete pelo carinho, amor e orações para que tudo caminhasse no sentido certo. Ao meu pai, Marcos pelo apoio. As minhas irmãs, Kelem, Juliana e Arligeny pelo carinho e incentivo. As minhas sobrinhas e meu sobrinho, a galerinha mais animada da família, Beatriz, Pabline, Luana, Marcos Emílio, Kerolayne, Amanda e Ketly. Sei que nos momentos mais complicados vocês estavam na torcida para que tudo ficasse mais tranquilo e mais fácil. Desde o começo de tudo sempre me apoiaram em todas as decisões. Por isso, sei que vocês tiveram uma participação mais do que importante para a concretização desse trabalho.

Aos meus avós, Geny e Waldir (Didi), pessoas que respeito e amo demais, sempre cuidaram de mim com muito amor e carinho. Sinto tanta falta de quando morávamos juntos, vocês juntamente com minha mãe são responsáveis por tudo que sou hoje. Amo vocês!!

Ao meu namorado, Paulo Ricardo, pessoa extraordinária, companheiro e incentivador.

Ao meu orientador, professor Juliano José de Resende Fernandes, pela paciência e por ter aceitado me orientar.

Ao meu co-orientador Victor Rezende Moreira Couto pela grande ajuda, principalmente na condução do experimento. Foram dias de grande aprendizagem e agradeço isso tudo a você.

À minha colega e amiga Barbara, que merece toda dedicação deste trabalho, foi com ela que passei dias e dias estudando e aprendendo juntas.

À empresa GRASP pelo apoio e principalmente ao representante da empresa Rafael Canonenco. Muito obrigada pelos conselhos, pelas críticas nas horas necessárias e pela confiança. Sei que tudo isso serão válidos para o meu crescimento profissional.

À melhor veterinária e colega de trabalho Jordana, que ajudou a cuidar dos meus animais e na condução e conclusão do experimento.

Ao professor Emmanuel Arnold, pela inestimável contribuição neste trabalho e em minha formação.

Ao professor Paulo Henrique, pela ajuda na concretização do trabalho.

Aos membros da banca examinadora pela atenção e dedicação no exame desse trabalho.

Aos meus colegas, companheiros e amigos, Fabíola, Kíria, Rayanne, Flávia, Marcondes, Tiago (da Kíria), Leonardo, Neto, Thiago (Ceará), Adesvaldo, Sérgio e Alex, pela amizade e que de alguma forma contribuíram para realização deste trabalho.

Aos estagiários sem os quais não seria possível a realização deste trabalho, Lídia, Amanda, Bárbara, Edilaine, Alexandre, Paulo, Marionones, Débora, Malu, Gisele, Jéssica, Jordana, Kelvin e Gabriel. Obrigada pela dedicação e ajuda na condução do experimento.

Ao Lucas, meu parceiro fiel de todos os dias no confinamento.

Aos Funcionários Eder, Hélio, Gerson, Dito e Reginaldo, pelo auxílio sempre que precisei.

Aos professores do departamento de Produção Animal (DPA)

À Universidade Federal de Goiás pela oportunidade de realização do curso de Doutorado.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) pela concessão da bolsa de estudos.

Aos Animais do experimento!

Muito Obrigada!!!

“A vida é uma peça de teatro que não permite ensaios. Por isso, cante, chore, dance, ria e viva intensamente, antes que a cortina se feche e a peça termine sem aplausos...”

Charles Chaplin

SUMÁRIO

LISTA DE FIGURAS

CAPÍTULO 2

CAPITULO 3

LISTA DE TABELAS

CAPÍTULO 2

CAPÍTULO 3

LISTA DE SIGLAS E ABREVIATURAS

ADF- Acid detergent fiber

ADG- carcass daily gain

AGV- Ácidos graxos voláteis

AGVR- Ácidos graxos cadeia ramificada

ALP- Alkaline phosphatase

ANOVA - Analysis of variance

AST- Aspartate aminotransferase

ATP- Adenosine triphosphate

BW- Body weight

CH_4- Metano

CMS - Consumo de matéria seca

CTL- Control

DM- Dry matter

DMI- Dry matter Intake

EA- Eficiência alimentar

EE- Ether extract

ENP- Encapsulated nitrate product

GGT- Gamma-glutamyl transferase

GMD- Ganho em peso médio diário

Hb- Hemoglobin

IMS- Ingestão matéria seca

MetHb- Metemoglobina

MS- Matéria seca

N- Nitrogênio

NDF- Neutral detergent fiber

NDT - Nutrientes digestíveis totais

NFC- Non-fibrous Carbohydrates

NH_3- Amônia

N-NH_3- Nitrogênio amoniacal

NNP: Nitrogênio não proteico

NO_2^- Nitrito

NO_3^- Nitrato

NP: Nitrogênio proteico

NPN- Non-protein nitrogen

NRC - National Research Council

OM- Organic matter

PB - Proteína bruta

PC - Peso corporal

PCF- Peso corporal final

PCI- Peso corporal inicial

PCQ- Peso carcaça quente

PDR- Proteína degradável rúmen

PM- Proteína metabolizável

Pmic- Proteína microbiana

PNDR- Proteína não degradável rúmen

RC- Rendimento carcaça

SD- Standard deviation

SEM- Average standard error

TDN- Total digestible nutrients

RESUMO GERAL

Objetivou-se avaliar a inclusão crescente de nitrato encapsulado (ENP) como fonte de nitrogênio não proteico em substituição do farelo de soja sobre variáveis de digestibilidade, desempenho, resíduos de nitrato e nitrito na carne, variáveis de comportamento ingestivo, metemoglobina e os constituintes sanguíneos de novilhos terminados em confinamento. Cento e vinte novilhos não castrados (339,5 ± 28,3 kg) foram divididos em grupos de 6 animais, alojados em 20 baias e distribuídos em um delineamento em blocos casualizados com 5 blocos e 4 tratamentos: Controle (CTL) - farelo de soja; ENP-1 - 1% ENP (0,65% NO_3^-) na MS da dieta; ENP-2 - 2% ENP (1,30% NO_3^-); ENP-3-3% ENP (1,95% NO_3^-). As dietas foram compostas com relação 90:10 concentrado: forragem (bagaço de cana *in natura*). O experimento durou 84 dias. O consumo de matéria seca diminuiu linearmente (9,6-8,4 kg / dia; $P<0,05$) com a adição de quantidades crescentes de nitrato encapsulado na dieta dos novilhos. No entanto, rendimento de carcaça e espessura de gordura não foram influenciados ($P>0,05$) pelos tratamentos. Houve tendência quadrática (0,121, 0,130, 0,128, 0,127, respectivamente; P = 0,07) para ganho em carcaça. A concentração de nitratos na carne aumentou linearmente (3,48, 2,73, 3,98, 6,59 mg NO_3^- / kg de carne fresca; $P = 0,02$) com a adição ENP, embora nitrito (NO_2^-) não foi detectado. Houve uma tendência para a redução quadrática ($P=0,07$) no tempo por unidade de ingestão de matéria seca e redução quadrática ($P<0,01$) quando expressa numa base de ruminação de FDN. Efeito quadrático na mastigação foram observados por unidade de ingestão de matéria seca ($P=0,03$) e consumo de FDN ($P<0,01$). A concentração sanguínea de metahemoglobina aumentou de forma linear ($P<0,05$) com a adição crescente do produto nitrato encapsulado, mas não atingiu valores que caracterizam risco de intoxicação. Para as variáveis sanguíneas (creatinina, fosfatase alcalina, proteína total, albumina, e glicose) não obtiveram efeitos ($P>0,05$) para os tratamentos. Entretanto, houve um efeito quadrático ($P<0,01$) para triglicérideos no sangue, e aumento linear para o colesterol total ($P=0,05$), as variáveis ureia ($P<0,01$), aspartato aminotransferase ($P <0,01$) e gama-glutamil transferase ($P=0,04$) resultaram em efeito linear decrescente. A digestibilidade aparente de nutrientes teve efeito quadrático para todos os nutrientes com os níveis crescentes de nitrato encapsulado substituindo farelo de soja nas dietas. Em conclusão, o produto nitrato encapsulado não mostrou riscos de desenvolvimento da metahemoglobinemia até a dosagem de 3% e pode ser utilizado como fonte de nitrogênio em dietas para terminação de bovinos. O nitrato encapsulado é seguro e aplicável na alimentação de ruminantes.

Palavras chave: Desempenho animal, metahemoglobinemia, nelore, proteína

Introdução

Nas dietas de bovinos, a proteína verdadeira pode ser substituída parcialmente por nitrogênio não proteico (NNP). Fontes de NNP possuem nitrogênio (N) não na forma polipeptídica das proteínas. Durante o metabolismo ruminal de fontes de NNP ocorre a produção de nitrogênio amoniacal ($N\text{-}NH_3$) que será usado na síntese de proteína microbiana.

Umas das fontes de nitrogênio não proteico usada hoje para animais ruminantes, é o nitrato, Hulshof et al[1] e Van Zijderveld et al[2]. O sucesso do uso do nitrato se encontra firmemente na mitigação de metano, devido o mesmo servir como dreno de hidrogênio. Pois sabe-se que animais ruminantes, são contribuintes de fontes de emissão de metano (CH_4) devido ao processo digestivo de fermentação entérica. Além disso, quando ha produção de gás no processo fermentativo, ocorrem perdas de energia do alimento consumido, podendo acarretar em ineficiência produtiva.

Vários estudos já revelaram o potencial de uso do nitrato na alimentação de animais ruminantes visando desempenho, Lewis[3]; Hulshof et al[1]; Van Zijderveld et al[2] e El Zaiat et al[4]. No rúmen o nitrato é reduzido a nitrito e este em amônia, Lewis[3]. A amônia gerada é utilizada no anabolismo de aminoácidos das bactérias ruminais. Desta forma, o nitrato pode substituir parte da proteína da dieta.

Entretanto, ressalta-se o risco de ocorrência da metahemoglobinemia causada pela intoxicação pelo nitrato. Por isso, a adaptação inadequada da microbiota ruminal e os erros no manejo alimentar dos animais podem favorecer a ocorrência desse distúrbio.

A intoxicação acontece quando a redução do nitrato a nitrito ocorre mais rapidamente que a conversão do nitrito em amônia. O nitrito acumulado no rúmen é absorvido através da parede ruminal e cai na corrente sanguínea onde se ligará à hemoglobina e oxidará os íons ferro, formando a metahemoglobina, que é incapaz de transportar oxigênio para as células, impedindo a geração de energia na cadeia respiratória, Cockburn et al[5] e Bruning-Fann & Kaneene[6].

O encapsulamento do nitrato torna seguro seu uso, permitindo que seja liberado lentamente no rúmen. O encapsulamento, associado à inclusão gradual e adequada de nitrato na dieta, permite aumentar a atividade e a quantidade de microrganismos redutores de nitrito à amônia, evitando o risco de intoxicação. O encapsulamento também minimiza os efeitos da baixa palatabilidade, em virtude do forte sabor amargo Farra e Satter[7], e também da alta higroscopicidade do nitrato na forma pura.

Objetivou-se avaliar a inclusão crescente de nitrato encapsulado como fonte de nitrogênio não proteico em substituição do farelo de soja sobre variáveis de desempenho, resíduos de nitrato e nitrito na carne, variáveis de comportamento ingestivo, metemoglobina, constituintes sanguíneos, e digestibilidade de nutrientes de novilhos terminados em confinamento.

Capitulo 1: Considerações iniciais

1.1 Nitrogênio não proteico

O uso de nitrogênio não protéico (NNP) na nutrição dos ruminantes iniciou-se na Alemanha em 1879. A ureia, considerada um NNP, começou a ser fabricada industrialmente em 1870 sintetisada por Bassarow a partir do gás carbônico e da amônia. No período de 1914 a 1918, durante a primeira guerra mundial, a Alemanha intensificou a utilização da uréia como fonte protéica na alimentação de ruminantes visando uma produção intensiva e de baixo custo de carne e leite Santos[8].

O NNP está presente em animais e plantas e não possui união de aminoácidos por meio de vínculos peptídicos, portanto, não é uma proteína. A ureia é um dos NNP mais utilizados em virtude do seu custo, disponibilidade e emprego, apesar de existir uma grande variedade de compostos (biureto, ácido úrico, glicosídeos nitrogenados, alcalóides, compostos de purinas e pirimidinas, sais de amônio e nitratos) Santos[8].

De acordo com Gallo[9], a necessidade de maximizar a eficiência da síntese microbiana estimulou o desenvolvimento de técnicas para sincronizar a liberação de NNP com a degradação de carboidratos no rúmen. Dentre estas técnicas citam se a amiréia, proteção com gordura, proteção com biureto, ureia com cloreto de Ca e uréia encapsulada por polímero, desenvolvidas a partir da ureia.

Segundo Gonçalves[10], a substituição da ureia tradicional por uréia de liberação lenta melhorou a digestibilidade aparente da proteína bruta, porem, resultou em poucos efeitos sobre o padrão de fermentação ruminal em bovinos submetidos a dietas à base de forragem de baixa qualidade. Sendo que o aumento no teor de NNP, com base na PB dietética pode comprometer a eficiência de utilização dos nutrientes em bovinos alimentados com forragens.

Uma das fontes que podem substituir a ureia é o nitrato. Nitratos e nitritos (NO_2^-) estão presentes em alguns alimentos fornecidos aos ruminantes, principalmente em gramíneas forrageiras no início do estágio vegetativo fertilizadas com grandes quantidades de nitrogênio, Leng[11].

O nitrato pode ser usado na alimentação de ruminantes como fonte de NNP para os microrganismos. Quando o nitrato chega ao rúmem, é reduzido primeiramente a nitrito e depois convertido a amônia, Leng & Preston[12]. Uma das vantagens de usar o nitrato é a utilização da amônia gerada no anabolismo de aminoácidos das bactérias, podendo assim, substituir parte da proteína da dieta, Van Zijderveld et al[2].

Outro benefício da utilização do nitrato que tem sido amplamente estudado é sua capacidade de reduzir a produção de CH_4 através da utilização de rota alternativa de escoamento do H_2, Van Zijderveld et al[2]; Silivong et al[13]; Van Zijderveld et al[14]; Hulshof et al[1]; Inthapanya et al[15] e El-Zaiat et al[4].

Estudos com uso de nitrato encapsulado na dieta de bovinos têm-se, intensificado nos últimos anos. El-Zaiat et al[4] e Lee et al[16]. O encapsulamento do nitrato pode melhorar a sincronia no momento da redução do nitrato a nitrito e favorecer a capacidade de capturar o H_2 disponível. Com isso diminui a redução de emissões de metano e excreção de nitrogênio, sem causar efeitos prejudiciais à saúde e desempenho animal, El-Zaiat [17] e Farra e Satter[7].

1.2 Efeitos na fermentação ruminal

O rúmen é um habitat microbiano que consiste em interações de bactérias, fungos e protozoários anaeróbios. A eficiência desse ecossistema depende da manutenção de um baixo potencial redox para fermentação. As reações que estão envolvidas na degradação de carboidratos e de proteínas geram cofatores reduzidos, que, se não oxidado na fermentação pode inibir o consumo animal. Os microrganismos metanogênicos utilizam o hidrogênio para reduzir o dióxido de carbono a metano permitindo maior rendimento de ATP para a manutenção e crescimento celular. A microbiota ruminal também é capaz de utilizar aceitadores de elétrons de alta afinidade, como nitrato e sulfato, gerando amoníaco e sulfureto de hidrogênio, respectivamente, Leng & Preston[12].

O nitrato quando convertido a amônia, por microrganismos anaeróbicos, é altamente competitivo com a produção de metano, funcionando como dreno de elétrons por consumir oito elétrons no processo, sendo considerado um potente inibidor da metanogênese, Van Zijderveld et al[2]. De acordo com Beauchemin et al18 e Van Zijderveld et al[2], essa rota é a principal via de eliminação de hidrogênio durante a fermentação ruminal, quando houver nitrato suficiente no rúmen.

Na conversão de nitrato à amônia, inicialmente ocorre a redução do nitrato (NO_3^-) à nitrito (NO_2^-) (equação 1 - $\Delta G0$ = -130 kJ / mol de H_2) e, posteriormente, a redução de nitrito a amônia (NH_3) (equação 2 - $\Delta G0$ = -124 kJ / mol de H_2), essas reações produzem mais energia do que a conversão de dióxido de carbono e água a metano ($\Delta G0$ = -16,9 kJ / mol de H_2). Parte dessa energia liberada pela fermentação é utilizada na formação de ATP microbiano, e a outra parte é dissipada na forma de produção de calor, Van Zijderveld et al [2].

$$NO_3^- + 2H^+ \text{---> } H_2O + NO_2^- \quad (1)$$

$$NO_2^- + 6H^+ \text{---> } H_2O + NH^3 \quad (2)$$

A amônia que é produzida no rúmen fica disponível para o anabolismo de aminoácidos. A amônia que não é absorvida pelos microrganismos é, em grande parte, absorvida através do epitélio ruminal e entra na circulação portal, chegando ao fígado onde é convertida em ureia que volta em parte ao trato gastrointestinal via saliva ou transepitelial, e o restante é eliminado, Kozloski[19].

1.3 Influência do nitrato na mitigação de metano

O metano (CH_4) é um gás inodoro e incolor, sua molécula é um tetraedro apolar com pouca solubilidade em água, Vieira et al[20].

Informações de Lipman e Delucchi[21]; Berchielli et al[22] mostram que o CH_4 é caracterizado como um importante gás de efeito estufa, contribuindo com cerca de 15% para o aquecimento global e vem aumentando 0,9% ao ano.

A produção de metano em bovinos adultos pode variar de 250 a 500 l/dia, Lascano et al[23]. O metano é importante para o bom funcionamento ruminal, pois é responsável por "drenar'' o hidrogênio livre, Johnson & Johnson[44]. Entretanto, isso gera perdas de 2% a 15% de energia para o animal, Johnson et al[24].

O metano é produzido em condições anaeróbias por bactérias metanogênicas presentes no ambiente ruminal e intestinal (*Methanobrevibacter* spp., *Methanomicrobium* spp., *Methanosarcina* spp., *Methanobacterium* spp.), Lassey et al[25] e Arcuri et al[26]. O excesso de hidrogênio produzido durante a fermentação de carboidratos e proteínas para subsequente formação de ácidos graxos de cadeia curta (acetato e butirato) é utilizado para o crescimento microbiano, biohidrogenação de ácidos graxos insaturados e produção de ácido graxo glicogênico (propionato e valerato), o restante seria completamente utilizado para a produção de metano, Benchaar et al[27].

A produção de metano é modulada principalmente pela presença de dióxido de carbono e de hidrogênio livres no ambiente ruminal e, a partir do hidrogênio livre, ocorre redução do dióxido de carbono por microrganismos metanogênicos, com conseqüente formação de metano, El- Zaiat[17].

A fermentação pode variar de alta emissão de metano, caracterizado por uma alta relação acetato:propionato, a uma baixa emissão de metano, caracterizado por uma baixa relação acetato:propionato, Johnson & Johnson[29]. Segundo Van Soest[30] para síntese de acetato (1), propionato (2) e butirato (3) tem- se o seguinte:

Piruvato: $C_2H_4O_2 + CO_2 + 4H$ (1)

Piruvato + CO_2: fumarato + 2H = $C_3H_6O_2 + CO_2$ (Via succinato) (2)

Piruvato: lactato + H_2O = acrilato + 2H = $C_3H_6O_2$ (Via acrilato) (2)

Piruvato: $C_4H_8O_2 + CO_2 + 2H$ (3)

Com isso observa-se que a produção de ácidos graxos de cadeia curta (exceto propionato) pode levar a uma alta produção de hidrogênio, e consequentemente levar a altas emissões de metano. Estratégias alimentares devem existir para diminuir a produção de metano entérica, Martin et al[30], sendo que Van Zijderveld,et al[14], relataram que a persistência na redução de metano é uma exigência para qualquer estratégia dietética a ser bem sucedida em diminuir as emissões de gases do efeito estufa provenientes de ruminantes.

Com isso fontes de nitrato pode ser uma alternativa interessante, pois alem de drenar o hidrogênio livre no meio ruminal, diminui a emissões de metano e pode levar a uma melhora na eficiência alimentar. Diversas fontes de nitrato já foram avaliadas e se mostraram eficientes na redução da produção de metano ruminal. Como o nitrato de potássio com redução de 23% na produção de metano entérico/kg de MS consumida Nolan et al[30], nitrato de sódio e de amônia (produção de 5,9% e 9,4% de metano respectivamente *versus* 20,2% da ureia) Huyen et al[32]e nitrato de cálcio, Silivong et al[33]; Li et al[13].

Pesquisa realizada por Hulshof et al[1], de dostraram que os animais que consumiram 22 g de nitrato por kg de matéria seca, comparada a uréia, possuíam potencial de diminuir a produção de 5,7 g de metano/kg/CMS. A mitigação de metano obtida foi de 5,0 g de metano/kg de CMS, resultando numa eficiência de 87%. Baseado neste estudo os pesquisadores relataram que a produção de metano em kg de MS ingerida foi 27% menor quando o nitrato foi adicionado na dieta.

Van Zijderveld et al[2] notaram redução na produção de metano de até 32% em dietas com o fornecimento de nitrato de cálcio (2,6% da MS) quando comparadas ao fornecimento de ureia (1,5% da MS). Resultados semelhantes foram obtidos por Inthapanya et al[15] em experimento avaliando a produção *in vitro* de metano, em que o nitrato de cálcio (5,5% da

MS) ou ureia (2% da MS) foram adicionados as dietas a base de mandioca (raiz e folhas). Os autores observaram redução de 32% na produção de metano com o uso de nitrato de cálcio como fonte de NNP.

No trabalho desenvolvido por Newbold et al[33] foi avaliada a influência de níveis de nitrato na dieta de trinta e seis bovinos da raça Holandês sobre a emissão de metano. Foram utilizados seis níveis de inclusão do nitrato (0,00; 0,60; 1,20; 1,80; 2,40 e 3,00% na matéria seca) e observado que a produção de metano diminuiu linearmente em até 49% conforme foi adicionado o nitrato.

Reduções de emissões de metano com uso de nitrato encapsulado foram comprovadas por El-Zaiat et al[4], substituindo a ureia na dieta de ovinos. Os tratamentos foram: grupo 1 - 1,5% de ureia na matéria seca, grupo II - 4,51% de nitrato encapsulado na matéria seca e grupo III - 4,51% de nitrato encapsulado na matéria seca e 2,96% de óleo de castanha de caju. Os autores concluíram que a produção de metano foi reduzida com a inclusão do nitrato na dieta (28,6, 19,1 e 19,5 l por kg ingestão de matéria seca, respectivamente).

1.4 Desempenho de ruminantes alimentados com nitrato

Hegarty et al[34] compararam o uso do nitrato de cálcio com ureia na dieta de bovinos confinados. Os tratamentos foram com duas fontes de nitrogênio não proteico e dois níveis de adição na dieta (baixa inclusão x alta inclusão). Observaram que tanto o consumo da ração, como o peso das carcaças dos novilhos, foram afetados ($P < 0,001$) com a inclusão do nitrato de cálcio na dieta. Sendo que neste experimento, o nitrato apresentou desvantagem ao ser substituído pela ureia. O mesmo comportamento ocorreu quando a inclusão de nitrato foi aumentada.

No experimento realizado por El-Zaiat et al[4] o nitrato encapsulado não afetou o ganho médio diário, peso vivo final e a ingestão de matéria seca ($P > 0,05$). Entretanto os autores afirmaram que o tratamento com nitrato encapsulado resultou em uma melhor eficiência alimentar ($P<0,01$).

Newbold et al[33] pesquisaram níveis de nitrato no desempenho de bovinos da raça nelore. Neste estudo, foram realizados dois experimentos. No experimento II usaram 300 bovinos com seis níveis crescentes de nitrato. Os autores avaliaram variáveis de desempenho e não houve diferença para ganho em peso diário, entretanto o consumo da ração diminui linearmente conforme adicionado o nitrato, resultando em uma melhor eficiência alimentar.

Com isso, o uso de nitrato na dieta de bovinos melhora o desempenho dos animais e pode ser usado sem efeito sobre a saúde do animal.

Hulshof et al[1] avaliaram o consumo de matéria seca de 16 novilhos alimentados com cana de açúcar em uma proporção volumoso:concentrado de 60:40, com as fontes de nitrogênio não proteico nitrato de cálcio e ureia (dieta controle). Observou-se uma tendência (P = 0,09) de redução de consumo de matéria seca de 6% nos animais alimentados com nitrato de cálcio quando comparados aos animais alimentados com ureia. Bruning-Fann & Kaneene[6] trabalhando com bovinos, também observaram redução na ingestão de alimentos para animais alimentados com inclusão de 10 g ou mais por kg de matéria seca.

Uso de doses crescente de nitrato encapulado foi testado por Lee et al[16] em novilhas. Neste trabalho, o peso vivo dos animais não foi afetado pela inclusão de nitrato encapsulado. Quando foi avaliado consumo da ração, apresentou-se uma tendência (P=0,06) decrescente linear com o aumento de doses de nitrato encapsulado.

Alguns ensaios de metabolismo também revelaram que a alimentação de ruminantes com sais de nitrato sustentam níveis de produção adequados, Farra e Satter [7]; van Zijderveld[2]; van Zijderveld[14] e Li et al[35].

1.5 Riscos associados à ingestão de nitrato

Uma das limitações do uso do nitrato está relacionada à sua conversão a amônia, pois se a redução de nitrato a nitrito for mais rápida que nitrito a amônia, ocorre o acúmulo de nitrito no rúmem. A taxa de redução de nitrato é 2,5 vezes maior que a de redução a nitrito, Kozloski[19].

Grande parte do oxigênio que é carreado pelo sangue está associada à hemoglobina e é transportado pelos eritrócitos. Assim, quando o nitrito está em excesso dentro do rúmen ele pode ser absorvido pela corrente sanguínea e se ligar a hemoglobina, promovendo a oxidação do ferro e a formação da metahemoglobina, que é incapaz de transportar oxigênio para os tecidos, impedindo assim, as células de gerar energia através da cadeia respiratória, Cockburn et al[5]e Bruning-Fann & Kaneene[6].

Segundo Camargo et al[54] valores elevados de metahemoglobina na corrente sanguínea podem indicar uma situação incompatível com a vida, impedindo o transporte de oxigênio dos pulmões aos tecidos periféricos, causando hipóxia e cianose. Sinais clínicos de intoxicação podem não ser visíveis quando a concentração sanguínea de MetHb é inferior a 60%, enquanto que valores superiores a 75% são considerados letais, Burrows et al[36].

Animais intoxicados com nitrato podem apresentar sintomas como anorexia, ranger os dentes, apatia, tremores musculares, contrações abdominais, salivação, corrimento nasal, andar cambaleante, mucosas cianóticas, olhar assustado, pêlos arrepiados e, finalmente, decúbito. Já quando estes apresentam intoxicações crônicas, pode haver prejuízos no desempenho dos animais quanto a produção de leite e carne. As fêmeas podem apresentar abortos, decorrentes da falta de oxigenação do feto, lesões uterinas e problemas nos ovários, Sezer et al[37].

Para confirmação do estado de intoxicação com nitrato dispõe-se de uma grande gama de exames, entretanto, alguns podem obter resultados diferentes.

Com relação ao hemograma, Silva[38] obteve queda dos valores de hemoglobinas em animais intoxicados com nitrato, já, El Zaiat[4] encontrou elevação dos teores de hemoglobinas nos animais intoxicados, demonstrando que somente os resultados do hemograma não são válidos para uma confirmação de intoxicação.

Alterações hepáticas podem ocorrer com o consumo de nitrato. Para diagnosticar a presença de alterações hepáticas nos animais, podem ser realizados alguns testes laboratoriais. Sezer et al[38] observaram que, quando vacas foram intoxicadas com nitrato, identificaram elevação dos níveis de AST e ALP. Já, Lee et al[16] trabalharam com níveis de nitrato de até 3% na ingestão de matéria seca e não encontraram alterações elevadas de AST, ALT e GGT.

El-Zaiat et al[4],fornecendo nitrato encapsulado a carneiros, notaram que a proteína total e a albumina não foram alteradas com adição de 4,51% nitrato encapsulado. Entretanto, Observaram que conforme foi adicionado o nitrato em substituição a ureia na ração resultou menores níveis de ureia sanguínea, para os mesmos as razões para esta alteração ainda são desconhecidas.

Exames de metahemoglobina tem grande importância na confirmação de animais intoxicados por nitrato. Vários trabalhos que utilizam nitrato na dieta não observaram valores altos de MetHb Nolan et al[31]; El-Zaiat et al[4]; Van Zijderveld et al[2]; Van Zijderveld et al[14].

Lewis[3], estudando efeitos do uso de nitrato no metabolismo ruminal de ovinos, observou que quando foi fornecido 25 ou 10 gramas de nitrato de potássio por inoculação ruminal ou 2 gramas de nitrato de potássio por via intravenosa, obtiveram valores próximos a 60% de metemoglobina (MetHb). Já, Nolan et al[31] também trabalharam com nitrato de potássio na dieta de ovinos na proporção de 4%, entretanto valores bem menores foram encontrados para MetHb de 0,62% ao longo de 18 dias de avaliação.

El-Zaiat et al[4], em seu estudo com fontes de nitrato encapsulado substituindo a ureia na dieta de ovinos, observaram que não houve diferenças entre os tratamentos e os valores

encontrados para metemoglobina foram de 1,08%. Em contrapartida Newbold et al[33] encontraram níveis de MetHb de mais de 20% no período de adaptação do uso de nitrato.

Uso de doses crescentes de nitrato encapsulado foi testado por Lee et al[16],. Estes autores relataram que níveis maiores de MetHb, ocorreu nas primeiras três horas de alimentação. No entanto, os níveis de MetHb em 3 e 6 h após a alimentação foram maiores para os animais que consumiram o doses de nitrato encapsulado com 2% dieta em comparação com 2,9-5,8%. Este resultado não foi surpreendente, porque quantidades crescentes de nitrato encapsulado diminuiu as taxas de consumo alimentar, especialmente nas primeiras horas após a alimentação.

De acordo com Jönck[39] diagnóstico da intoxicação por nitrato/nitrito pode ser efetuado através da epidemiologia, sinais clínicos, teste de difenilamina, lesões encontradas na necropsia, porem as alterações de necropsia são sugestivas, desde que feita logo após a morte do animal, pois algumas horas após a morte do animal a metemoglobina retorna a hemoglobina, desaparecendo a coloração escura do sangue, da carcaça e musculatura. Fatores esses que dificultam a formação do diagnóstico, principalmente se a necropsia não for realizada rapidamente após a morte.

A decisão terapêutica deve ser orientada primariamente pela gravidade do quadro clínico sendo que o nível sangüíneo da MetHb tem importância secundária na definição da conduta. O tratamento terapêutico realizado a estes animais pode ser com uso do azul-de-metileno. O azul-de-metileno é o antídoto especifico usado para tratamentos com intoxicação por nitrato, considerado com propriedade anti-séptica e oxidante dose-dependente. Seu subproduto metabólico é leucoazul de metileno que transforma a MetHb em hemoglobina reduzida já que reduz o ferro da hemoglobina do estado ferroso. Porem deve haver cautela em seu uso, já que tanto o azul quanto o leucoazul-de-metileno são lentamente excretados pelos rins Nascimento et al[40]

Bucaretchi e Baracat[41] propõem como proposta terapêutica adjuvante à administração do azul de metileno, a administração de doses múltiplas de carvão ativado. O emprego de métodos mais invasivos e de alto custo, tais como exsanguíneotransfusão, plasmaferese e infusão contínua de azul de metileno, hemodiálise e hemoperfusão, tem sido citado em casos isolados de intoxicações agudas.

Com isso nota-se que o uso de nitrato em rações para animais não é uma prática comum por apresentar riscos de intoxicação e levar o animal a morte Mcallister et al[42]. Apesar de alguns trabalhos provarem ao contrário, alguns cuidados devem ser realizados para evitar que não ocorra intoxicação, Nolan et al[31]; El-Zaiat et al[4].

Para evitar a intoxicação por nitrito é necessário a adaptação da microbiota ruminal com inclusão gradual do nitrato, bem como concentrações de enxofre, energia e nitrogênio equilibrados na dieta Leng[11]. A inclusão gradual de nitrato aumenta a população de bactérias redutoras de nitrato e nitrito, bem como as suas capacidades redutoras, Alaboudi e Jones[43]; Iwamoto et al[44]. Desta forma, em boas condições de manejo, ruminantes podem utilizar nitrato como fonte de N sem que concentração sanguínea de MetHb caracterize risco para a saúde animal (Farra e Satter[7]; Alaboudi e Jones[43]; van Zijderveld et al[2]; van Zijderveld et al[14]; Li et al[35].

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Running head: Encapsulated nitrate for feedlot bulls

Capítulo 2- Encapsulated nitrate replacing soybean meal on growth, eating behavior, and blood metabolites of feedlot finishing young bulls

ABSTRACT: This study addressed the effects of an encapsulated nitrate product (**ENP**) replacing soybean meal in the finishing diets (84-d period) of feedlot young bulls. One hundred twenty non–castrated Nellore bulls (339.5 ± 28.3 kg of BW) were allotted to 20 outdoor pens (6 animals each) and assigned to a complete randomized block design with 5 blocks (Initial BW was used for blocking) and 4 dietary treatments: **Control (CTL)** – soybean meal; **ENP-1** – 1% ENP (0.65% NO_3^-) in the dietary DM; **ENP-2** – 2% ENP (1.30% NO_3^-); **ENP-3** – 3% ENP (1.95% NO_3). Diets were formulated to (14% CP; 75% TDN and 29% NDF) with 90:10 concentrate:roughage (sugarcane bagasse) ratio. Animals were gradually and simultaneously adapted over 14 d to ENP and concentrate. The DMI reduced linearly (from 9.6 to 8.4 kg/d; $P < 0.05$) with ENP inclusion. Final BW, BW ADG, carcass ADG, HCW, dressing percentage, and fat thickness were not affected ($P > 0.05$) by treatments. There was a quadratic tendency (0.121, 0.130, 0.128, 0.127, respectively; $P = 0.07$) for carcass gain:feed. Nitrate concentration in meat increased linearly (3.48, 2.73, 3.98, 6.59 mg NO_3^-/kg fresh meat; $P = 0.02$) with ENP addition, although nitrite (NO_2^-) was not detected. Time spent eating (min/d) was similar ($P > 0.10$) among treatments, although linear increases ($P = 0.02$) were obtained for time spent eating per unit of DMI and NDF intake. There was a tendency for quadratic reduction ($P = 0.07$) in ruminating time per unit of DMI and a significant quadratic decrease ($P < 0.01$) when

expressed in a NDF intake basis. Quadratic effects were observed for chewing time expressed per unit of DMI ($P = 0.03$) and NDF intake ($P < 0.01$). Blood methemoglobin increased linearly ($P < 0.05$) as more ENP was added, not representing risks of intoxication because values remained < 4% of total hemoglobin. No effects ($P > 0.05$) were detected in blood variables (e.g., creatinine, alkaline phosphatase, total protein, albumin, and glucose). There was a quadratic response ($P < 0.01$) in blood triglycerides, whilst a linear increase for total cholesterol ($P = 0.05$) and linear decreases for urea ($P < 0.01$), aspartate aminotransferase ($P < 0.01$), and gamma-glutamyl transferase ($P = 0.04$) were obtained. In conclusion, encapsulated nitrate product is a possible nitrogen source replacing soybean meal with no risks of nitrate intoxication. There were evidences of a possible improvement in feed efficiency, mainly caused by DMI reduction.

Key words: feedlot performance, methemoglobin, nitrite, non-protein nitrogen, slow-release nitrate

INTRODUCTION

In ruminants, non-protein nitrogen (**NPN;** e.g., urea) is important N sources for rumen microbes and microbial protein synthesis and can partially replace true protein (e.g., soybean meal) aiming to achieve reduced dietary costs. Recently, nitrate as a NPN source has gained attention due to its capacity to mitigate methane consistently in beef cattle (Hulshof et al., 2012; Velazco et al., 2014; Lee et al., 2015a), dairy cattle (van Zijderveld et al., 2011; Veneman et al., 2013; Lund et al., 2014), and sheep (Nolan et al., 2010; van Zijderveld et al., 2010; Li et al., 2012, 2013; El-Zaiat et al., 2014; de Raphélis-Soissan et al., 2014).

Nitrate is still believed as a potentially toxic feed component. During the ruminal reduction of nitrate to ammonia, nitrite accumulation may occur and, absorbed into blood, causes hypoxia by conversion of hemoglobin (**Hb**) into methemoglobin (**MetHb;** Cockburn et al., 2013). Adaptation by a stepwise exposure of rumen microbes to nitrate reduces the risk of nitrite accumulation and poisoning. Despite this, poisoning may still occur due to reasons previously reviewed (Leng, 2008), such as feed intake rate (Lee et al., 2015b), amount of highly fermentable carbohydrates (Burrows et al., 1987) and level of nitrate ingested.

To improve safety, a slow-release encapsulated nitrate product (**ENP**) was developed. It was reported that feeding a diet supplemented with ENP ad libitum mitigated enteric methane production without elevated MetHb to risk levels for animal health (El-Zaiat et al., 2014; Lee et al., 2015b), resulting at least in similar growth performance in sheep compared with a urea-based diet (El-Zaiat et al., 2014; Freire et al., 2013, 2014). In a metabolism study, beef heifers fed ENP replacing slow-release urea had lower total-N excretion and increased retained energy (Lee et al., 2015a).

Although few studies have evaluated the long-term effects of dietary nitrate on feedlot performance (Hegarty et al., 2013, Newbold et al., 2014; Velazco et al., 2014), the effects of nitrate as a replacement for true protein source (e.g., soybean meal) have not been examined. Therefore, the aim of this study was to evaluate the dose-response effect of ENP replacing soybean meal on growth, eating behavior, and blood metabolites of feedlot young bulls.

MATERIAL AND METHODS

This study was conducted from July to October 2012 in the Experimental Beef Cattle Feedlot of the Veterinary and Animal Science School of the Federal University of Goiás, Goiânia, State of Goiás, Brazil. Experimental protocol (n^{o} 054/12) and animal-use procedures

were approved and followed guidelines recommended by the Animal Care Committee at the same institution.

Animals and Housing*, *Experimental Design and Treatments

One hundred twenty non–castrated Nellore young bulls, 339.5 ± 28.3 kg of BW (mean ± SD); averaging 20 mo old) were allotted to 20 outdoor pens (7.7 m feedbunk line × 9.7 m width). All bulls had the same origin and were selected from a 600-animals herd. At the onset of the experiment, animals were vaccinated against clostridioses (Poli-Star, Vallée S.A., São Paulo, SP, Brasil), injected with A, D, and E vitamins supplement (ADE Injetável Emulsificável Pfizer, Zoetis, Morris County, NJ), and dewormed depending on BW with a subcutaneous injection of 15% albendazole sulfoxide (Agebendazol, Agener União, Embu-Guaçu, SP, Brazil) and 1% ivermectin (Absolut, Vallée S.A., São Paulo, SP, Brazil).

Animals were assigned to a complete randomized block design with 4 dietary treatments and 5 blocks. Pen, containing 6 bulls each, was considered the experimental unit for growth and eating behavior variables. Pen, containing 2 bulls each was considered the experimental unit for blood variables. For carcass, animal was considered as replicate. Initial BW was used for blocking the pens and treatments defined as follows: **Control (CTL)** – soybean meal as supplemental protein source; **ENP-1** – 1% ENP in the dietary DM; **ENP-2** – 2% ENP in dietary DM; **ENP-3** – 3% ENP in dietary DM.

The experimental ENP is protected by an international patent and manufactured by GRASP Ind. e Com. LTDA (Curitiba, PR, Brazil) using the double salt of calcium and ammonium nitrate decahydrate coated with a slow release matrix. Product composition was (% of DM): 85.3% DM in as-fed basis, 16.0% N, 65.1% NO_3^-, and 19.1% Ca.

Feeding Management and Data Collection

Total feedlot period lasted for 84 d, including a 13-d adaptation. Despite the protection derived from encapsulation, adaptation to ENP was performed to minimize possible neophobia

(aversion to nitrate bitter taste), and risks of poisoning. Young bulls were gradually and simultaneously adapted to ENP and concentrate as follows: d 1 – 4, all animals fed a 70:30 concentrate:roughage (sugarcane bagasse *in natura*) diet using CTL concentrate; d 5 – 8, all animals fed a 75:25 concentrate:roughage diet, with all nitrate-fed animals receiving ENP-1 concentrate; d 9 – 13, all animals fed a 80:20 concentrate:forage, with ENP-2 concentrate fed for treatments ENP-2 and ENP-3; d 14 onward, all animals fed their final experimental diets with 90:10 concentrate:roughage ratio (Table 1).

Finishing diets were formulated to (14% CP; 75% TDN and 29% NDF) according to NRC (2000) recommendations aiming 1.5 kg/d of ADG. Composition and chemical the ingredients analyses of experimental diets are shown in Table 1. To maintain similar TDN levels, ground corn was included (from 10.69% to 56.38% in DM basis) at the expense of ground sorghum (from 46.5% to 0% in DM basis) according to ENP inclusion replacing soybean meal. It was reported in Brazilian conditions that feedlot bulls fed a 80:20 concentrate:roughage diet performed similarly when 70% fine ground corn (in DM basis) was totally replaced by fine ground sorghum (Clarindo et al., 2008) or when 44% high-moisture corn was replaced by the same amount of high-moisture sorghum (Igarasi et al., 2008). Due to a slightly greater NDF content in sorghum than corn, soybean hulls were also added (from 5.6 to 9.23% in DM basis) according to ENP addition to adjust NDF levels among treatments. It is known that soybean hulls can be used as a grain or forage replacer with minimum interference on performance within the range used here in the experimental diets (Grigsby et al., 1992; Ipharraguere and Clark, 2003).

Total mixed rations were fed once daily at 0700 h. Animals were allowed ad libitum access to feed and fresh water. At the time of feeding, concentrate was mixed with sugarcane bagasse using a 3 m^3 capacity mixer (Siltomac 203, São Carlos, SP, Brazil) and dietary DM content was adjusted to 64% by water addition. Amounts of feed offered was adjusted daily for

orts not to exceed 5% of daily intake. Orts were recorded weekly to determine DMI and sampled. Feed, orts, and individual ingredients were sampled weekly and frozen at -20°C for further analyses.

To determine ADG and feed efficiency, animals were weighed at the beginning and end of experimental period after a 12-h fast of feed and water. The ADG in carcass and carcass gain:feed (feed efficiency based on carcass gain) were obtained considering an initial carcass dressing of 52%.

Determination of Eating Behavior

Observations for each pen (n = 20) were visually recorded every 5 min for 24 h on d 44 and d 74. Five observers were used for each 4-h interval, each one responsible for recording the eating behavior of animals in 4 pens (24 animals). This was accomplished by making point-in-time observations of each pen sequentially, as described by Araujo et al. (2008). Eating, ruminating, and total chewing times were expressed as minutes per day. Time (expressed in minutes) expended in each activity was calculated by the number of observations recorded multiplied by 5. Total chewing time was considered the sum of eating and ruminating times (Weidner and Grant, 1994). Eating, ruminating, and total chewing times were also expressed as minutes per kg of DMI and NDF intake.

Determination of Blood Metabolites

Two bulls per pen (n = 40) were used as tracer animals for all blood variables, with samples collected from these animals throughout experimental period. For methemoglobin (**MetHb**) determination, blood was sampled during adaptation on d 1 and always 24 h after each ENP increment, corresponding to d 6, 10, and 15. Blood collection was performed early in the morning prior to feed delivery. This protocol was taken to collect blood samples during adaptation in a moment of expected high levels of MetHb with minimum interference on normal feedlot conditions. Collection from 3 to 6 h after feeding would be preferred, but it

would cause an intensive change in animal behavior. After adaptation, blood was obtained on d 28, 56, and 84 during weighing. Samples (5 mL) were collected from jugular vein before morning feeding using evacuated K_2 EDTA tubes (Becton Dickinson & Co., Franklin Lakes, NJ). Methemoglobin was determined colorimetrically as described by Sato (2005) immediately after blood sampling using a spectrophotometer (SP 220 model, Bioespectro, Curitiba, PR, Brazil).

For biochemical analyses, blood samples (5 mL) were obtained on d 28, 56, and 84 from jugular vein at weighing using evacuated tubes without anticoagulant agents (Becton Dickinson & Co., Franklin Lakes, NJ.). Serum was separated after centrifugation at 1395 *g* (Q222TM-16 model, Quimis, Diadema, SP, Brazil) for 15 min and stored at -20°C. Analyses were performed using spectrophotometric commercial kits (Labtest Diagnóstica S.A. Lagoa Santa, MG, Brazil) and an automatic chemistry analyzer (Cobas Mira Plus model, F. Hoffmann-La Roche, Basel, Switzerland): Glucose was measured by the end point method (Reference 133); Cholesterol (Reference 76) and triglycerides (Reference 87) were measured by the enzymatic colorimetric method with Trinder end-point reaction; Total protein was determined by biuret colorimetric end-point reaction (Reference 99); Albumin was analyzed by bromocresol green colorimetric method (Reference 19); Urea was determined by the enzymatic UV photometric method using fixed time kinetic (Reference 104); Creatinine was measured colorimetrically by end-point reaction and alkaline picrate – Jaffe´s method (Reference 35); Aspartate aminotransferase (**AST;** also called aspartate transaminase was determined using the AST/GOT Liquiform kit (Reference 109) by the UV-IFCC continuous kinetic method; The gamma glutamyltransferase (**GGT**; also called gamma-glutamyl transpeptidase) was quantified by the kinetic method with a modified Szasz procedure using the GamaGT Liquiform kit (Reference 105); Alkaline phosphatase was analyzed by the continuous kinetic method with a

modified Bowers and McComb procedure using the Alkaline Phosphatase Liquiform kit (Reference 79).

Feed Analyses

Feed samples were thawed, composited by pen, dried in a forced-air oven at 55°C for 72 h, and ground with a Wiley mill (R-TE-650 model, Tecnal, Piracicaba, SP, Brazil) to pass a 1-mm screen. Dry matter was determined by oven drying at 105°C for 24 h, and OM was determined by difference after heating at 550°C for 4 h (AOAC, 1990). Nitrogen content was determined using a micro Kjeldahl apparatus (TE- 036/1 model, Tecnal, Piracicaba, SP, Brazil) according to AOAC (1990). Ether extract (**EE**) was also measured according to AOAC (1990) methods using a TE-044 extractor (Tecnal, Piracicaba, SP, Brazil). Sequential detergent fiber analyses were used to determine NDF and Lignin was determined by cellulose solubilization with 72% sulfuric acid (Van Soest et al., 1991) and ADF (Goering and Van Soest, 1970) concentrations of the experimental diets with a TE-149 Fiber Analyzer (Tecnal, Piracicaba, SP, Brazil). The NDF was determined using heat-stable α-amylase (A3306, Sigma Chemical. Co., St. Louis, MO) without sodium sulfite. The NDF concentrations were ash- and protein-corrected. The TDN was calculated according Weis et al. 1992.

Slaughtering and Carcass and Meat Assessment. After d 84, animals were transported to a commercial slaughter house (JBS, Goiânia, GO, Brazil; 12 km from feedlot facility) and slaughtered according to its standard procedures. Hot carcasses were separated into 2 symmetrical sections, weighed to obtain HCW, and identified individually. Carcass dressing percentage was determined using HCW and final BW after fasting. After chilling for 24 h at 4°C, left-side carcasses were ribbed between the 12th and 13th ribs to expose the LM. The 12th-rib fat thickness was measured using an outside digital caliper (150 mm, model HT0403-A1, Jiangsu, China) Masseter muscle was sampled from 1 animal per pen (n = 20) and frozen at -16ºC. Nitrate and nitrite residues in meat were determined spectrophotometrically at the

Instituto de Tecnologia de Alimentos (ITAL, Campinas, SP, Brazil) according to Brazilian Official Methods (Brasil, 2005a,b).

Statistical Analyses

Growth, carcass, eating behavior, and blood data were analyzed using the R software (R Development Core Team, 2013) for a randomized complete block design with 4 treatments and 5 blocks, according to the model:

$$Y = \mu + Bi + Tj + eij,$$

where μ = overall mean, Bi = block effect (i = 1 to 5), Tj = treatment effect (j = 1 to 4), and eij = residual error, being assumed to be normally distributed with mean = 0 and constant variance. For all analyzes pen was considered the experimental unit. Polynomial regression equations were used to evaluate linear, quadratic, and lack of adjustment for fixed models. Models were adopted when regression effects were significant at $P < 0.05$, followed by a non-significant lack of adjustment. Differences between treatments with $0.05 < P < 0.10$ were considered as a trend toward significance.

The mean DMI of each pen was calculated on d 1 and for each week repeatedly over the test period (12 weeks). Data were analyzed as repeated measures on R software (R Development Core Team, version 2.0) using the easyanova package (Arnhold, 2013). The model used for this analysis was as follows: $Y = \mu + Bi + Tj + Sij + Dk + (TD)\,jk + eijk$, in which μ = overall mean, Bi = block effect (i = 1 to 5), Tj = treatment effect (j = 1 to 4), and Sij = random residual error (block × treatment), Dk = time effect (k = 1 to 4), (TD) = jk interaction treatment x time and eijk = random residual error. Effects of treatment, time and interaction treatment × time were tested with F test of variance analyses. The Tukey test ($\alpha = 0.05$) was applied to compare treatments on weeks with P value < 0.05 of F test for treatment effect.

RESULTS

Growth Perfomance, Carcass, and Meat

Dry matter intake, growth, and carcass and meat variables of feedlot young bulls fed ENP replacing soybean meal are shown in Table 2. There was a linear reduction ($P = 0.02$) in DMI according to soybean meal replacement by ENP. Dry matter intake during the 84-d feedlot period, including the adaptation phase (14 d) and the remaining 70 d (10 wks) when animals were fed their final experimental diets are shown in Figure 1.

Final BW, ADG, carcass daily gain, and BW gain:feed (feed efficiency expressed per unit of BW gain) were not affected ($P > 0.10$) by inclusion of ENP. Notwithstanding, it was observed a tendency for a quadratic increase ($P = 0.08$) in carcass gain:feed (feed efficiency expressed per unit of carcass gain) with increasing doses of ENP.

Hot carcass weight, dressing percentage, and fat percentage were similar ($P > 0.10$) among treatments. The NO_3^- concentration in meat (masseter muscle) increased linearly ($P = 0.02$) with ENP inclusion in the diet, whereas meat NO_2^- was not detected in any sample.

Eating Behavior

Eating behavior data collected at d 44 and d 74 are shown on Table 3. In agreement with the results of DMI considering the whole experimental period, DMI obtained during eating behavior measurements was reduced linearly ($P = 0.02$) with increasing doses of ENP, whereas a quadratic effect ($P < 0.01$ with a $P = 0.06$ for a lack of adjustment to the quadratic model) was observed for NDF intake. Time spent eating (min/d) was similar ($P > 0.10$) among treatments, although a linear increase ($P = 0.02$) was obtained for time spent eating expressed per kg of DMI and per kg of NDF intake. Ruminating time (min/d) did not adjust to linear or quadratic models (lack of adjustment with $P < 0.01$). Despite this, there was a tendency for quadratic reduction ($P = 0.07$) in ruminating time expressed as kg DMI and a significant

quadratic ($P < 0.01$) when expressed in a NDF intake basis. Chewing time (min/d) also did not adjust to linear or quadratic models (lack of adjustment with $P \leq 0.01$). Nevertheless, significant quadratic effects were observed for chewing time expressed per unit of DMI ($P = 0.03$) and NDF intake ($P < 0.01$).

Blood MetHb and other Metabolites

Overall means and maximum individual values of blood MetHb concentration (% of total Hb) of feedlot bulls during ENP adaptation (d 1, d 6, d 10, and d 15) and throughout experimental period (d 28, d 56, and d 84) are shown on Table 4. Data were not statistically analyzed during adaptation phase because animals were not in their final experimental diets. For all treatments, mean and maximum blood MetHb concentrations obtained during adaptation remained below 3.33% of total Hb. There was a linear increase ($P < 0.01$) in blood MetHb at d 28 and d 56, but data collected at d 84 did not adjust to linear or quadratic models ($P < 0.001$ for lack of adjustment).

Blood biochemical metabolites are presented in Table 5. Glucose was not affected ($P > 0.10$) by supplementary ENP. Total cholesterol increased linearly ($P = 0.05$) and triglycerides increased quadratically ($P = 0.05$) with increasing dosage of ENP. No effects were observed ($P > 0.10$) on total protein and albumin, whereas a linear decrease ($P < 0.01$) was obtained for urea as more soybean meal was replaced by ENP. Creatinine was not influenced ($P > 0.10$) by ENP supplementation, whereas a linear reduction was verified for the liver enzymes AST ($P < 0.01$ for linear effect and tendency for quadratic effect with $P = 0.07$) and GGT ($P = 0.04$). Finally, ALP was similar ($P > 0.10$) among treatments.

DISCUSSION

Growth Performance and Carcass

Supplementary nitrate to ruminants has gained attention due to its consistent and persistent methane mitigating properties (Lee and Beauchemin, 2014) combined with the fact that once reduced to ammonia in the rumen, nitrate can be a source of NPN. Even considering intoxication risks, dietary nitrate has been listed as a promising methane mitigation strategy (Hristov et al., 2013). It is accepted that following feeding practices like stepwise dietary adaptation, adequate feeding management to avoid feed restriction, and adequate dietary formulation (level of fermentable carbohydrates, RDP, sulfur, vitamin A etc) there are low risks for animal health (Leng et al., 2008; Lee and Beauchemin, 2014).

In the current experiment, there was a significant linear decrease in DMI with increasing doses of ENP. Loss of appetite is a common effect in animals under chronic nitrate intoxication (MetHb > 20% of total Hb; Cockburn et al., 2013). However, the current study decreased DMI is not supported by mild nitrate poisoning, because MetHb levels < 4.07% of total Hb were very low in all tracer animals.

There are strong indications that nitrate-fed cattle or sheep reduced feed intake when long-term feedlot trials with large number of animals were conducted (Sokolowski et al., 1969; Weichenthal et al., 1963; Freire et al., 2013; Hegarty et al., 2013; Newbold et al., 2014; Velazco et al., 2014). Although other performance or metabolism studies have showed tendencies or numerical decreases in DMI (Hulshof et al., 2012; Li et al., 2012; El-Zaiat et al., 2014; Freire et al., 2014; Lund et al., 2014; Lee et al., 2015a), some others have not observed the same (Nolan et al., 2010; van Zijderveld et al., 2010, 2011; Veneman et al., 2013; de Raphélis-Soissan et al., 2014). Differences appear to be related to the low number of replicates and/or intensive animal interference for data collection (metabolism vs. performance trials), experimental design (change-over vs. complete randomized or complete block designs), short-term feeding period, and feeding protocol (restricted vs. ad libitum). Inconsistencies may come from nitrate dosages and dietary variations as well.

The first explanation for the reduced DMI relies on neophobia (feed aversion) due to nitrate bitterness (Weichenthal et al., 1963; Jones et al., 1966). Supporting this, Farra and Satter (1971) and Lee et al. (2015b) gradually increased pure nitrate (from 0.67 to 5.81% NO_3^- in DM basis) or ENP (from 0.11 to 4.9% NO_3^- in DM basis), respectively, observing a concomitant DMI reduction as more nitrate was added. Especially under high inclusion rates, changes in feeding behavior (e.g. lowered feed consumption rate, greater number of meals per day, and smaller size of meals) have been documented in cattle, responses likely attributed to nitrate taste reducing organoleptic dietary characteristics (Lichtenwalner et al., 1973; Lund et al., 2014; Velazco et al., 2014; Lee et al., 2015b). Supplemental ENP used in the current study possibly could have minimized organoleptic issues due to coating, a topic that deserves future investigation under a direct comparison with pure nitrate. On the other side, the use of soybean meal as a control may have exacerbated this response when compared with urea-based controls. Supplemental urea, which is widely used as a control against supplemental nitrate, also presents organoleptic limitations and causes lower feed intake compared to soybean meal (Huber and Kung Jr., 1981).

Little is known about nitrate-mediated effects on physiological pathways regulating feed intake in ruminants, but their existence cannot be discounted. Nitric oxide (**NO**) has been demonstrated as a key signaling messenger involved in feeding control in chicken (Choi et al., 1994; Khan et al., 2007) and mice (Morley et al., 1995; Morley et al., 2011; Han et al., 2013). Increased blood circulating NO is suggested in cattle receiving nitrate, because NO-mediated effects like peripheral vasodilation, elevated skin surface temperature, and increased wool growth have been demonstrated in nitrate-fed sheep (Sokolowski et al., 1969; Li et al., 2013; de Raphélis-Soissan et al., 2014). It is known that blood nitrite can be reduced to NO by deoxyhemoglobin in humans (Cosby et al., 2003), and that intravenous injections of nitrite resulted in a sudden drop of sheep blood pressure, a response strongly supporting NO effects

(Whatman et al., 2013). Finally, NO is an inhibitory neurotransmitter of nerves intrinsic to the forestomach of ruminants (Schneider and Eades, 1998; Onaga et al., 2001), allowing the speculation that decreased rumen motility may help to explain DMI reduction (Chen and Godwin, 2013).). Therefore, studies investigating physiological effects of nitrate in ruminants are encouraged for better understanding responses of supplemental nitrate on feed intake.

Final BW, ADG (expressed per unit of BW and carcass gains), and HCW were not affected by ENP supplementation, although numerical decreases were observed. These numerical reductions are attributed to the lower DMI, an issue that may have been intensified by the short feeding period (84 d), once adaptation phase represented 17% of the entire feedlot period (14 d of a total 84 d). Feed intake of ENP-fed bulls was especially low during weeks 3 and 6 of feeding, a period that maximum feed intake is desired to achieve nutrient requirements for optimum ADG (Loerch and Fluharty, 1999). This speculation is supported by the numerical lower ADG at 28 d (calculated using full BW without fasting) for ENP treatments compared with CTL (1.59, 1.28, 1.45, 0.98 kg/d for CTL, ENP-1, ENP-2, and ENP-3, respectively; data not shown), with the opposite occurring at d 56 (1.56, 1.73, 1.78, 1.68 kg/d for CTL, ENP-1, ENP-2, and ENP-3, respectively; data not shown). A better comprehension of ruminal and physiological adaptations to nitrate are necessary to minimize possible negative effects on feed intake and growth during receiving phase.

As observed in the current study, others also reported similar reductions (statistically or numerically) in final BW and ADG in feedlot cattle or sheep fed nitrate, results commonly accompanied by a decrease in feed intake (Freire et al., 2013; Hegarty et al., 2013; El-Zaiat et al., 2014; Freire et al., 2014; Velazco et al., 2014). However, even with a significant DMI reduction, Newbold et al. (2014) found numerical increases in final BW and ADG in feedlot cattle comparing different doses of pure nitrate with pure urea.

Due to methane mitigation, it was hypothesized that supplemental nitrate could enhance growth or feed efficiency by increasing retained energy (Leng, 2008). Nevertheless, meta-analysis using available data has not proved growth benefits (Lee and Beauchemin, 2014), with recent studies also showing no improved feed efficiency or performance (van Zijderveld et al., 2011; Freire et al., 2013; Hegarty et al., 2013; El-Zaiat et al., 2014; Freire et al., 2014; Velazco et al., 2014). In fact, decreased feed efficiency was also observed (Weichenthal et al., 1963; Sokolowski et al., 1969; Lichtenwalner et al., 1973), but usually linked with a strong DMI depression probably caused by nitrate overdose.

Increased hydrogen production and escape to the rumen gaseous phase of animals fed nitrate (Zijderveld et al., 2011; Lund et al., 2014) may also be at least partially involved in the lack of positive effects on performance, because it represents a loss of energy. Changes in ruminal degradation of nutrients by nitrate inclusion, mainly structural polysaccharides, should also be considered. Nitrate (or nitrite and its other intermediates) can decrease fiber degradability (Marais et al., 1988; Lee et al., 2015a) and fiber-degrading bacteria (Asanuma et al., 2014), although increased starch degradation (Lee et al., 2015a) and elevated population of starch-utilizing organisms (Asanuma et al., 2014) have been observed.

Notwithstanding, a significant improvement in feed efficiency was obtained by Lichtenwalner et al. (1973) and Newbold et al. (2014), which supports the tendency for quadratic increase observed in the current study. When compared with coated urea, ENP improved total tract DM digestibility (mainly derived from increased starch digestibility), enhanced retained energy, and lowered N excretion in beef heifers (Lee et al., 2015a) as well as resulted in higher rumen pH and lower time with pH < 6.0 (Lee et al., 2015b). It was also reported that pure nitrate compared with urea increased in vitro microbial growth (Guo et al., 2009) with in vivo supporting evidences (Li et al., 2012). All these results provide explanations that could support a better feed efficiency when feeding nitrate. As observed by Newbold et al.

(2014), the lower feed intake likely caused by dietary nitrate may be another contributor to the improved feed efficiency, because it was estimated that each 1% reduction in DMI improves efficiency in 0.6% (Sainz, 1995).

Nitrate and Nitrite in meat

Vegetables and drinking water are the most important exogenous nitrate sources for humans. At the same time, meat curing traditionally uses nitrite and nitrate salts as preservatives (Sindelar and Milkowski, 2012). Acceptable daily intakes (**ADI**) are 0 to 3.7 mg NO_3^-/kg BW and 0 to 0.07 mg NO_2^-/kg BW (FAO/WHO, 2003a,b), which could reach an intake of 259 mg NO_3^-/d and 4.9 mg NO_2^-/d for a 70-kg adult. In the current experiment, there was a linear increase in fresh meat NO_3^-, reaching a maximum concentration of 6.59 mg $NaNO_3$/kg (equivalent to 4.81 mg NO_3^-/kg) for ENP-3. Considering the average US bovine meat consumption of 37 kg per year in 2011 (101 g/d; FAO/STAT, 2015), it is calculated that ingestion of meat from cattle fed ENP-3 would represent 0.19% of ADI for a 70-kg man.

In a recent European survey, 8 from 50 analyzed bovine fresh meat samples obtained in the market were positive for nitrate, with concentration range of 10.8 to 15.0 mg NO_3^-/kg. Nitrite residues were not detected in all samples (Iammarino and Taranto, 2012). In Turkey, another survey obtained 110.0 mg NO_3^-/kg of cow meat and 79.5, 92.9, 106.1, 108.2, and 122.9 mg NO_3^-/kg of cow heart, lung, kidney, liver, and spleen, respectively (Sungur and Atan, 2013). The presence of nitrate and nitrite in fresh meat is a consequence of their natural ingestion through forages and water, at the same time being intermediates involved in the NO tissue metabolism.

This demonstrates that nitrate is a natural substance found in bovine meat and, although levels were increased by supplemental ENP they did not represent concentrations that could be detrimental to human health. Although controlled by legal authorities due to its proposed deleterious effects on human health (e.g. potential development of cancer and

methaemoglobinemia), emerging scientific studies are now bringing evidences that nitrate has potential positive effects on cardiovascular function, blood pressure control and others (Weitzberg and Lundberg, 2013).

Eating Behavior

As observed for the 84-d period, DMI during eating behavior observations linearly reduced with ENP supplementation. Interestingly, the decrease in DMI was not followed by a lower time spent eating, which in fact showed a numerical increase for ENP-3 compared with the other treatments (160 min/d for ENP-3 vs. 143, 137, 146 min/d for CTL, ENP-1, and ENP-2, respectively). As a result, eating time per unit of DMI and NDF intake increased linearly with ENP addition.

It was reported previously that beef heifers had lower feed consumption rate (kg of feed per time) in the first hours after feeding with increasing dosages of ENP (Lee et al., 2015b). This allows the hypothesis that animals may spend more time for eating when supplemented with nitrate. In the current experiment, this was especially valid for ENP-3, suggesting that the feasible upper limit of ENP might have been achieved in terms of organoleptic characteristics. As previously discussed, the bitter taste of nitrate can alter dietary organoleptic properties (Weichenthal et al., 1963; Jones et al., 1966; Farra and Satter, 1971), being observed that feedlot beef calves spent more time eating and visited feedbunk more frequently when fed urea + KNO_3 versus urea only (Lichtenwalner et al., 1973). In this referred study, these effects were more evident in the latter hours after feeding (animals fed once daily) and less pronounced with the course of experimental days. Respectively, it shows that cattle ate less in the first hours after feeding, attempted to compensate this in the latter hours, and that this interference was minimized as animals adapted to nitrate taste. A similar effect of greater intake in the latter hours after feeding has been confirmed in beef heifers (Lee et al., 2015b) and observed in dairy cows (Lund et al., 2014) fed ENP and pure nitrate, respectively. It has also been reported that

nitrate-fed feedlot cattle had greater numbers of meals per day and smaller in size when compared with a urea-fed animals (Velazco et al., 2014).

At a glance, overall nitrate effects on feeding behavior may be viewed as negatives. However, this potentially lower palatability of nitrate-based diets resulting in more time spent eating and more number of meals with smaller sizes could be explored positive side-effects to achieve a more uniform feed ingestion over time. It is something always pursued by nutritionists to stabilize rumen conditions, aiming at reducing digestive disorders (e.g. acidosis) and maximizing DM degradation when high concentrate diets are fed.

Blood MetHb and Other Metabolites

Blood MetHb is a key variable to identify the risk of nitrate toxicity. It has been reported that mild poisoning occurs with blood MetHb > 20% of total Hb, when oxygen supply to tissues becomes limited, causing physiological discomfort and reducing feed intake and performance (Cockburn et al., 2013). Usually only concentrations > 75% are lethal and clinical signs of acute toxicity may not be apparent if blood MetHb is < 60% (Burrows et al., 1987).

In this experiment, blood MetHb was monitored during the 14-d ENP adaptation, because intoxication risks were generally expected in this period. Blood was taken 24 h after each ENP increment, a moment likely to observe increased MetHb at the same time allowing blood collection with minimal interference on animal behavior. Levels of MetHb peak at 3 to 6 h after nitrate ingestion (Leng, 2008; Lee et al., 2015b), but unfortunately blood samples were not taken during this interval due to unfeasibility under the feedlot conditions of the current study. During the first 14 d, MetHb numerically increased over time according to ENP addition, but maximum concentrations in tracer bulls always remained < 4.07% of total Hb. This indicates that animals were not at risk of methemoglobinemia.

After adaptation, blood MetHb at d 28, 56, and 84 showed an overall linear increase with ENP inclusion, showing a maximum individual concentration of 3.57% of total Hb at d

84. However, those levels were far below the threshold limits for chronic or acute intoxications. Slight increases in blood MetHb in the current study were expected as observed when ENP was fed to sheep (El-Zaiat et al., 2014) and beef heifers (Lee et al., 2015b). A review by Lee and Beauchemin (2014) demonstrated that adaptation is able to avoid poisoning risks, because the magnitude of MetHb responses to supplementary nitrate was different between non-adapted and adapted animals. Besides that, Newbold et al. (2014) and de Raphélis-Soissan (2014) have reported cases of intoxication using pure calcium nitrate even after adaptation. Several other nutritional and management factors are known to influence nitrite accumulation, such as level and source of nitrate, consumption rate, N and S levels, feeding frequency, dietary composition and others (Leng, 2008; Lee and Beauchemin, 2014; Lee et al., 2015b). Thus, the use of ENP constitutes another strategy to avoid health issues.

There was a linear increase in total cholesterol and a quadratic effect in triglycerides according to ENP inclusion. Liver damage is known to result in altered lipid metabolism such as high levels of triglycerides and low levels of cholesterol (Cornelius, 1987). However, this inverse relationship between triglycerides and cholesterol was not observed in the current study, which resulted in no metabolic changes. Ingested nitrate can promote hypothyroidism in animals by inhibition of iodine uptake, with increased total cholesterol and triglycerides being common responses (Kaneko et al., 2008). Notwithstanding, in the current experiment, the concentrations of triglycerides were close to the standard reference (Kaneko et al., 2008) and no changes in glucose concentration have been observed were within normal reference values (Amorim et al., 2007). Effects of dietary nitrate on thyroid function deserve further investigation, mainly motivated because it may help to explain DMI responses.

Blood total protein, albumin, and urea have been widely used to monitor protein nutritional status and hydration level (Knowles and Warriss, 2007), as well as infection, inflammation, or injury processes (Fleck et al., 1985). No differences in total protein and

albumin among treatments indicate that bulls were in adequate protein balance, supporting that ENP was used effectively as a NPN source. El-Zaiat et al. (2014) also reported no differences in total protein and albumin between an ENP- and urea-based diet in sheep.

Blood urea, as an indicator of N excretion, linearly decreased with ENP. It is noted that in the current study the diets were formulated to provide similar amounts of N and TDN. Blood urea concentrations were within normal reference values (Amorim et al., 2007). El-Zaiat et al. (2014) and Lee et al. (2015a) reported similar reductions in rumen ammonia-N and blood urea, but in these studies ENP was compared with pure urea or coated urea, respectively, and not with soybean meal. Changes in DMI and eating behavior caused by ENP may also have influenced responses on blood urea. Finally, improved N retention feeding ENP was documented (Lee et al., 2015a). As suggested by Newbold et al. (2014), experiments designed to determine the effects of nitrate on N utilization, microbial synthesis, and growth, such as using diets limited in protein, are needed.

Blood creatinine, which is an indicator of muscle protein mobilization, did not differ among treatments. The adequate DMI and growth irrespective of treatments support the lack of effects on creatinine. Liver-related enzymes were evaluated as other health indicators. In animals, nitrate and nitrite may be converted into N-nitrous compounds, nitric oxide, and other reactive substances (Gangolli et al., 1994) able to promote lipid peroxidation (Salvemini and Cuzzocrea, 2002) and altered liver function in mice (Raat et al., 2009; El-Kott et al., 2012) and poultry (Atef et al., 1991). Surprisingly, inclusion of ENP linearly decreased AST and GGT, with no differences observed for ALP (Table 5). Values were within concentrations reported in cattle (Amorim et al., 2003; Moreira et al., 2009), indicating no liver injuries. El-Zaiat et al. (2014) in sheep and Lee et al. (2015b) in cattle did not report differences in liver enzymes when ENP was fed.

In conclusion, the ENP as a non-protein nitrogen was effective to replace soybean meal source in the finishing diet of feedlot bulls. Taking adequate feeding practices (total mixed ration, stepwise adaptation, and ad libitum intake), no risks of intoxication were observed for bulls fed ENP as determined by blood MetHb and other blood metabolites. A linear reduction in dry matter intake was observed followed by a possible improvement in feed efficiency. Additional feedlot studies are still necessary to confirm or not the possibility of improved feed efficiency observed in the current study. No significant levels of nitrate and nitrite have been detected in fresh meat. Furthermore, a better comprehension of nitrate effects on mechanisms controlling feed intake, neophobia (initial feed aversion due to nitrate bitterness), and changes in eating behavior is encouraged.

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Table 1. Ingredients and chemical composition of experimental diets (%; DM basis)

Item	Experimental diets[1]			
	CTL	ENP-1	ENP-2	ENP-3
Ingredient				
Sugarcane bagasse in natura	10.00	10.00	10.00	10.00
Cottonseed cake	13.54	13.54	13.54	13.54
Ground corn	10.69	25.92	41.15	56.38
Ground sorghum	46.50	31.00	15.50	0.00
Soybean hulls	5.60	6.81	8.02	9.23
Soybean meal	11.27	9.13	6.99	4.85
ENP[2]	-	1.00	2.00	3.00
Magnesium sulfate ($MgSO_4.7H_2O$)	-	0.20	0.40	0.60
Mycotoxin binder[3]	0.20	0.20	0.20	0.20
Mineral[4]	2.20	2.20	2.20	2.20
Chemical composition[5]				
DM, as-fed basis	85.4	85.3	85.2	85.2
OM	94.8	93.6	92.4	91.2
CP	13.9	14.1	14.2	14.4
EE[6]	3.3	3.3	3.3	3.3
NDF	29.0	29.0	29.0	29.1
ADF	17.2	16.8	16.5	16.2
Lignin	2.47	2.40	2.32	2.25
Calculated concentration[7]				
TDN	75.88	75.35	74.81	74.28

[1]CTL: control: soybean meal as protein source; ENP-1, 1% encapsulated nitrate product (0.65% NO_3^- of feed DM) in the dietary DM; ENP-2, 2% encapsulated nitrate product (1.3% NO_3^- of feed DM) in the dietary DM; ENP-3, 3% encapsulated nitrate product (1.95% NO_3^- of feed DM) in the dietary DM.

[2]Encapsulated nitrate product (% of DM): 85.3% DM in as-fed basis, 16.0% N, 65.1% NO_3, 19.1% Ca.

[3]Mastersorb FM®, GRASP Ind. e Com. LTDA, Curitiba, Paraná, Brazil.

[4]Composition: Ca, 130 g/kg DM; Na, 111 g/kg DM; P, 60 g/kg DM; S, 20 g/kg DM; Mg, 6 g/kg DM; Zn, 4000 mg/kg DM; Cu, 1000 mg/kg DM; Mn, 600 mg/kg DM; F, 600 mg/kg DM; Co, 80 mg/kg DM; I, 80 mg/kg DM; and Se, 8 mg/kg DM (Ganho 60 corte, Ganho Nutrição Animal, Goiânia, Goiás, Brazil).

[5]Dietary DM adjusted to 64% using tap water at the moment of feeding.

[6]Ether extract.

[7]TDN calculated according to Weiss et al. (1992). Data of sugarcane bagasse and cottonseed cake were obtained in Valadares Filho et al. (2006).

Table 2. Dry matter intake, growth, and carcass characteristics of Nellore feedlot young bulls fed encapsulated nitrate product as soybean meal replacer

Item	Experimental Diets[1]				SEM	Reg[2]	*P*-value[3]		
	CTL	ENP-1	ENP-2	ENP-3			α	β	LA
No. of pens (animals)	5 (30)	5 (30)	5 (30)	5 (30)	-	-	-	-	-
Initial BW, kg	339.7	339.6	339.4	339.4	-	-	-	-	-
Final BW, kg	479.3	471.1	472.4	466.8	5.0	L	0.13	-	0.75
						Q	0.49	0.79	0.49
DMI, kg/d[4]	9.6	8.7	8.8	8.4	0.3	L	0.02	-	0.36
						Q	0.12	0.39	0.25
BW ADG, kg	1.605	1.512	1.529	1.464	0.055	L	0.13	-	0.73
						Q	0.49	0.80	0.46
Carcass ADG, kg	1.004	0.985	0.985	0.928	3.082	L	0.12	-	0.72
						Q	0.92	0.56	0.59
BW gain:feed	0.168	0.174	0.173	0.174	0.005	L	0.46	-	0.80
						Q	0.48	0.61	0.69
Carcass gain:feed[5]	0.121	0.130	0.128	0.127	0.002	L	0.21	-	0.12
						Q	0.04	0.07	0.31
Hot carcass									
Weight, kg	270.3	268.3	268.2	262.5	3.2	L	0.13	-	0.74
						Q	0.94	0.58	0.61
Dressing percentage	56.42	56.94	56.75	56.28	0.33	L	0.69	-	0.36
						Q	0.22	0.17	0.78
Fat thickness, mm	4.16	3.96	3.76	3.57	0.35	L	0.23	-	0.99
						Q	0.71	0.99	0.98
Meat (as is basis)									
$NaNO_3$, mg/kg[6]									
Mean	3.48	2.73	3.98	6.59	0.84	L	0.02	-	0.17
						Q	0.29	0.07	0.87
Maximum	4.85	5.37	5.99	10.22	-	-	-	-	-

$NaNO_2$, mg/kg	n.d.[8]	n.d.	n.d.	n.d.	-	L	-	-	-
						Q	-	-	-

[1]CTL: control: soybean meal as protein source; ENP-1, 1% encapsulated nitrate product (0.65% NO_3^- of feed DM) in the dietary DM; ENP-2, 2% encapsulated nitrate product (1.30% NO_3^- of feed DM) in the dietary DM; ENP-3, 3% encapsulated nitrate product (1.95% NO_3^- of feed DM) in the dietary DM.

[2]Regression; L: Linear; Q: Quadratic.

[3] β_1: *P*-value of F test in ANOVA for the coefficient associated to linear effect; β_2: *P*-value of F test in ANOVA for the coefficient associated to quadratic effect; LA: *P*-value of F test in ANOVA for the lack of adjustment of linear and quadratic models.

[4]DMI: $y = 9.3842 - 0.3345x$ ($R^2 = 0.78$)

[5]Carcass gain:feed: $y= 0.1217 +0.0092x – 0.0026x^2$ ($R^2 = 0.83$)

[6]$NaNO_3$: $y = 2.609 - 1.057x$ ($r^2 = 0.66$)

[7]non-detected (detection limit for nitrate and nitrite was 5 mg of $NaNO_3$ or $NaNO_2$ per kg of fresh meat).

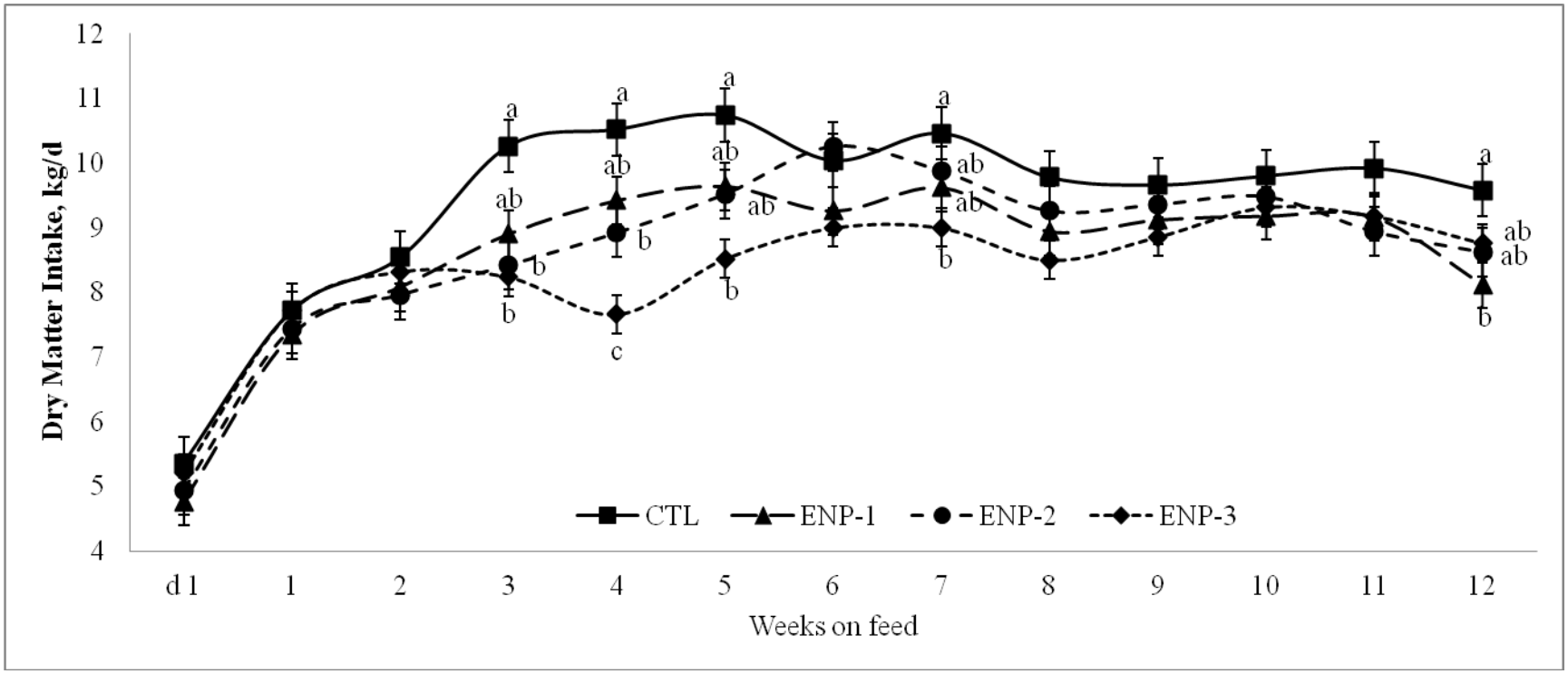

Figure 1. Dry matter intake (mean ± SEM) of Nellore feedlot young bulls fed soybean meal (CTL; ■), 1% encapsulated nitrate product (ENP-1;▲), 2% encapsulated nitrate product (ENP-2; ●), and 3% encapsulated nitrate product (ENP-3; ♦) during a 84-d feedlot period. d 1 – 4: all animals fed a 70:30 concentrate:forage (sugarcane bagasse *in natura*) diet using CTL concentrate; d 5 – 8: all animals fed a 75:25 concentrate:forage diet and with all nitrate-fed animals moved to ENP-1 concentrate; d 9 – 13: all animals fed a 80:20 concentrate:forage and with ENP-2 concentrate fed for treatments ENP-2 and ENP-3; d 14 onward: all animals fed the final 90:10 concentrate:forage diet and all animals fed their respective concentrates (SEM = 0.28; treatment $P = 0.08$; time $P < 0.01$; treatment x time $P < 0.01$).

Table 3. Eating behavior of Nellore feedlot young bulls fed encapsulated nitrate product as soybean meal replacer (data obtained in d 44 and d 74)

Item	Experimental diets[1]				SEM	Reg[2]	*P*-value[3]		
	CTL	ENP-1	ENP-2	ENP-3			β_1	β_2	LA
DMI, kg/d[4]	10.5	9.3	10.4	9.0	0.3	L	0.02	-	0.18
						Q	0.18	0.49	0.09
NDFI, kg/d[5]	4.4	4.4	4.7	4.0	0.1	L	0.80	-	<0.01
						Q	<0.01	<0.01	0.06
Eating[6]									
min/d	143	137	146	160	8.8	L	0.16	-	0.56
						Q	0.56	0.30	0.81
min/kg DMI	14	15	14	17	0.9	L	0.02	-	0.22
						Q	0.85	0.34	0.14
min/kg NDFI	30	32	30	39	2.0	L	0.02	-	0.10
						Q	0.43	0.12	0.12
Ruminating[7]									
min/d	300	230	256	237	9.5	L	<0.01	-	<0.01
						Q	<0.01	0.02	<0.01
min/kg DMI	29	25	25	26	1.1	L	0.09	-	0.17
						Q	0.03	0.07	0.62
min/kg NDFI	63	54	53	57	2.3	L	0.09	-	0.03
						Q	<0.01	<0.01	0.69
Chewing[8]									
min/d	443	368	402	397	11.9	L	0.07	-	<0.01
						Q	<0.01	0.01	0.01
min/kg DMI	42	40	39	43	1.3	L	0.78	-	0.08
						Q	0.05	0.03	0.49
min/kg NDFI	93	85	83	96	2.8	L	0.65	-	<0.01
						Q	<0.01	<0.01	0.40

[1]CTL: control: soybean meal as protein source; ENP-1, 1% encapsulated nitrate product (0.65% NO_3^- of feed DM) in the dietary DM; ENP-2, 2% encapsulated nitrate product (1.30% NO_3^- of feed DM) in the dietary DM; ENP-3, 3% encapsulated nitrate product (1.95% NO_3^- of feed DM) in the dietary DM.

[2]Regression; L: Linear; Q: Quadratic.

[3] β_1: *P*-value of F test in ANOVA for the coefficient associated to linear effect; β_2: *P*-value of F test in ANOVA for the coefficient associated to quadratic effect; LA: *P*-value of F test in ANOVA for the lack of adjustment of linear and quadratic models.

[4]DMI**:** $y = 9.74 – 0.36x$ ($R^2 = 0.35$)

[5]NDF intake, kg/d: $y = 4.16 – 0.33x + 0.75x^2 – 0.22x^3$ ($R^2 = 1.00$)

[6]Eating (min/kg DM): $y = 13.456 + 1.06x$ ($R^2 = 0.66$). Eating (min/kg NDF): $y = 28.96 + 2.46x$ ($R^2 = 0.57$)

[7]Ruminating (min/d): $y = 300.2 – 165.0x + 118.5x^2 – 23.5x^3$ ($R^2 = 0.44$). Ruminating (min/kg NDF):$y = 63.11 – 12.56x + 3.56x^2$ ($R^2 = 0.98$)

[8]Chewing (min/d): $y = 443.20 – 181.13x + 130.70x^2 – 25.17x^3$ ($R^2 = 1.00$). Chewing (min/kg DM): $y = 42.48 – 4.40x + 1.52x^2$ ($R^2 = 0.90$). Chewing (min/kg NDF): $93.20 – 15.12x + 5.23x^2$ ($R^2 = 0.95$).

Table 4. Blood methemoglobin (% of total Hb) of Nellore feedlot young bulls fed encapsulated nitrate product as soybean meal replacer

Item	Experimental diets[1]				SEM	Reg[2]	*P*-value[3]		
	CTL	ENP-1	ENP-2	ENP-3			β_1	β_2	LA
Adaptation[4]									
d 1									
Mean	0.60	0.38	0.62	0.49	-	-	-	-	-
Max.	1.16	0.97	1.25	0.98	-	-	-	-	-
d 6									
Mean	0.86	0.75	0.75	0.56	-	-	-	-	-
Max.	1.23	1.23	1.62	0.92	-	-	-	-	-
d 10									
Mean	1.25	1.13	1.20	1.23	-	-	-	-	-
Max.	3.27	4.07	3.21	3.23	-	-	-	-	-
d 15									
Mean	0.51	0.77	1.05	1.58	-	-	-	-	-
Max.	0.91	1.32	1.88	3.33	-	-	-	-	-
In treatments									
d 28[5]									
Mean	0.58	0.75	0.99	1.59	0.14	L Q	<0.001 0.98	- 0.15	0.31 0.63
Max.	0.88	1.20	1.65	2.39	-	-	-	-	-
d 56[6]									
Mean	0.68	1.00	1.02	1.26	0.12	L Q	<0.01 0.232	- 0.74	0.58 0.33
Max.	1.06	1.83	1.74	2.39	-	-	-	-	-
d 84[7]									
Mean	0.60	1.17	2.61	1.50	0.17	L Q	<0.001 <0.001	- <0.001	<0.001 <0.001
Max.	1.46	1.90	3.57	2.38	-	-	-	-	-

[1]CTL: control: soybean meal as protein source; ENP-1, 1% encapsulated nitrate product (0.65% NO_3^- of feed DM) in the dietary DM; ENP-2, 2% encapsulated nitrate product (1.30% NO_3^- of feed DM) in the dietary DM; ENP-3, 3% encapsulated nitrate product (1.95% NO_3^- of feed DM) in the dietary DM.

[2]Regression; L: Linear; Q: Quadratic.

[3] β_1: *P*-value of F test in ANOVA for the coefficient associated to linear effect; β_2: *P*-value of F test in ANOVA for the coefficient associated to quadratic effect; LA: *P*-value of F test in ANOVA for the lack of adjustment of linear and quadratic models.

[4]d 1: first day animals fed a 70:30 concentrate:forage (sugarcane bagasse in natura) diet using CTL concentrate; d 6: 24 h after all animals fed a 75:25 concentrate:forage diet and with all nitrate-fed animals moved to ENP-1 concentrate; d 10: 24 h after all animals fed a 80:20 concentrate:forage and with ENP-2 concentrate fed for treatments ENP-2 and ENP-3; d 15: 24 h after all animals fed the final 90:10 concentrate:forage diet and with all animals fed their respective concentrates.

[5]d 28: $y = 0.4849 + 0.3284x$ ($R^2 = 0.91$)

[6]d 56: $y = 0.7279 + 0.1754x$ ($R^2 = 0.90$)

[7]d 84: $y = 0.6010 – 0.9890x + 2.1265x^2 – 0.5655x^3$ ($R^2 = 1.00$)

Table 5. Blood metabolites in Nellore feedlot young bulls fed encapsulated nitrate product as soybean meal replacer

Item	Experimental diets[1]				SEM	Reg[2]	*P*-value[3]		
	CTL	ENP-1	ENP-2	ENP-3			β_1	β_2	LA
Glucose, mg/dL	82.67	90.67	96.17	88.67	7.3	L	0.49	-	0.56
						Q	0.25	0.31	0.75
Total cholesterol[4], U/L	136.43	146.78	160.78	160.36	8.68	L	0.05	-	0.75
						Q	0.24	0.55	0.65
Triglycerides[5], mg/dL	15.44	18.69	21.58	17.62	0.99	L	0.06	-	<0.01
						Q	<0.01	<0.01	0.17
Total protein, g/dL	7.64	7.56	7.64	7.58	0.11	L	0.84	-	0.81
						Q	0.90	0.94	0.52
Albumin, g/dL	2.80	2.85	2.91	2.84	0.06	L	0.52	-	0.55
						Q	0.26	0.32	0.65
Urea[6], mg/dL	40.89	38.28	36.99	34.27	1.16	L	<0.01	-	0.96
						Q	0.19	0.83	0.86
Creatinine, mg/dL	1.53	1.50	1.53	1.45	0.04	L	0.29	-	0.55
						Q	0.69	0.47	0.42
AST[7], U/L	101.77	85.00	78.28	81.90	5.19	L	0.01	-	0.19
						Q	0.02	0.07	0.99
GGT[8], U/L	24.28	22.30	21.33	19.82	1.36	L	0.04	-	0.95
						Q	0.42	0.87	0.80
ALP[9], U/L	332.55	369.57	335.82	315.56	24.37	L	0.45	-	0.40
						Q	0.38	0.26	0.45

[1]CTL: control: soybean meal as protein source; ENP-1, 1% encapsulated nitrate product (0.65% NO_3^- of feed DM) in the dietary DM; ENP-2, 2% encapsulated nitrate product (1.30% NO_3^- of feed DM) in the dietary DM; ENP-3, 3% encapsulated nitrate product (1.95% NO_3^- of feed DM) in the dietary DM.

[2]Regression; L: Linear; Q: Quadratic.

[3] β_1: *P*-value of F test in ANOVA for the coefficient associated to linear effect; β_2: *P*-value of F test in ANOVA for the coefficient associated to quadratic effect; LA: *P*-value of F test in ANOVA for the lack of adjustment of linear and quadratic models.

[4]Total cholesterol: $y = 138.2225 + 8.5763x$ ($R^2 = 0.89$)

[5]Triglycerides: $y = 15.1152 + 6.3545x - 1.8040x^2$ ($R^2 = 0.89$)

[6]Urea: $y = 40.7209 - 2.1741$ ($R^2 = 0.98$)

[7]AST: Aspartate aminotransferase, also called aspartate transaminase: $y = 96.6867 – 6.6332x$ ($R^2 = 0.68$)

[8]GGT: Gamma-glutamyl transferase or gamma-glutamyl transpeptidase: $y = 24.0865 – 1.4355x$ ($R^2 = 0.98$)

[9]ALP: Alkaline phosphatase.

O conteúdo deste capítulo segue as normas de formatação da Revista Tropical Animal health and Production (http://www.springer.com/life+sciences/animal+sciences/journal/11250)

Capítulo 3- Nellore bulls fed finishing diets containing encapsulated nitrate replacing soybean meal: Nutrients intake and digestibility

Abstract: This study was designed to investigate to the dose-response effect of encapsulated nitrate replacing soybean meal in a finishing diet on nutrients intake and digestibility of 120 Nellore bulls (mean ± SD, 339.5 ± 28.3 kg of BW) allotted to 20 pens (6 bull each). Bulls were assigned to a randomized complete block design (by initial BW) within 5 blocks and 4 dietary treatments: CTL (Control) – soybean meal; ENP-1 – 1% ENP (0.65% NO_3^-) in the dietary DM; ENP-2 – 2% ENP (1.30% NO_3^-); ENP-3 – 3% ENP (1.95% NO_3). Diets contained 90:10 concentrate:roughage (sugarcane bagasse) and similar contents of N (140 g k^{-1} CP), energy (750 g k^{-1} TDN), and fiber (290 g k^{-1} NDF). Bulls were gradually and simultaneously adapted over 14 d to ENP and concentrate. Digestibility evaluation was performed from the d 70 to 73 of feedlot period (84d) and iNDF was used as the internal marker to estimate fecal excretion. There was a linear decrease ($P<0.05$) on non-fibrous carbohydrates (NFC) intake and a tendency to linear decrease ($P<0.10$) on DMI according to soybean meal replacement by ENP. Intake of organic matter (OM), ether extract (EE), crude protein (CP) and neutral detergent fiber (NDF) were not affected ($P>0.10$) by inclusion of ENP. A consistent quadratic response ($P<0.02$) were observed to digestibility of DM, OM, EE, CP and NDF with the increasing levels of ENP replacing soybean meal. The maximum values of nutrients digestibility were achieved with 1.6, 1.5, 1.8, 2.0 and 1.6% of ENP inclusion for DM, OM, EE, CP and NDF, respectively. The NFC was not affected ($P>0.10$) by inclusion of ENP. Encapsulated nitrate product may reduced the nutrients intake, but enhanced the digestibility and, consequently, decreased the fecal output of nutrients when replacing soybean meal up to 2% in finishing diets fed to feedlot young Nellore bulls.

Key words: beef cattle, nitrite, non-protein nitrogen, slow-release nitrate, toxicity

Introduction

Rumen microorganisms require nitrogen (N) for growth. Sources of dietary N are based on true protein (i.e., polypeptides; e.g., cottonseed meal and soybean meal) or non-protein nitrogen (NPN; e.g., urea and nitrate). True protein can be partially replaced by NPN, which releases ammonia ($N\text{-}NH_3$) used on microbial protein synthesis.

Nitrate salts have been used on ruminant nutrition due the activity of rumen microorganisms of reducing nitrate to ammonia. Nitrite is an intermediate compound produced by a minor group of rumen microbes which reduces nitrate and oxidizes sulphide (Leng, 2008). The conversion of nitrite to ammonia occurs slower than the reduction of nitrate to nitrite. Thus, nitrite may accumulate in the rumen and, be absorbed into bloodstream, where it oxidizes Fe ions converting hemoglobin into methemoglobin (MetHb), causing hypoxia (Cockburn et al., 2013). Nitrite accumulation also was reported in an *in vitro* study as the main factor reducing digestibility of DM in the rumen environment, prior to nitrate and ammonia levels (Marais et al., 1988).

Blood MetHb has been evaluated in most of the nitrate studies as indicative of toxicity (Leng, 2008; Cockburn et al., 2013; El-Zaiat et al., 2014; Lee et al., 2015b). However, due to the sensitivity of some rumen microorganisms to high concentration of nitrite (Marais et al., 1988), digestibility of nutrients might be altered prior the increasing MetHb blood levels. Lee et al. (2015b) findings support this hypothesis. The authors observed quadratic response on VFA concentration with increasing levels of encapsulated nitrate to restricted-fed heifers (75% of ad libitum intake) compared to encapsulated-urea based diet. Thus, the diet digestibility decisively affects energy supply and animal performance.

Few studies show the dose-response effects of nitrate-based diets on *in vitro* (Shi et al., 2012) and total-tract nutrients digestibility (Lee et al., 2015a), and most reported no effects on apparent digestibility of dry matter compared to urea-based diets (Nolan et al., 2010; van

Zijderveld et al., 2011; Li et al., 2012; El-Zaiat, 2013). Thereby, the effects of encapsulated nitrate replacing true protein on apparent digestibility of nutrients are unknown.

A slow-release encapsulated nitrate product (ENP) was developed aiming improve the safety of nitrate inclusion on ruminant diets. Study to monitoring the blood levels of MetHb reveal that feeding ENP prevents the nitrite accumulation in the rumen, which represents no risks of toxicity (Silva, 2014). Therefore, this study was designed to investigate to the dose-response effect of encapsulated nitrate replacing soybean meal in a finishing diet on intake and digestibility output of nutrients of young Nellore bulls.

Material and Methods

The experiment of performance was conducted from july to october 2012 in the Veterinary and Animal Science School of the Federal University of Goiás (UFG), at the Experimental Beef Cattle Feedlot (Goiânia, Goiás, Brazil). The Ethics Committee on Use of Animals of the same institution (CEUA/UFG) reviewed and approved (No. 054/12) the experimental procedures, which were performed according to Brazilian Guidelines for the Care and Use of Animals for Scientific and Educational Purposes – DBCA (RN No. 12 of september 20th, 2013).

Animals, Housing, Experimental Design and Treatments

One-hundred twenty Nellore bulls (initial BW 339.5 ± 28.3 kg) averaging 20 months old were selected from a 600-animals herd. Bulls were blocked by initial BW and assigned randomly to 1 of 4 dietary treatments within block. Treatments were replicated in 5 outdoor pens (n = 20) with 6 bulls per pen. The size of the pens was 7.7 m feedbunk line × 9.7 m width, providing 1.28 m of bunk per bull and 12.4 m^2 per bull. Before start the experiment, bulls were vaccinated against clostridioses (Poli-Star, Vallée S.A., São Paulo, SP, Brazil), injected with A, D, and E vitamins supplement (A-D-E Injetável Emulsificável Pfizer, Zoetis, Morris County,

NJ), and dewormed (based on BW) with a subcutaneous injection of 15% albendazole sulfoxide (Agebendazol, Agener União, Embu-Guaçu, SP, Brazil) and 1% ivermectin (Absolut, Vallée S.A., São Paulo, SP, Brazil).

Dietary treatments were defined as follows: CTL, control = no inclusion of ENP, and soybean meal as supplemental protein source; ENP-1 = 10 g kg^{-1} of ENP (6.5 g kg^{-1} NO_3^-) in the dietary DM; ENP-2 = 20 g kg^{-1} of ENP (13.0 g kg^{-1} NO_3^-) in dietary DM; ENP-3 = 30 g kg^{-1} of ENP (19.5 g kg^{-1} NO_3) in dietary DM.

The experimental ENP is protected by an international patent and manufactured by GRASP Ind. e Com. LTDA (Curitiba, Paraná, Brazil) using the double salt of calcium and ammonium nitrate decahydrate [$5Ca(NO_3)_2 \cdot NH_4NO_3 \cdot 10H_2O$] coated with a slow release matrix. ENP composition, DM basis: 853 g kg^{-1} of DM in as fed basis, 160 g kg^{-1} of N, 651 g kg^{-1} of NO_3^-, and 191 g kg^{-1} of Ca.

Feeding Management

Bulls were finished over a period of 84 days on feed (DOF), including 14 d of adaptation. Even though the protection derived from the encapsulation process, bulls were gradually and simultaneously adapted to ENP to avoid the occurrence of feed aversion due bitter taste of nitrate salts, and risks of intoxication. Adaptation to ENP and concentrate was conducted as follows: d 1 to 4: all bulls fed a 70:30 concentrate:roughage (C:R ratio; sugarcane bagasse *in natura*) diet using concentrate of CTL treatment; d 5 to 8: all bulls fed 75:25 C:R diet, and bulls of treatments ENP-1, ENP-2 and ENP-3 receiving the ENP-1 concentrate; d 9 to 13: all bulls fed 80:20 C:R diet, and bulls of treatments ENP-2 and ENP-3 receiving the ENP-2 concentrate; d 14: all bulls fed the finishing diets with 90:10 C:R, and bulls of treatments ENP-3 receiving the ENP-3 concentrate (i.e., all bulls fed their respective treatments).

Finishing diets were formulated according to NRC (2000) to achieve (DM basis) 140 g kg^{-1} of CP, 750 g kg^{-1} of TDN, and 290 g kg^{-1} of NDF, aiming 1.5 kg d^{-1} of average daily gain

(ADG, on live weight basis). Composition and chemical analysis of experimental diets are shown in Table 1. Total mixed rations (TMR) were fed once daily at 0700 h. Bulls were allowed *ad libitum* access to feed and fresh water. At the time of feeding the concentrate was mixed with sugarcane bagasse using a 3 m^3 capacity mixer (Siltomac 203, São Carlos, SP, Brazil) and dietary DM content was adjusted to 630 g kg^{-1} of DM by sprinkling water on top of the TMR into the mixer. The amount of TMR offered was adjusted daily for orts not to exceed 5% of daily intake, aiming to avoid feed waste and diet sorting by the bulls.

Data Collection

Data and samples used on digestibility evaluation were collected from the 70th to 73rd DOF. The weight of the diets offered was recorded and samples were taken daily on d 70, 71 and 72. The weight of the orts was recorded and samples were taken daily (10% of total amount of each pen) on d 71, 72 and 73. Fecal samples were collected on the rectum of each one of the 120 bulls (approximately 200 g per bull) once on d 73 at 0600 h, before feeding. Individual daily samples were frozen (-20 ºC).

Diet, orts and fecal samples were thawed, homogenized and dried at temperature of 55 ºC in a forced-air oven for 72 h. Dried samples were ground with a Wiley mill (R-TE-650, Tecnal, Piracicaba, SP, Brazil) to pass a 1-mm screen. The dried and ground samples were proportionally composited, and later used for chemical analyses. Diet samples were composited by treatment using similar amounts of daily samples (three days of collection). Orts samples were composited by pen using 10% of the total amount of each one of the three individual samples (three days of collection). Fecal samples were composited by pen using 40 g of each one of the six individual samples (six bulls into each pen).

The nutrients intake was calculated as the amount of nutrient contained in the diet offered to the bulls minus the amount of nutrients in the orts. The fecal output was calculated using the concentration of indigestible neutral detergent fiber (iNDF) in the feces and the total intake by

animals as an internal marker. The amount of nutrients consumed in the diet offered and excreted in feces were used to calculate the apparent total-tract digestibility coefficients [(nutrient intake – nutrient output in feces) ÷ nutrient intake] on a DM basis.

Laboratorial Analyses

Analytical DM contend was determined by oven drying at 105 °C for 24 h, and OM was determined by difference after heating at 550 °C for 4 h (AOAC, 1990). Nitrogen content was determined using a micro Kjeldahl apparatus (TE- 036/1, Tecnal, Piracicaba, SP, Brazil) according to AOAC (1990) and the concentration of CP calculated by multiplying the N content x 6.25. Ether extract (EE) was also measured according to AOAC (1990) methods using a TE-044 extractor (Tecnal, Piracicaba, SP, Brazil). Sequential detergent fiber analyses were used to determine NDF (Van Soest et al., 1991). Concentrations of the experimental diets with a TE-149 Fiber Analyzer (Tecnal, Piracicaba, SP, Brazil). The NDF was determined using heat-stable α-amylase (A3306, Sigma Chemical. Co., St. Louis, MO, US) and, without add sodium sulfite. The NDF concentrations were ash- and protein-corrected.

The concentration of non-fibrous carbohydrates (NFC) was calculated as the OM concentration minus the concentrations of EE, CP and NDF.

The concentration of iNDF was determined by *in situ* incubation of 0.8 g of sample (triplicates) into non-woven textile bags (5 x 5 cm) with porosity of 100 μm (Casali et al., 2009) over 264 h (Casali et al., 2008). After incubation, the bags were rinsed on washing machine until the water became clear. Rinsed bags were analyzed for NDF content using the autoclave procedure described by Detmann et al., 2012.

Statistical Analyses

Data were analyzed using the epr package (R Development Core Team, version 2.0) using the epr package (Arnhold, 2013). The model was: $Y = \mu + Bi + Tj + eij$, in which μ = overall mean, Bi = block effect (i = 1 to 5), Tj = treatment effect (j = 1 to 4), and eij = residual

error, being assumed to be normally distributed with mean = 0 and constant variance. For nutrients intake, digestibility and fecal output data, pen was considered the experimental unit (n = 20) and the initial BW the effect of block. Polinomial regression equations were used to evaluate linear, quadratic, and lack of fit for fixed models. Models were adopted when regression effects were significant at $P<0.05$, followed by a non-significant lack of fit. Differences between treatments with $0.05<P<0.10$ were considered as a tendency towards significance.

Results

The responses of increasing levels of ENP on growth performance, carcass characteristics, eating behavior, and blood metabolites of young Nellore bulls were reported by Pereira et al. (2015). The composition of the experimental diets is presented Table 1. During the digestibility evaluation the diference between the analyzed composition of diet offered and refused (orts) suggests there was no sorting activity of the diets with encapsulated nitrate product (ENP) replacing partially soybean meal (Figure 1).

Nutrients Intake

The nutrients intake of feedlot young Nellore bulls fed ENP replacing soybean meal are shown in Figure 2. There was a linear reduction ($P<0.05$) in non-fibrous carbohydrates (NFC) intake according to soybean meal replacement by ENP, achieving 10.7%, 15.1%, and 15.5% lower NFC intake for ENP-1, ENP-2, and ENP-3 when compared with CTL, respectively. Intake of NFC followed the trend of linear decrease in dry matter intake (DMI, 9.70, 9.28, 8.84, and 8.75 kg d^{-1} for CTL, ENP-1, ENP-2, and ENP-3, respectively; SEM = 0.41, $P<0.10$) with increasing doses of ENP. Although, intake of organic matter (OM), ether extract (EE), crude protein (CP) and neutral detergent fiber (NDF) were not affected ($P>0.10$) by inclusion of ENP.

Apparent Digestibility of Nutrients

Apparent total-tract digestibility of experimental diets, based on fecal output estimate using iNDF as internal marker, is presented in Table 2. A consistent quadratic response ($P<0.02$) were observed to all nutrients with the increasing levels of encapsulated nitrate product replacing soybean meal in the diets. The digestibility coefficients retreated to lower values on ENP-3 diet (DM 0.687, 0.715, 0.744 and 0.688; OM 0.700, 0.731, 0.759 and 0.696; EE 0.843, 0.881, 0.901 and 0.869; CP 0.679, 0.719, 0.769 and 0.723 and NDF 0.516, 0.602, 0.640. The maximum values of nutrients digestibility were achieved with 1.6, 1.5, 1.8, 2.0 and 1.6 of ENP inclusion for DM, OM, EE, CP and NDF, respectively. Although, digestibility of NFC was not affected ($P>0.10$) by inclusion of ENP.

Discussion

Recent reviews and studies (Hristov et al., 2013; Lee and Beauchemin, 2014) have shown that feeding nitrate to ruminants positively affect rumen fermentation mainly due to be able to reduce energy losses caused by methane production (van Zijderveld et al., 2010, 2011; Li et al., 2012; Newbold et al., 2014; Velazco et al., 2014; Lee et al., 2015a). Most of the studies reported a decrease on DM and nutrients intake in response to nitrate inclusion. However, some studies reported no negative effects of nitrate on DMI (Nolan et al., 2010; van Zijderveld et al., 2010, 2011).

As observed for the 84-d period, the DMI decrease. This response may be mainly due the replacement of soybean meal, which has a great acceptance by ruminants than encapsulated nitrate product. Regardless if the encapsulation process used in the nitrate source in the current study possibly could have minimized organoleptic issues, negative effects of low acceptance of nitrate salts by ruminants due the strong bitter taste are reported in the literature (Farra and

Satter, 1971; Lichtenwalner et al., 1973; Lund et al., 2014; Velazco et al., 2014; Lee et al., 2015b).

The range of decrease on DMI varies largely between studies which reported statistical effects due many reasons such as the number of experimental units, source and nitrate doses, feeding management, concentrate:roughage ratio of experimental diets, nitrogen source on control diet, species and animal category evaluated. Hulshof et al. (2012) reported a tendency to reduce 7% the DMI of steers fed 22 g kg^{-1} of nitrate, and Farra e Satter (1971) reported a dramatic decrease (26%) on DMI of cows fed 58 g kg^{-1} of NO_3^- on dietary DM. In our digestibility study, compared to CTL, the DMI tended to decrease 4.3%, 8.9% and 9.8% with ENP-1, ENP-2 and ENP-3 diets (6.5, 13.0 and 19.5 g kg^{-1} of NO_3^- on dietary DM, respectively).

Few studies have shown the effects of dietary nitrate on nutrients digestibility, and most of them reported no effects on DM apparent digestibility with the nitrate inclusion ranging from 19 to 27.4 g kg^{-1} compared to urea-based diets, using non-protected sources of urea (Nolan et al., 2010, 24.4 g kg^{-1}; van Zijderveld et al., 2011, 21 g kg^{-1}; Hulshof et al., 2012, 22 g kg^{-1}; Li et al., 2012, 19 g kg^{-1}) or encapsulated nitrate (El-Zaiat, 2013, 27.4 g kg^{-1}). In other hand, Lee et al. (2015a) reported linear increase on DM digestibility and a tendency to increase OM digestibility feeding up to 21.4 g kg^{-1} of supplemental encapsulated nitrate [5Ca(NO3)2·NH4NO3·10H2O] compared to an encapsulated urea. In contrast to all previous results, in our study a consistent quadratic response was observed to DM, OM, EE, CP and NDF when soybean meal was being replaced by nitrate.

The reasons for the quadratic responses of nutrients digestibility may be partially explained due a better synchronization between carbohydrates and N in the rumen (Lee at al., 2015a), microbial growth (Guo et al., 2009; Li et al., 2012) and mainly due the rumen passage rate, which increases with the increasing volume of rumen content (Schettini et al., 1999). Lee

et al. (2015b) reported a linear decrease on feed consumed within 6 h after feeding and consequential feed consumption rates (72.3%, 71.4%, 63.5% and 64.5% of total feed consumed on an as-fed basis for 0, 10, 20 and 30 g kg^{-1} of ENP, respectively) with increasing dietary encapsulated nitrate replacing encapsulated urea. These results indicate that there should be an adequate inclusion of ENP, from which the rumen concentration of nitrate and nitrite may inhibit the microbial fermentation activity (Marrais et al., 1988). According to the observations under the conditions of this study, the adequate inclusion to achieve a maximum nutrient digestibility might be close to 13 g of NO_3^- kg^{-1} DM (20 g kg^{-1} ENP) when replacing soybean meal on 90:10 concentrate: roughage finishing diets.

Digestibility of NDF seemed to be the most affected by ENP inclusion. Digestibility of NDF enhanced 17%, 24% and 3% from CTL to ENP-1, ENP-2 and ENP-3, respectively. However, NDF digestibility from ENP-2 (0.64) to ENP-3 (0.53) decreased by 17%. It was demonstrated that cellulolytic bacteria seems to be sensitive to nitrite rumen concentration (Marrais et al., 1988; Zhou et al., 2011; Asanuma et al., 2014), and a slow passage rate of nitrate-based diets (due lower DMI and feed consumption rate as reported by Lee et al., 2015b) can cause more nitrate release in the rumen. The nitrate available is rapidly reduced to nitrite reducing the fermentation of fiber components of the diet. Even though feeding nitrate may be able to increase starch digestibility (Lee et al., 2015b) and increase rumen population of starch-utilizing microorganisms (Asanuma et al., 2014), in this study the NFC digestibility was slightly increased up to 13 g kg^{-1} (i.e, ENP-2) of supplementary nitrate (0.806, 0.815, 0.831 and 0.782 g g^{-1} for CTL, ENP-1, ENP-2 and ENP-3 diets). In fact is desired achieve the maximum nitrate release in the rumen. However, the lowest rate of reduction of nitrite to ammonia can cause nitrite accumulation. The goal of feeding an encapsulated nitrate source (ENP) is achieve a balance between both processes.

Encapsulated nitrate product may reduced the nutrients intake, but enhanced the digestibility when replacing soybean meal up to 2% in finishing diets fed to feedlot young Nellore bulls. Therefore, it was effective to replace soybean meal source in the finishing diet of feedlot bulls.

Acknowledgments

First author thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/CNPq, Brazil) of Ministry of Education (MEC, Brazil) for the scholarship. Funding for this study was provided by GRASP Ind. e Com. LTDA (Curitiba, PR, Brazil).

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Table 1 - Ingredients and chemical composition of experimental diets with encapsulated nitrate product (ENP) as soybean meal replacer

Item	Experimental diets[1]			
	CTL	ENP-1	ENP-2	ENP-3
Ingredient, g kg^{-1} of DM				
Sugarcane bagasse *in natura*	100.0	100.0	100.0	100.0
Cottonseed cake	135.4	135.4	135.4	135.4
Ground corn	106.9	259.2	411.5	563.8
Ground sorghum	465.0	310.0	155.0	00.0
Soybean hulls	56.0	68.1	80.2	92.3
Soybean meal	112.7	91.3	69.9	48.5
ENP[2]	-	10.0	20.0	30.0
Magnesium sulfate ($MgSO_4.7H_2O$)	-	2.0	4.0	6.0
Mycotoxin binder[3]	2.0	2.0	2.0	2.0
Mineral[4]	22.0	22.0	22.0	22.0
Chemical composition (g kg^{-1} of DM)				
Dry Matter, DM[5] (g kg^{-1} as fed)	854	853	852	852
Organic Matter, OM	948	936	924	912
Ether Extract, EE	33	33	33	33
Crude Protein, CP	139	141	142	144
Neutral Detergent Fiber, NDF	290	290	290	291
Non-fibrous Carbohydrates, NFC	486	472	459	444

[1]CTL: control, soybean meal as protein source; ENP-1, 10 g kg^{-1} of encapsulated nitrate product (6.5 g kg^{-1} of NO_3^- of feed DM) in the dietary DM; ENP-2, 20 g kg^{-1} of encapsulated nitrate product (13.0 g kg^{-1} of NO_3^- of feed DM) in the dietary DM; ENP-3, 30 g kg^{-1} of encapsulated nitrate product (19.5 g kg^{-1} of NO_3^- of feed DM) in the dietary DM.

[2]Encapsulated nitrate product (g kg^{-1} of DM): 853 g kg^{-1} of DM in as-fed basis, 160 g kg^{-1} of N, 651 g kg^{-1} of NO_3^-, 191 g kg^{-1} of Ca.

[3]Mastersorb FM®, GRASP Ind. e Com. LTDA, Curitiba, Paraná, Brazil.

[4]Composition : Ca, 130 g kg^{-1} of DM; Na, 111 g kg^{-1} of DM; P, 60 g kg^{-1} of DM; S, 20 g kg^{-1} of DM; Mg, 6 g kg^{-1} of DM; Zn, 4000 mg kg^{-1} of DM; Cu, 1000 mg kg^{-1} of DM; Mn, 600 mg kg^{-1} of DM; F, 600 mg kg^{-1} of DM; Co, 80 mg kg^{-1} of DM; I, 80 mg kg^{-1} of DM; and Se, 8 mg kg^{-1} of DM (Ganho 60 corte, Ganho Nutrição Animal, Goiânia, Goiás, Brazil).

[5]Dietary DM adjusted to 630 mg kg^{-1} of DM in as-fed basis using tap water at the moment of feeding.

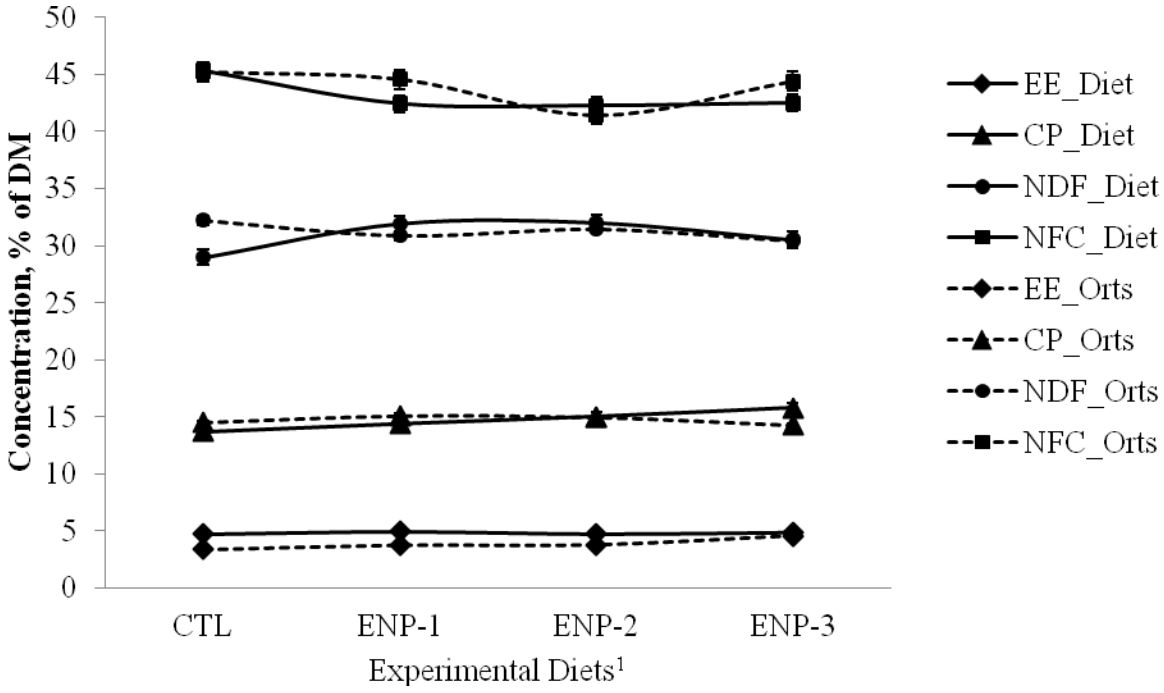

Figure 1 - Analyzed composition during the digestibility evaluation of diet offered and refused (orts) by young Nellore bulls fed finishing diets with encapsulated nitrate product (ENP) as soybean meal replacer ([1]CTL: control: soybean meal as protein source; ENP-1, 10 g kg^{-1} of encapsulated nitrate product (6.5 g kg^{-1} of NO3- of feed DM) in the dietary DM; ENP-2, 20 g kg^{-1} of encapsulated nitrate product (13.0 g kg^{-1} of NO3- of feed DM) in the dietary DM; ENP-3, 30 g kg^{-1} of encapsulated nitrate product (19.5 g kg^{-1} of NO_3^- of feed DM) in the dietary DM).

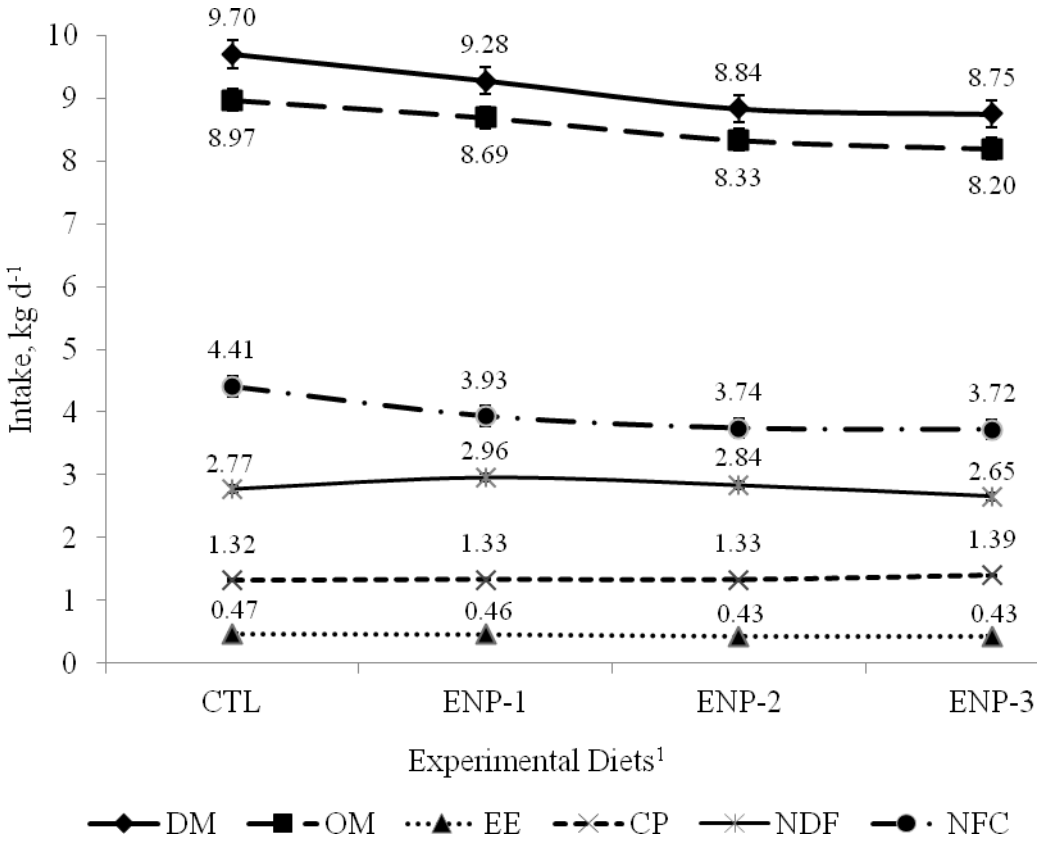

Figure 2 - Nutrients intake (mean ± SEM) of Nellore bulls fed encapsulated nitrate product (ENP) as soybean meal replacer. [1]CTL: control, soybean meal as protein source; ENP-1, 10 g kg^{-1} of encapsulated nitrate product (6.5 g kg^{-1} of NO3- of feed DM) in the dietary DM; ENP-2, 20 g kg^{-1} of encapsulated nitrate product (13.0 g kg^{-1} of NO3- of feed DM) in the dietary DM; ENP-3, 30 g kg^{-1} of encapsulated nitrate product (19.5 g kg^{-1} of NO_3^- of feed DM) in the dietary DM. Intake of NFC: $y = 4.288 - 0.225x$ ($R^2 = 0.83$, SEM = 0.18, linear effect, $P<0.05$); DM: $y = 9.636 - 0.328x$ ($r^2 = 0.94$, SEM = 0.41, linear effect, $P<0.10$). There were no linear or quadratic effects ($P>0.05$) on intake of OM, EE, CP and NDF.

Table 2 – Apparent digestibility of nutrients of finishing diets with encapsulated nitrate product (ENP) as soybean meal replacer in feedlot young Nellore bulls

Item	Experimental diets[1]				SEM[2]	Reg[3]	*P*-value[4]		
	CTL	ENP-1	ENP-2	ENP-3			α	B	LA
No. of pens (No. of bulls)	5 (30)	5 (30)	5 (30)	5 (30)	-	-	-	-	-
Apparent digestibility coefficients, g g^{-1}									
DM	0.687	0.715	0.744	0.688	0.01	L	0.63	-	0.02
						Q	0.01	0.01	0.20
OM	0.700	0.731	0.759	0.696	0.01	L	0.78	-	<0.01
						Q	<0.01	<0.01	0.16
EE	0.843	0.881	0.901	0.869	0.01	L	0.09	-	0.03
						Q	<0.01	0.01	0.57
CP	0.679	0.719	0.769	0.723	0.01	L	<0.01	-	<0.01
						Q	<0.01	<0.01	0.08
NDF	0.516	0.602	0.640	0.530	0.02	L	0.39	-	0.01
						Q	<0.01	<0.01	0.29
NFC	0.806	0.815	0.831	0.782	0.01	L	0.44	-	0.15
						Q	0.15	0.09	0.32

[1]CTL: control: soybean meal as protein source; ENP-1, 10 g kg^{-1} of encapsulated nitrate product (6.5 g kg^{-1} of NO3- of feed DM) in the dietary DM; ENP-2, 20 g kg^{-1} of encapsulated nitrate product (13.0 g kg^{-1} of NO3- of feed DM) in the dietary DM; ENP-3, 30 g kg^{-1} of encapsulated nitrate product (19.5 g kg^{-1} of NO_3^- of feed DM) in the dietary DM.

[2]SEM = Standard error of the mean.

[3]Regression; L: Linear; Q: Quadratic.

[4]α: *P*-value of F test in ANOVA for the coefficient associated to linear effect; β: *P*-value of F test in ANOVA for the coefficient associated to quadratic effect; LA: *P*-value of F test in ANOVA for the lack of adjustment of linear and quadratic models.

Dry matter (DM, g g^{-1}): $y = 0.682 + 0.066x - 0.021x^2$ ($R^2 = 0.94$)

Organic matter (OM, g g^{-1}): $y = 0.695 + 0.0734x - 0.024x^2$ ($R^2 = 0.85$)

Ether extract (EE, g g^{-1}): $y = 0.842 + 0.062x - 0.017x^2$ ($R^2 = 0.97$)

Crude protein (CP, g g^{-1}): $y= 0.674 + 0.080x - 0.020x^2$ ($R^2 = 0.86$)

Neutral detergent fiber (NDF, g g^{-1}): $y = 0.511 + 0.155x – 0.049x^2$ ($R^2 = 0.95$)

Capítulo 4- Considerações finais

No presente estudo, a substituição parcial do farelo de soja por nitrato encapsulado na dieta de terminação melhorou a eficiência alimentar de novilhos confinados. O consumo de matéria seca diminuiu em até 12,5% com a inclusão crescente do nitrato encapsulado, sem afetar o ganho em peso vivo e as características de carcaça dos novilhos.

A substituição entre 1 e 2% do farelo de soja por nitrato encapsulado aumentou a digestibilidade aparente da matéria seca, matéria orgânica, extrato etéreo, proteína bruta e da fibra em detergente neutro.

Nas inclusões estudadas, as analises de sangue evidenciaram que não existe risco de intoxicação dos animais e também não foram detectados concentrações de nitrato e nitrito na carne dos novilhos que poderiam ser prejudiciais para a saúde humana.

Dessa forma, o nitrato encapsulado se mostrou como uma opção segura para terminação de novilhos, capaz de promover resultados similares á dietas a base de farelo de soja.

Ressalta-se a importância da adaptação da microbiota ruminal com inclusão gradual do nitrato, manejo adequado de cocho (consumo à vontade, fornecido sempre no mesmo horário do dia), homogeneidade na mistura da ração total e equilíbrio nas concentrações de enxofre, energia e nitrogênio na dieta. Pesquisas avaliando maior quantidade de animais, dietas com diferentes relações concentrado:volumoso e períodos mais longos em alimentação são necessárias para auxiliar a interpretação das respostas uso de nitrato em substituição ao farelo de soja sobre o desempenho de animais ruminantes.