UNIVERSIDADE FEDERAL DA BAHIA
PROGRAMA DE DOUTORADO EM ZOOTECNIA

ÁCIDOS GRAXOS DE CADEIA MÉDIA NA ALIMENTAÇÃO DE RUMINANTES

JOCELY GOMES DE SOUZA

SALVADOR – BA
OUTUBRO- 2017

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Tese apresentada ao Programa de Pós-Graduação em Zootecnia, da Universidade Federal da Bahia, como requisito parcial para a obtenção do título de Doutor em Zootecnia.

Área de Concentração: Produção Animal

Orientador: Dr. Cláudio Vaz Di Mambro Ribeiro

Coorientador: Dr. João Paulo Ismério dos Santos Monnerat

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ÁCIDOS GRAXOS DE CADEIA MÉDIA NA ALIMENTAÇÃO DE RUMINANTES

Jocely Gomes de Souza

Tese defendida e aprovada para obtenção do grau de Doutor em Zootecnia

Salvador, 26 de outubro de 2017

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DADOS CURRICULARES DA AUTORA

JOCELY GOMES DE SOUZA, filha de Joana Gomes de Oliveira, nasceu em Cuiabá-MT, no dia 04 de junho de 1986. Em Agosto de 2006, iniciou o curso de Zootecnia na Universidade Federal de Mato Grosso- Campus Universitário Sinop, finalizando o mesmo em fevereiro de 2011. Em fevereiro de 2011, iniciou o curso de Pós-Graduação Mestrado em Ciência Animal, na Universidade Federal de Mato Grosso- Cuiabá, sob a orientação do professor Dr. André Soares de Oliveira. Em Agosto de 2011, submeteu-se à banca examinadora para defesa da dissertação de mestrado. Em 2013, iniciou o curso de Pós-graduação Doutorado em Zootecnia pela Universidade Federal da Bahia- UFBA, na área de produção animal no qual foi bolsista da Fundação de Amparo ao Pesquisador do Estado da Bahia (FAPESB) desenvolvendo sua pesquisa com Nutrição de Ruminantes sob a orientação do Dr. Cláudio Vaz Di Mambro Ribeiro, em 2015 foi bolsista do Programa de Doutorado Sanduíche no Exterior (PDSE) da CAPES, pelo qual passou o período de um ano na Pennsyvalnia State University nos EUA sob a orientação do Dr. Kevin J. Harvatine e apresentou a defesa de tese na UFBA em Outubro de 2017.

Epígrafe

"When the dreams you are dreaming come to you, and when the work you put in is realized, let yourself feel the pride, but always stay humble and kind."

Lori McKenna

Dedico:

Este trabalho é dedicado à minha mãe Joana, minha avó Marta, aos meus irmãos Glória, Kleberson e Alessandro (*in memorian*). A minha família pela extrema importância na minha vida.

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LISTA DE ABREVIATURAS

AGCC – Ácido graxo de cadeia curta
AGCL – Ácido graxo de cadeia longa
AGCM – Ácido graxo de cadeia média
AGNE – Ácido graxo não esterificado
AGS – Ácido graxo saturado
AGV – Ácidos graxos voláteis
AL - ácido linoleico
CLA- Ácido linoleico conjugado
CMS - Consumo de matéria seca
CNF - Carboidratos não-fibrosos
FDN - Fibra em detergente neutro
g - Gramas
LDL – Low density lipoprotein
MFD - depressão da gordura do leite
MO – Matéria orgânica
MS - Matéria seca
NDT - Nutrientes digestíveis totais
PB - Proteína bruta
R^2 - Coeficiente de determinação
RA - ácido rumenico
SA – ácido esteárico
Spp. – Spécie

LIST OF ABBREVIATIONS

°C – Celsius degrees
ADF – Acid detergente fiber
ADICP - Acid detergent insoluble crude protein
AL – allantoin
AOAC - Association of analytical chemists
AP - Absorbed purines
apNDF – neutral detergent fiber corrected for ash and protein
AUTO – Automatic
BH – biohydrogenation
BHBA – Beta- hydroxybutyrate
BW – Body weight
CAD - apparent digestibility coefficient
CLA – conjugated linoleic acid
CON – control
COO – coconut oil
CP – crude protein
d - Day
dL – Deciliter
DM – Dry matter

DMI - Dry matter intake
DOMR – Digestible organic matter fermented in the rumen
EE – Ether extract
FA – fatty acids
FAME - Fatty acid methyl esters
FFA – Free fatty acids
GC – Gas chromatography
h – hours
HDL – High density lipoprotein
HMTBa - 2-hydroxy-4-(methylthio) butanoate
Kg - kilogram
L – Liters
LA – Linoleic acid
m – Meters
MCFA – Medium chain fatty acid
MFD - Milk fat depression
mg - Milligrams
min – Minutes
mm- Millimiters
mmol – Millimoles
MN – microbial nitrogen
NB – Nitrogen balance
NDF - Neutral detergente fiber
NDICP - Neutral detergent insoluble crude protein
NE – Net energy
NEFA – non esterified fatty acid
NFC – non fiber carbohydrate
NRC - Nutrient Research Council
OM - Organic matter
PCA – Principal components analysis
PD - Purine derivatives
peNDF – Physically effective neutral detergent fiber
PUFA – polyunsaturated fatty acid
R^{2-} Coefficient of determination
RA- Rumenic acid
RMSE – root mean square error
SA- Stearic acid
SD – Standard deviation
SEM – Stardard error mean
TDN - - Total digestible nutrientes
TFA – Total fatty acids
TNT – Tissue non tissue
TP - Total purine derivatives
UA –Uric acid
UFA – Unsaturated fatty acids
UV – Urinary volume
VA – Vaccenic acid; ácido vacênico
VO – visual observation

SUMÁRIO

Ácidos graxos de cadeia média na alimentação de ruminantes

RESUMO GERAL

Com o objetivo de se avaliar os efeitos da dieta sobre a ruminação e, da ruminação sobre a gordura do leite, uma meta-análise foi conduzida. E com o intuito de se estudar o efeito dos ácidos graxos de cadeia média sobre o metabolismo e produção animal, dois experimentos foram conduzidos. No primeiro experimento foi conduzida uma meta-análise com o objetivo de entender a variação do tempo de ruminação e comportamento alimentar e a relação com a gordura do leite. A meta-análise incluiu 115 artigos em inglês entre os anos de 1986 e 2017, que reportaram dados de ruminação com vacas lactantes da raça holandesa. A média para o tempo total de ruminação foi de 449 min/d. Foi observada uma relação quadrática entre o tempo de ruminação (min/d) e a produção de leite (kg/d; R^2= 0,22), %gordura no leite (R^2=0,08). O modelo da análise multivariada para predição do tempo total de ruminação incluiu produção de leite, concentração de gordura e CMS (Tempo total de ruminação, min/d= 96,73 + 9,96 x leite + 64,10 x % gordura – 4,86 x CMS; total R^2= 0,37e RMSE = 79,72). O segundo experimento teve como objetivo avaliar o efeito da suplementação de óleo de coco sobre as taxas de biohidrogeção (BH) do ácido linoleico (AL) utilizando uma abordagem *in vivo*. Foram utilizadas quatro vacas da raça holandesa em um delineamento cross over com dois tratamentos. No tratamento controle foi infundido uma única dose de 200 g de óleo de cartámo e 12 g de C17:0 via canula ruminal. O tratamento com óleo de côco (COO) foi infundido 430 g de óleo de côco via canula ruminal + uma única dose de 200 g de óleo de cartámo e 12 g de C17:0 via canula ruminal. O COO não afetou o consumo de matéria seca (CMS), a produção de leite e a gordura do leite. O COO não afetou a taxa de desaparecimento do C:17:0 com média de 6,9%/h e 7,5%/h para os tratamento controle e COO respectivamente, mas aumentou o pH ruminal (6,6 vs 6,5) e a concentração de C18:1 *trans*-11 no leite. O terceiro experimento foi conduzido utilizando 20 borregos confinados, e foi avaliada a substituição da torta de girassol pela torta de dendê sobre o consumo de nutrientes, digestbilidade, parâmetros sanguíneos, balanço de nitrogênio e síntese de proteína microbiana. Os tratamentos foram: 100% torta de girassol; 66% de torta de girassol e 33%

torta de dendê; e 33% torta de girassol e 66% torta de dendê e 100% de torta de dendê. A inclusão de torta de dendê afetou cúbicamente (P≤0,05) o consumo de todos os nutrientes, a digestibilidade da MO e NDT e o nitrogênio retido. A substituição da torta de girassol pela torta de dendê aumentou linearmente a concentração sanguínea de NEFA.

PALAVRAS CHAVE: bio-hidrogenação, gordura, ruminação

Medium chain fatty acids in ruminant feed

ABSTRACT

In order to evaluate the effects of diet on rumination and rumination on milk fat, a meta-analysis was performed. And in order to study the effect of medium chain fatty acids on metabolism and animal production, two experiments were performed. In the first experiment, a meta-analysis was conducted to understand the variation of rumination time and feeding behavior and its relationship with milk fat. The meta-analysis included 115 peer reviewed papers in English between 1986 and 2017, which report rumination data with Holsteins lactating cows. The total rumination time averaged 449 min/d. A quadratic relationship between rumination time (min/d) and milk yield (k/d; R^2 = 0.22), milk fat concentration (R^2 = 0.08) was observed. The final multivariant model to predict total rumination time included milk yield, milk fat concentration and DMI (Total rumination time, min/d = 96.73 + 6.96 × milk + 64.10 × fat% – 4.86 × DMI; Total R^2 = 0.37 and RMSE = 79.72). The objective of the second study was to evaluate the effect of coconut oil (COO) on the on biohydrogetion rates (BH) of linoleic acid (LA) using an *in vivo* approach. There were four Holsteins cows in a cross over desing with two treatments. The control treatment was infused in a single bolus dose of 200 g of safflower oil and 12 g C17:0 via ruminal cannula. The treatment with coconut oil (COO) was infused 430 g of coconut oil via ruminal cannula + a single dose of 200 g of safflower oil and 12 g C17:0 via ruminal cannula. The COO did not affect DMI, milk yield and milk fat. The COO did not affect the disappearance rate of C17:0 and averaged 6.98% / h and 7.54% / h for the control and COO treatments respectively. It increased ruminal pH (6.56 vs 6.47) and C18:1 *trans*-11 concentration in milk. The third experiment was conducted using 20 feedlot lambs, to evaluate effect of the replacement of sunflower cake for palm cake on nutrients intake and digestibility, blood parameters, nitrogen balance and microbial protein synthesis. The treatments were: 100% sunflower cake; 66% sunflower cake and 33% palm cake; 33% sunflower cake 66% palm cake and, 100% of palm cake. The Palm cake inclusion cubically affected ($P \leq 0.05$) all the nutrients intake, digestibility of OM and TDN and the nitrogen retained. The replacement of sunflower cake by palm cake linearly increased NEFA blood concentration.

The inclusion of palm cake decreased the intake of EE, non fiber carbohydrate (NFC) and total digestible nutrients (TDN). Also decreased the organic matter, crude protein, NFC and TDN digestibility. The nitrogen retained and NEFA concentration in blood were decreased.

KEY WORDS: bohydrogenation, fat, rumination

1.0 INTRODUÇÃO GERAL

Os ácidos graxos são componentes energéticos na nutrição animal e sua ingestão é na forma esterificada principalmente de triglicerídeos, a qual também é a forma de reserva em que se encontram os ácidos graxos no tecido adiposo do animal. Os triglicerídeos são formados por três moléculas de ácidos graxos condensadas a uma molécula de glicerol. No rúmen os triglicerídeos precisam ser hidrolisados primeiramente para serem biohidrogenados. Na bio-hidrogenação (BH) ocorre a isomerização das duplas ligações e adição de hidrogênio nas ligações duplas, o que caracteriza o rúmen como o maior determinante da composição de ácidos graxos nos produtos de ruminantes.

A regulação da composição lipídica do tecido adiposo e muscular (carne e do leite de ruminantes) tem sido revisada em vários trabalhos. É conhecido que o perfil de ácidos graxos da carne e do leite de ruminantes podem ser manipulados pelas fontes de ácidos graxos dietéticos para obter produtos ricos em específicos ácido graxos (GIVENS et al., 2006), dentre estes o ácido linoleico conjugado (CLA).

O CLA refere-se a um grupo de isômeros posicionais e geométricos do ácido linoleico, porém com ligações duplas conjugadas. Dois isômeros de CLA, o *cis*-9, *trans*-11 e o *trans*-10, *cis*-12, foram descritos como os isômeros mais ativos biologicamente (BAUMAN et al., 2001), provenientes da BH parcial de ácidos graxos no rumen (BERNARD et al., 2009).

O CLA *cis*-9, *trans*-11 é o isômero majoritário em produtos e derivados de ruminantes, com potencial para promover melhorias na saúde humana (PARIZA et al., 2001). Geralmente, a isomerização do ácido linoleico produz *cis*-9 *trans*-11 CLA e *trans*-11 18:1 (ácido rumênico; PALMQUIST et al., 2005), mas sob específicas dietas, o ambiente ruminal é alterado e uma parte da BH ocorre por uma via que produz o CLA *trans*-10, *cis*-12 e o *trans*-10 18:1. O CLA *trans*-10, *cis*-12 têm efeito sobre a depressão da gordura do leite (BAUMGARD et al., 2000) e é comumente relatado quando ocorre o fornecimento de dietas mais fermentáveis, diminuindo o pH ruminal e a ruminação.

A alteração nas rotas de BH podem resultar em mudanças nas taxas de BH ou inibição de um passo específico (GRIINARI e BAUMAN, 1999). Várias manipulações dietéticas podem resultar em alteração de rota, tais como: aumento da disponibilidade ruminal de ácidos graxos insaturados, aumento da fermentação da dieta através de

concentrados, fontes de amido rapidamente fermentável, diminuição do fornecimento de fibra efetiva com forragem de partículas pequenas, fornecimento de ionoforos, dentre outros (VAN SOEST, 1994). Muitas dessas modificações dietéticas podem causar impacto na ruminação, e é bem reconhecida a importância do funcionamento normal do rúmen no desempenho animal.

Os ácidos de cadeia média (AGCM) exercem efeito antimicrobiano (Hristov et al., 2011), provavelmente pela dissociação desses ácidos graxos na célula bacteriana (GOEL et al., 2012). Altos níveis de ácido linoleico inibem a BH do *trans*-11 18:1 (McKNAIN et al., 2010; POLAN et al., 1964), sugerindo que as bactérias do segundo passo da BH são mais sensíveis aos ácidos graxos insaturados. As propriedades físicas dos ácidos graxos insaturados e de cadeia média são similares. Dessa forma, é possível que possam atuar de maneira semelhante sobre os microrganismos ruminais, aumentando o fluxo de intermediários da BH para o duodeno.

O fornecimento de AGCM poderia reduzir a BH total de fontes de ácidos graxos insaturados, resultando em um possível aumento de CLA nos produtos de ruminantes. Porém pouco se sabe sobre a interação entre diferentes perfis de ácidos graxos. Assim, destaca-se a necessidade de estudar o fornecimento de fontes AGCM e seu efeito no metabolismo ruminal, bem como o efeito da alteração das dietas na gordura do leite.

2.0 REVISÃO DE LITERATURA GERAL

2.1 Metabolismo de ácidos graxos no rúmen

2.1.1 Mecanismos de Hidrólise e bio-hidrogenação

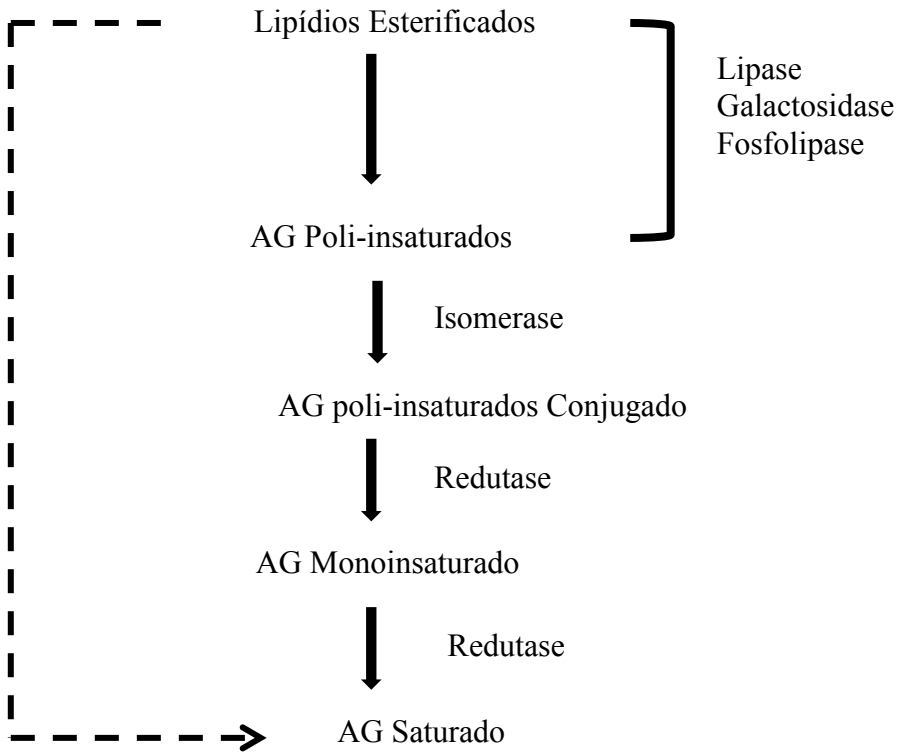
O ambiente ruminal é caracterizado por conter 10^{10} de bactérias, 10^{7} protozoários, 10^{6} de fungos e leveduras /mL de líquido ruminal, temperatura entre 38 a 39 ºC, pH entre 6,0 e 6,7 e potencial redox de -150 a -350 mV. Alterações destas condições podem influenciar a população microbiana e os seus produtos de fermentação (BUCCIONI et al., 2012).

No ambiente ruminal existem dois grandes grupos de bactérias, as Gram positivas (+) e Gram negativas (-). As bactérias Gram negativas possuem membrana externa, as tornando mais resistente aos componentes do ambiente ruminal.

Determinados ácidos graxos, especialmente os poli-insaturados, são tóxicos aos microrganismos ruminais. Os mais susceptíveis são as bactérias Gram (+), metanogênicas e protozoários. A toxicidade é relacionada à natureza anfipática dos ácidos graxos, isto é, àqueles que são solúveis tanto em solventes orgânicos como em água, são mais tóxicos. Tais ácidos incluem AGCM e ácidos graxos poli-insaturados de cadeia longa. Assim a toxicidade parece estar naqueles ácidos graxos que tem solubilidade maior em água e membranas celulares, com potencial para romper as estruturas das membranas. Ácidos graxos se associam com superfícies hidrofóbicas das partículas de alimento, o que explica a baixa toxidade das gorduras quando o animal é alimentado com rações ricas em volumosos (BERCHIELLI et al., 2011).

O primeiro passo do metabolismo de lipídios no rúmen é a hidrólise (figura 1), que é realizada majoritariamente pelas bactérias. Os protozoários não estão envolvidos em grande parte na hidrólise, com exceção em fosfolípidos (DOREAU e FERLAY, 1994). A produção das enzimas lipolíticas se dá pela bactéria ruminal *Anaerovibrio lipolytica* (HARFOOT, 1978).

Figure 1. Esquema de lipólise e bio-hidrogenação. Adaptado de Jenkins (1993)



A bio-hidrogenação ruminal pode ser simplesmente descrita como uma função da disponibilidade de ácido graxo, tempo de retenção ruminal e capacidade de hidrogenação (HARVATINE e ALLEN, 2006). Embora a bio-hidrogenação seja alta acima de 90%, a intensidade desse processo depende das características das fontes de ácidos graxos, tempo de retenção dessas fontes no rúmen e características da população microbiana (ALLEN, 2000).

É ainda sustentada a teoria de que a bio-hidrogenação é uma forma de controle da concentração ruminal de H^+ (ULYATT et al., 2002). Porém Jenkins et al. (2008) demonstraram que a remoção de hidrogênio através da síntese de metano é cerca de 25 vezes mais eficiente do que via bio-hidrogenação, e que embora as bactérias necessitem de hidrogênio para realizar a bio-hidrogenação, somente 1 a 2% do hidrogênio metabólico é usado para este processo (CZERKAWSKI, 1984).

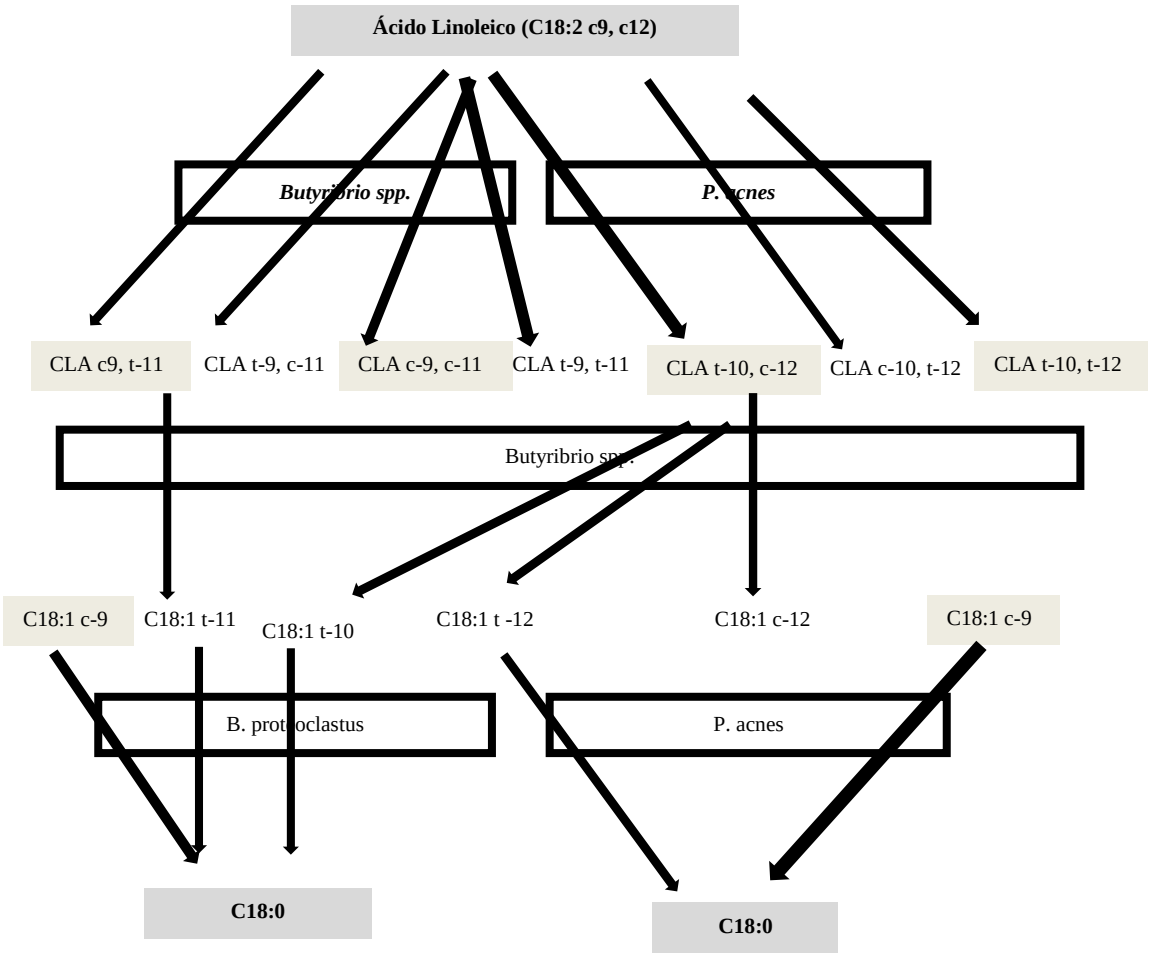
Porém a teoria mais aceita atualmente afirma que a hidrogenação dos ácidos graxos atua como uma ferramenta de controle da fluidez das membranas celulares dos microrganismos e, consequentemente, da sua permeabilidade (DEHORITY, 2003). Nessa teoria é sugerido que os ácidos gráxos insaturados inibem o crescimento bacteriano e a biodrogenação reverte esse efeito, e tem sido desenvolvida uma série de experimentos baseado nessa teoria.

Estudos de Mckain et al. (2010) indicam que há provavelmente três mecanismos para o metabolismo de ácidos graxos insaturados nas bactérias ruminais que fazem a bio-hidrogenação, um que reduz os isômeros geométricos de 9,11 de CLA para *trans*-11-18:1, outro que reduz isômeros geométricos de 10,12 de CLA a uma mistura de 10 e de 12-18:1 de ácidos graxos, e uma terceira, apenas encontrado em *Butyrivibrio proteoclasticus*, que reduz uma gama de ácidos graxos monoenóicos incluindo *trans*-10 C18:1, a C18:0. Os estudos indicaram também que *Propionibacterium acnes* hidrogena C18:1 para obter substratos de C18:0 como um produto final (Figura 2). Estes estudos do rúmen também fornecem informações sobre os prováveis caminhos no metabolismo de ácidos graxos (DEVILLARD et al., 2007).

As espécies de bactérias ruminais mais ativas envolvidas na bio-hidrogenação do C18 bio-hidrogenação pertencem ao grupo "Butyrivibrio", onde todas as bactérias formam o ácido linoleico conjugado (CLA) a partir de ácido linoleico, enquanto que apenas *Clostridium proteoclasticum* é capaz de converter C18:1 *trans*-11 a C18:0 (KEMP et al.,

1975; POLÁN et al., 1964). *C. proteoclasticum* foi reclassificado como *Butyrivibrio proteoclasticus* (MOON et al., 2008).

Figure 2. Papel da Butyrivibrio spp., Propionibacterium acnes e Butyrivibrio proteoclasticum sobre o metabolismo dos ácidos graxos insaturados linoleico e oleico ácidos. Fonte: McKain et al. (2010) e Wallace et al. (2006), modificado por Buccioni et al. (2012).



2.3 Cinética de bio-hidrogenação

Em física, a cinética é um termo utilizado no ramo da mecânica clássica que se refere à relação entre o movimento e suas causas, ou seja, forças e torques.

Estimativas das taxas de bio-hidrogenação ruminal pode ser uma ferramenta útil para quantificar relação entre fatores dietéticos ou ruminal e o perfil de ácidos graxos que chegam ao duodeno dos ruminantes (RIBEIRO et al., 2007).

A via principal para a bio-hidrogenação do ácido linoleico é a seguinte (HARFOOT e HAZLEWOOD, 1997):

1) Acido linoleico (LA): é primeiro isomerizado para produzir *cis*-9, *trans*-11 C18:2 ou Ácido linoléico conjugado, também conhecido como ácido rumenico (AR);

2) Acido rumenico é biohidregenado para produzir *trans*-11 C18: 1 ou ácido vacênico (AV);

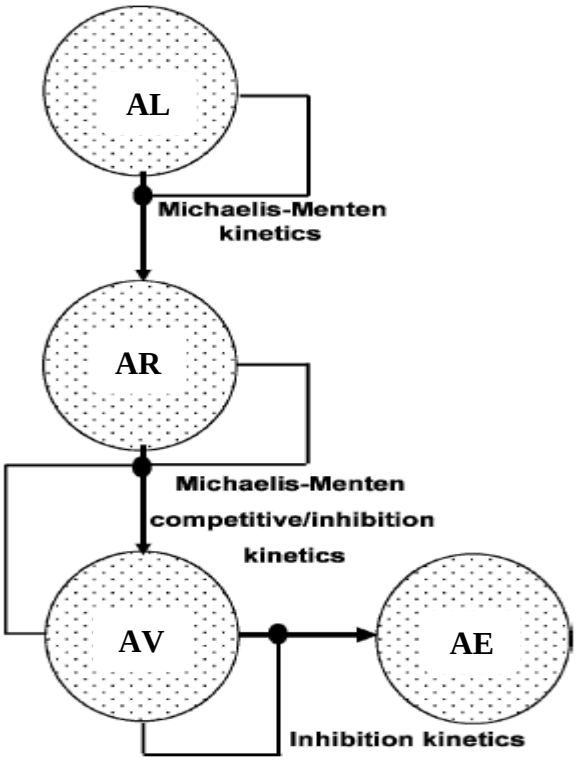
3) Acido vacênico é biohidrogenado a C18: 0 ou ácido esteárico (AE).

O modelo da cinética de bio-hidrogenação do ácido linoleico á ácido esteárico descrito por Moate et al. (2008) na Figura 3 impõe conceito de que a matéria é conservada e conhecimentos sobre estequiometria nos processos cinéticos, onde LA (t), AR (t), AV (t) e AE (t) são as concentrações (Mg / L) de AL, AR, AV e AE respectivamente em tempo (horas após o início da incubação). As constantes de Michaelis-Menten Vmax e constantes de afinidade são constantes de primeira ordem, que ocorre enquanto que uma inibição constante com unidades de mg / L. Quando a quantidade de AV (t) é muito pequena com respeito constante de inibição, então AV é biohidrogenado para AE Em um processo de quase primeira ordem. Contudo, uma vez que a AV aumenta, inibe sua própria bio-hidrogenação. Sucintamente, quando a concentração do AV é igual ou superior a constante de inibição, a bio-hidrogenação do AV é completamente inibida.

Krueger et al. (2010) em dois experimentos avaliando os efeitos do glicerol (0, 2 e 20% MS) sobre a produção de ácido graxo voláteis (AGV) e taxas *in vitro* cinética da fermentação de feno de alfafa, demonstraram 48% e 77% de redução nas taxas de acumulo de incubações de ácidos graxos livres em relação aos suplementados com 2% e 20% de glicerol e comparação aos controles. Incubações com feno de alfafa *in vitro* demonstraram que níveis crescentes de glicerol (0, 5, 10, 20 e 40% Glicerol/ %MS) não afetam a digestibilidade da FDN do feno (45,73; 53,48; 51,20; 50,15 e 48,40%). Além disso, quantidades crescentes de glicerina diminuíram a relação acetato e propionato no rúmen. Estes resultados sugerem que a inibição da degradação bacteriana do glicerol pode

promover passagem ruminal de lipídios totais, proporcionando assim maior proporção de gordura insaturada benéfico para incorporação em produtos de carne bovina.

Figure 3. Esquema da isomerização do ácido linoleico (AL), seguido pela fase de bio-hidrogenação do ácido rumenico (AR) à ácido vacênico (AV), e depois ácido esteárico (AE) a etapa final da bio-hidrogenação. Fonte: Moate et al. (2008)



2.4 Ácido Linoleico conjugado (CLA)

Há cerca de 40 anos começou a ser dada maior ênfase ao papel da dieta na saúde e em doenças crônicas (tabela 1), sendo que considerável interesse foi voltado para os lipídios dos alimentos (LIMA et al., 2000).

Existem evidências de que O CLA *cis*-9, *trans*-11 18:2 suprime quimicamente o desenvolvimento do tumor induzido em animais (PARODI, 1999). Alimentos derivados de ruminantes têm uma importante contribuição para o consumo total de gordura

(GIVENS e SHINGFIELD, 2004) e é a principal fonte de CLA na dieta humana (LAWSON et al., 2001).

O CLA foi encontrado em maior concentração em uma ampla variedade de alimentos com carne e leite de ruminantes por Chin et al. (1992). Kepler e Tove (1971), descobriram que o primeiro intermediário na bio-hidrogenação do ácido linoleico pelas bactérias ruminais *Butyrivibrio fibrisolvens*, é o C18:2 *cis*-9, *trans*-11, o que sugere que os ruminantes podem converter o ácido linoleico em CLA explicando maior concentração de CLA em produtos de ruminantes.

O primeiro grupo a relatar o efeito anticarcinogênico do CLA foi o grupo do Dr. Pariza's da universidade de Wisconsin-Madison, nos Estados Unidos (HA et al., 1987; PARIZA e HARGRAVES, 1985; YAVARI et al., 2010).

O CLA tem demonstrado potencialidade para reduzir o câncer em vários órgãos de animais, como a pele, o estômago, o cólon, a glândula mamária e o fígado (BHATTACHARYA et al., 2006; KELLEY et al., 2007)

Uma explicação para a variedade de atividades biológicas do CLA é que o CLA é uma mistura de isômeros geométricos e posicionais, com duplas ligações em [9,11], [10,12], [8,10], [7,9] e [11,13]. Embora uma série de isômeros do CLA sejam encontrados em alimentos (KRAMER et al., 1998), o principal foco de pesquisa está nos dois principais isômeros, *cis*-9, *trans*-11 e *trans*-10, *cis*-12. É importante ser apontado que o CLA ocorre naturalmente e consiste principalmente no isômero *cis*-9, *trans*-11 (> 80%) presentes nos alimentos, tais como carne, leite e lácteos (CHIN et al., 1992).

Estes dois isômeros podem ter diferentes efeitos sobre o metabolismo e funções celulares e podem atuar através de diferentes vias de sinalização celular (WAHLE et al., 2004). A maioria dos estudos dos efeitos do CLA são sobre a perda ou ganho de gordura e composição corporal, pois o CLA supostamente melhora a oxidação de gordura, a termogênese; reduz a lipogênese e a diferenciação e proliferação dos pré-adipócitos (WANG e JONES, 2004). O CLA ainda têm efeitos anticarcinogênicos, diminuição de risco às doenças cardiovasculares, atua sobre a sensibilidade à insulina e sobre a função imunitária em estudos com animais (WAHLE et al., 2004).

Além de o CLA ser produzido naturalmente no rúmen por bactérias fermentativas, como explicado acima. Os ruminantes também sintetizam o CLA a partir de trans-11-18:1 (ácido vacênico) pela enzima delta-9-dessaturasse, e este é o ácido graxo

monoinsaturado predominante no tecido adiposo de ruminantes (DHIMAN et al., 2005; PARODI, 2003).

Table 1. Histórico dos estudos com ácidos graxos e seus efeitos na saúde humana

Estudo	Período	Resultados	Autores
Ausência de Gordura	1929-19230	Diminuição do crescimento, reprodução, doença renal, fígado gorduroso	Bur et al. (1989)
Ácidos graxos Saturados	1956	Aumento do colesterol sérico, redução do consumo de gordura. Ácidos graxos insaturados não promoviam hipercolesterolemia	Ahrens et al. (1957)
PUFA : AGS	1956 a 1966	Dietas com baixa proporção P:S se associava à alta incidência de doenças cardiovasculares	Grundy e Bonanome (1987)
AGS e Colesterol	1959 a 1971	AGS e colesterol aumentam doenças cardiovasculares e PUFA exercia diminuição	Tuperinen et al. (1979)
PUFA : AGS	1980	O risco de morte era inversamente proporcional à ingestão de PUFAs e diretamente proporcional a ingestão de colesterol	Shekelle et al. (1981)
n-3 oriundo de óleo de peixe e dieta normal	1989	Redução da mortalidade em 29% consumindo óleo de peixe (200 a 400g/semana)	Burr et al. (1989)
Extratos de carne moída (CLA) em ratos	1985-1987	Primeiros relatos que o CLA exerce efeitos anticarcinogênico	Pariza e Hargraves (1985) Ha et al. (1987)
c9,t11 CLA, 3g/d 13 semanas em homens com obesidade abdominal	2004	CLA afeta negativamente a resistência à insulina e a peroxidação lipídica	Riserus et al. (2004)
ALA, CLA e VA Homens e mulheres com Hipercolesterolemia (5,68–7,49 mmol/L)	2013	A atuação do CLA é sinérgica com outros AG. A ingestão de um alimento é capaz de afetar o sistema endocanabinóide	Pintus et al. (2013)
AGS do leite	2004 a 2013	ÁGS não influenciam no aumento de LDL e estão inversamente relacionados com doenças cardíacas	Patterson et al. 2013; Sjogren et al. 2004

PUFA = Ácido graxo polinsaturado; AGS= Ácido graxo saturado; PUFA:AGS = relação PUFA:AGS; CLA= Ácido linoleico conjugado; ALA= Ácido alfa linolênico; VA= Ácido vacênico

Elaboração: Próprio autor

2.5 Suplementação de Ácidos Graxos em dietas de ruminantes

No rúmen, os AGV são produzidos por fermentação da matéria orgânica (MO). Os AGV´s predominantes no fluido ruminal são os ácidos acético, propiônico e butírico, e presente em quantidades relativamente pequenas o isobutírico, valérico, isovalérico, 2-metilbutírico e outros geralmente presente em pequenas quantidades relativas (DIJKSTRA, 1994). Sendo que o ácido acético (C2), ácido propiônico (C3) e ácido butiríco (C4) produzidos em proporções molares de 6: 2: 1, são oriundos principalmente da fermentação ruminal de forragem e outros substratos celulósicos (SINGHANIA et al., 2013).

Estes ácidos graxos com menos de 12 átomos de carbonos são classificados como ácidos graxos de cadeia curta (AGCC) (DUBOIS et al., 2007). O comprimento da cadeia carbônica, ligações saturadas ou insaturadas, são diferenças estruturais e afetam a solubilidade de ácidos graxos em água (PAPAMANDJARIS et al., 1998).

Devido ao menor peso molecular de AGCM em relação aos AGCL ocorre ação mais rápida da lipase pancreática e no intestino delgado, e consequentemente, aumenta a taxa de digestão de AGCM (BACH e BABAYAN, 1982). Além de que a oxidação dos AGCM é rápida e pouco influenciada por hormônios e status nutricional do animal (McGARRY e FOSTER, 1971). Isso ocorre porque ácidos graxos com comprimento de cadeia de 12 carbonos ou menos entram nas mitocôndrias sem a ajuda de transportadores de membrana (NELSON e COX, 2014).

Estudos indicam a utilização dos AGCM como potentes redutores de metano em proporções inferiores a 3,0% de AGCM (C12:0 e C14: 0), podem reduzir em 50% a emissão de metano *in vivo* (MACHMULLER, 2006). Da mesma forma, os fornecimentos de ácidos graxos insaturados também estão associados à redução na produção de metano no rúmen, por exercer ação deletéria sobre as metanogênicas e protozoários e também por consumir hidrogênio no processo de bio-hidrogenação (MACHMÜLLER et al., 1998).

Outro efeito dos lipídeos sobre a fermentação ruminal é sobre a redução da fermentação dos carboidratos estruturais. Sendo que o grau de redução depende das fontes de fibra e de lipídeos (VALADARES FILHO et al,. 2006).

2.5.1 Fontes de AGCM

O óleo de coco (*Cocus nucifera*) e óleo de dendê (*Elaeis guineenses*) têm um perfil de ácidos graxos semelhante (Tabela 2), incluindo uma maioria de ácidos graxos saturados (AGS), principalmente o ácido láurico (C12:0) (DUBOIS et al., 2007).

O objetivo de se concentrar em aumento dos intermediários e evitar a completa bio-hidrogenação dos ácidos graxos insaturados, levou à persistência de pesquisas sobre muitas prováveis rotas de bio-hidrogenação (JENKINS et al., 2007) e experimentos com diferentes dietas ao longo das décadas (tabela 3). Porém ainda existem grandes lacunas sobre o efeito das dietas na composição do perfil de ácidos graxos em produtos de ruminantes.

Table 2. Perfil de ácidos graxos em óleos vegetais.

	AGCL					**AGCM**		
Ácidos graxos (%)	OCa	OG	OS	OCz	Ooli	OC	ODe	Opalm
C8:0	-	-	-	-	-	7,6	4,1	0,1
C10:0	-	-	-	0,01	-	5,5	3,7	0,1
C12:0	-	0,02	-	-	-	47,7	46	0,4
C14:0	0,1	0,09	0,1	-	-	19,9	17,8	1,1
C16:0	6,7	6,2	10,8	4,6	16,5	-	8,4	43,8
C18:0	2,4	2,8	3,9	1,7	2,3	2,7	1,6	4,4
C22:0	-	-	0,3	-	0,15	-	-	0,1
C18:1 (n-9)	11,5	28	23,9	63,4	66,4	6,2	16,4	39,1
C18:2 (n-6)	79	62,2	52,1	19,6	16,4	1,6	3,1	10,2
C18:3 (n-3)	0,15	0,16	7,8	1,2	1,6	-	-	0,3
C18:3 (n-6)	-	-	-	-	-	-	-	-
AGS	9,3	9,4	15,7	6,3	19,4	92,1	81,9	50,4
MUFAs	11,6	28,3	24,2	72,8	68,2	6,2	16,4	39,4
PUFAs	79,1	62,4	59,8	20,9	18	1,6	3,1	10,5

AGCL= fontes de ácidos graxos de cadeia longa; AGCM= fontes de ácidos graxos de cadeia média; Oca= óleo de cártamo; OG= óleo de girassol; OS= óleo de soja; OCz= óleo de colza; Ooli= óleo de oliva; OC= óleos de coco; ODe= óleo de dendê; Opalm= óleo de palma. Fonte: Dubois et al. (2007) e Orsavova et al. (2015).

A inclusão de AGCM em dietas de ruminantes vêm sendo explorado pelo potencial significativo de reduzir a emissão metano entérico e aumentar eficiência da utilização do Nitrogênio (N) (HRISTOV e JOUANY, 2005).

Table 3. Efeito dos ácidos graxos da dieta sobre o produto final de animais ruminantes.

Fonte de Lípidio	**Resultados**	**Autor**
Óleo de cártamo	Aumento do CLA em vários tecidos de Cordeiros	Mir et al. (2000), Boles et al. (2005)
Óleo de soja	Mais efetivo no aumento do CLA no músculo de cordeiros	Bessa et al. (2005), Santos-Silva et al. (2004)
Óleo de girassol e óleo de linhaça	Ambos aumentaram o CLA na gordura do músculo mas maior aumento com óleo de girassol	Bessa et al. (2007)
Óleo de girassol e óleo de linhaça	Substituição de óleo de girassol por óleo de linhaça reduziu CLA, mas aumento níveis de n-3 no tecido adiposo de cordeiros	Jerônimo et al. (2009)
Óleo de peixe	Aumento dos níveis de ácidos graxos PUFA de cadeia longa (EPA e DHA) no músculo de cordeiros	Cooper et al. (2004), Demirel et al. (2004)
Óleo de palma	Aumento de ácidos graxos indesejáveis no músculo de cordeiros	Castro et al. (2005)
Óleo de coco	Redução de AGCC, C16:0 e C18:0 no leite de vacas	Hristov et al. (2009)
Óleo de canola	Aumentou o teor de ácidos graxos n-3 muscular, mas reduziu a oxidação lipídica no sangue e no músculo LL de cabritos.	Karami et al. (2013)
Óleo de Linhaça	Aumento de 18:3, n-3 e CLA na carne de bovinos	Nassu et al. (2011)
Óleo de linhaça (OL), girassol (OG) e soja (OS)	Aumento de n-3 (PUFA) no Longissimus dorsi com óleo de linhaça, aumento de CLA com OL e OS na carne de bovinos	González et al. (2014)
Óleo rico em n-3 ou n-6	Aumento de ácidos graxos biologicamente ativos, diminuição da relação n-6:n-3 na carne de bovinos	He et al. (2011)
Óleo Vegetal e óleo de peixe	Aumento de n-3 e CLA na carne de bovinos	Ducket e Gillis (2010)

CLA = Ácido linoleico conjugado; EPA= Ácido eicosapentanóico; DHA = Ácido docosapentanóico; OL= Óleo de linhaça; OG= Óleo de girassol; OS= Óleo de soja.

Adaptado de: Ribeiro et al. 2011

Os AGCM, tal como ácido láurico, demonstram ter potentes efeitos de defaunação (FACIOLA e BRODERICK, 2013; HRISTOV et al., 2004; HRISTOV et al., 2011; MATSUMOTO et al., 1991; NEWBOLD e CHAMBERLAIN, 1988; SOLIVA et al., 2003).

Hollmann e Beede (2012) observaram dois efeitos majoritários quando suplementaram o óle de coco, como fonte de ácido láurico, para vacas de leite. Primero, a introdução abrupta de grande concentração de óleo de coco, diminuiu a ingestão de matéria seca e energia (DE e DEL) e consequente a reduziu os sólidos totais corrigidos para a produção de leite total.

Diferentemente, Faciola e Broderick (2014) quando suplementaram ácido láurico e óleo de coco (1,3%, base de matéria seca) não encontraram diferenças no consumo de matéria seca, mas os protozoários foram reduzidos em 40%, ocasionando pequenas reduções na concentração de AGVs, aumentando a proporção molar de propionato, reduzindo a amônia ruminal e AGVs de cadeia ramificada, o que sugere que ocorreu redução da degradação proteica e redução do N ureico do leite e sangue, devido a uma provável melhora na eficiência proteica.

O ácido láurico também apresenta efeitos sobre a redução a digestibilidade aparente do trato total da fibra em detergente neutro (FDN; FACIOLA e BRODERICK, 2014). Sendo que essa redução da digestidade da fração fibrosa, está associada a supressão dos protozoários ruminais causada pelo ácido láurico (JOUANY et al., 1988).

A suplementação de ácidos graxos saturados (C12:0, C14:0 e C18:0), podem aumentar a composição de ácidos graxos saturados depositados na carne e leite. Sendo que a gordura da carne já contém uma elevada proporção de AGS, mas também contem alguns ácidos graxos com efeitos anticarcinogênico como o ácido vacênico que é o resultado da bio-hidrogenação ruminal dos ácidos graxos insaturados da dieta (DUCKETT e GILLIS, 2010).

A formação de intermediários da bio-hidrogenação ruminal e do CLA é dependente da quantidade de ácido linoleico dietético (BAUMAN et al., 1999). Pesquisas anteriores mostraram que as extensões da bio-hidrogenação ruminal aumentam com o acréscimo de ácidos graxos insaturados na dieta (DUCKETT et al., 2002), indicando que maior escape ruminal (34%) ocorreria para o ácido oleico (C18:1) (DUCKETT e GILLIS, 2010).

Bernard et al. (2009) suplementaram óleo de girassol (6,1% MS), óleo de linhaça (6,2% MS), e dieta controle sem óleo para cabras alpinas, e as dietas com óleo aumentou a produção de leite (3,4; 3,6 e 3,5 kg/d) e a concentração de *cis*-9, 18:1 no leite em

comparação a dieta sem óleo (14,1; 17,6 e 18,5 g/d) respectivamente (BERNARD et., 2009).

2.5.2 Depressão da gordura do leite

A depressão na gordura do leite induzida por dieta (DGL) ou síndrome do baixo teor de gordura foi descrita pela primeira vez há mais de 150 anos atrás, é caracterizada pela diminuição na produção de gordura do leite em até 50%, sem qualquer alteração no rendimento de leite ou no rendimento de outros componentes do leite (BAUMAN e GRIINARI, 2001), envolve uma inter-relação entre fermentação ruminal e metabolismo do tecido mamário (HAVARTINE et al., 2009).

A DGL é causada por intermediários da bio-hidrogenação ruminal (BAUMGARD et al., 2000). E é caracterizada por uma redução de até 50% na produção de gordura láctea em resposta a dietas com alto teor de concentrado e de baixa forragem ou dietas suplementadas com óleos vegetais ou de peixe (BAUMAN e GRIINARI, 2003). Nos ruminantes, os fatores dietéticos que causam DGL estão associados a alterações nos padrões de fermentação ruminal, microbiota e à produção de ácidos graxos bioativos a partir do metabolismo dos ácidos graxos poli-insaturados dietéticos, resultando em uma mudança no metabolismo tecidual (RICO e HARVATINE, 2013).

Estudos com vacas em lactação forneceram evidências de que a redução na síntese de gordura do leite está relacionada com o aumento do fluxo ruminal de ácidos graxos *trans* específicos, que exercem efeitos anti-lipogênicos (SHINGFIELD et al., 2010).

A DGL induzida pela dieta, a concentração do CLA *trans*-10, *cis*-12 são correlacionadas, através de estudos onde este era infundido no abomaso (BAUMAN et al., 2008) e conhecidos por inibir a síntese de gordura do leite na glândula mamária e seu mecanismo de ação envolve a regulação negativa de genes relacionados ao transporte e síntese de ácidos graxos (RICO e HARVATINE, 2013).

Almeida et al. (2013), avaliaram níveis crescentes (30, 60 ou 90 g/dia) de infusão via oral de óleo de soja em cabras leiteiras, sendo que o consumo e a digestibilidade da matéria seca foram afetados negativamente. A concentração de ácido vacênico no sangue arterial aumentou com a infusão oral do óleo de soja, sendo 127% com 90 g/d. O CLA

trans-10, *cis*-12 no leite aumentou com a infusão do óleo e causou redução na gordura do leite que atingiu a 31%, enquanto que o CLA *cis*-9, *trans*-1 foi reduzido.

Ebrahimi et al. (2017) em experimento com caprinos, recebendo diferentes proporções de PUFA n-6: n-3 de 2,3:1 (razão baixa), 5,0:1 (razão média) e 10,4:1 (Razão elevada), observou que as cabras alimentadas com dieta RE apresentaram menor propionato e ácidos graxos voláteis totais e maior butirato. As dietas com alta proporção de n-6: n-3 aumentaram a concentração de CLA *cis*-9, *trans*-11, ácido vacenico e C18:2n-6 e diminuíram a concentração de C18:0 no rúmen de caprinos. A dieta com baixa relação n-6:n-3 diminuiu a população de *B. fibrisolvens*, que está envolvida no processo de bio-hidrogenação no rúmen.

A alteração da proporção dietética do n-6:n-3 também tem como objetivo melhorar o desempenho animal. Sendo que Greco et al. (2015), avaliou o fornecimento de ácidos graxos com três razões diferentes de n-6 a n-3 (3,9:1 4,9:1 ou 5,9:1). O fornecimento da proporção dietética aproximadamente 4:1 de n-6:n-3 resultou em maior CMS, produção de leite e de componentes do leite. Não houve diferenças hormonais, peso corporal e condição corporal e aproximadamente 1,3 kg de resposta do leite não pode ser explicado somente por diferenças na ingestão de nutrientes, o que sugere que a redução da proporção dietética de ácido graxo de 6:1 para 4:1 pode influenciar a partição de nutrientes para favorecer uma maior proporção da energia líquida total consumida.

2.5.3 Suplementação de tortas e farelos

A suplementação através de tortas e farelos vem sendo cada vez mais utilizados, haja visto que estes são coprodutos da indústria de óleo e biodiesel, sendo encontrado em grande escala, grande disponibilidade e geralmente a baixo custo. Através do fornecimento de tortas a taxa de liberação de ácidos graxos livres é reduzida, pois estes ácidos graxos se encontram na forma complexada (triglicerídeos/galactolipideos), podendo restultar em menor taxa de bio-hidrogenação dos ácidos graxos poli-insaturados, aumentando os intermediários para serem absorvidos pelo intestino.

As tortas e farelos são comumente testados por apresentarem alguns fatores antinutricionais (ex. ricina, glicosinolatos) ou fatores limitantes (ex. lignina, cutina), por

isso é necessário a experimentação para identificar os componentes limitantes no metabolismo e desempenho animal.

Abubakr et al. (2014) testando o fornecimento da torta de dendê e torta de palma decantada e resultou num aumento das populações de bactérias celulolíticas (*Fibrobacter succinogenes*, *Ruminococcus flavefeciens*, e *Ruminococcus albus*) e diminuição da densidade de arqueas metanogênicas no rúmen de cabras, embora essas duas tortas tenham perfil de AG similares.

Palmieri et al. (2012) não encontrou diferença sob características quantitativas da carcaça em borregos Boer, quando substituiu até 100% do farelo de soja pela torta de girassol.

A fase em que os animais recebem a dieta é outro fator que pode afetar o rendimento de carcaça, segundo, Bessa et al. (2008), cordeiros alimentados com concentrado na fase inicial apresentam maior peso de carcaça quente (11,2 vs 9,6 kg), menor proporção de CLA (18:2 *cis*-9, *trans*-11) (0,98% vs 1,38% de ácidos graxos totais), maior proporção de CLA 18:2 *cis*-9, *trans*-11 na gordura intramuscular e a maioria dos ácidos graxos poli-insaturados n-3 do que os cordeiros alimentados inicialmente com alfalfa. O aumento da duração do período de acabamento reduziu a porcentagem de carcaça muscular (57,4% vs 55,5%) e aumentou a porcentagem de gordura subcutânea (5,67% vs 7,03%).

Borregos confinados alimentado com dietas contendo 3% de óleo de canola ou óleo de palma não apresentaram diferenças no desempenho. Porém a incorporação de óleo de canola na dieta das cabras aumentou o teor de ácidos graxos ômega-3, mas a oxidação lipídica foi reduzida no sangue e no músculo *Longissimus lumborum*. A dieta de óleo de palma aumentou o teor de ácido mirístico e palmítico no sangue, mas isso não alterou os ácidos graxos no músculo *Longissimus lumborum*, e aumentou as substâncias lipídicas oxidativas no fígado e *Longissimus lumborum* (KARAMI et al., 2013).

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CAPÍTULO I

Meta-Analysis of rumination behavior and its relationship with milk fat in lactating dairy cows

Meta-analysis of rumination behavior and its relationship with milk fat in lactating dairy cows

ABSTRACT

Time spent ruminating is affect by diet, has direct impacts on the rumen environment, and has been associated with milk fat production. The objective of the current study was to conduct a meta-analysis to better understand the variation in rumination time and behavior and its relationship with milk fat. The analysis included English articles from the literature published between 1986 and 2017. The final dataset included 115 papers reporting 447 treatment means from lactating Holsteins cows during established lactation. The average diets composition was 17.4% CP, 31.9% NDF, 19.8% roughage NDF, 27.3% starch, and 3.89% EE on a DM basis. Milk yield averaged 34.4 kg/d (14.2 to 52.1 kg/d), milk fat averaged 3.47% (2.20 to 4.60%), and rumen pH averaged 6.09 (5.3 to 7.00). Rumination observation systems were categorized into six groups. Visual observation was the most frequently used system (n = 217) and automated systems were assigned to 5 categories based on principle of the mechanized system. There was little difference in total rumination time between systems. The total time spent ruminating averaged 449 min/d (151 to 638 min/d), there were on average 13.7 bout/d (7.8 to 17.4 bout/d), and average bout length was 31.1 min/d (20.0 to 48.1 min/d) min. A quadratic relationship was observed between rumination time (min/d) and milk yield (kg/d; R2= 0.22), milk fat concentration (R2= 0.08), and NDF total tract digestibility (R2= 0.07) ($P < 0.05$). Principle component analysis was also conducted to explore the relationship between variables. The first principal components explain 41.9% of the 38 variables evaluated and includedruminal VFA and pH, milk composition (*trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA; fat concentration), total tract digestibility (NDF, FA), diet composition, rumination behavior. Total chewing (min/d), total rumination (min/kg DM) and total rumination (min/kg NDF) were highly correlated. Milk fat *cis*-9, *trans*-11 CLA, total chewing (min/d), and total rumination (min/kg DM) were negatively correlated. The final multivariant model to predict total rumination time included milk yield, milk fat concentration and DMI (Total rumination time, min/d = 96.73 + 6.96 × milk + 64.10 × fat% – 4.86 × DMI; model $R^2 = 0.37$ and RMSE = 79.72). Milk fat yield was quadratically affected by rumination time (min/kg DM), and was not related to total rumination time

(min/d) and rumination (min/kg NDF). In conclusion, rumination time was related to rumen pH and milk fat, but explained only a limited portion of the variation in milk fat.

Keywords: correlation, meta-regression, milk fat, neutral digestible fiber, rumination time

1.0 INTRODUCTION

Milk is an important component of the diets of ~6 billion people worldwide (FAO, 2012) and milk fat is a major part of the energy value of milk and important to the production of many dairy products. Milk fat is also a major energy cost to the cow and is one of the most variable component that is modified by many factors, including robust responses to diet composition and rumen fermentation (BAUMAN et al., 2006; BAUMAN and GRIINARI, 2001). Ruminants are effective at digestion of low quality feed and conversion of human inedible feedstuffs to high value food, but management of rumen function is essential to optimal animal health, milk production, and feed efficiency. Rumination is a key part of rumen function and is readily observable.

Milk fat is affected by numerous physiological and environmental factors. Nutrition is the main environmental factor impacting milk fat and provides the opportunity to increase milk fat yield (BAUMAN and GRIINARI, 2001). Diet-induced milk fat depression (MFD) is a condition where milk fat is rapidly decreased because changes in the diet resulted in ruminal production of bioactive *trans* FA, including *trans*-10, *cis*-12 conjugated linoleic acid (CLA). However, predicting MFD is difficult and several dietary manipulations can result in MFD, including increasing rumen available unsaturated fatty acids, increasing diet fermentability through concentrates, lush pasture, or rapidly digested starch sources, decreasing effective fiber with forages with small particle size, feeding ionophores, and a number of other factors that disrupt normal fermentation (VAN SOEST, 1994). Many of these dietary modifications are also expected to impact rumination.

Rumination is the rhythmic regurgitation and remastication of rumen digesta that occurs between meals and during rest periods, especially at night. The goal of rumination is to break apart large particles to allow increased microbial attachment and digestion and allow passage from the rumen. Saliva flow is also greatly increased during rumination and provides important buffers to increase rumen pH. Regulation of rumination is complex, but has been correlated to forage NDF level and particle size (ALLEN, 1997).

Activity and rumination monitoring systems are growing in popularity in the global dairy industry and are mostly focused on management of reproduction and health (SJOSTROM et al., 2016). On-farm rumination observation could also aid nutritional management and optimization of milk fat, but information is lacking on the expected variation in rumination time and the relationship between rumination and milk fat.

Rumination has been investigated in many individual studies and the meta-analysis approach allows aggregation of the results, which improves the ability to detect treatment effects, and increases the capacity to explore sources of variation in responses (GLASS, 1976; HIGGINS, 2008).

The objective of the current study was to conduct a meta-analysis to better understand rumination behavior and its relationship with milk fat. The first objective was to benchmark expected rumination time and behavior and the variation of these parameters across diverse diets and conditions. The second objective was to understand the relationship between rumination behavior and milk fat using both simple and multiple regression. In this course of this work we also evaluated the differences between various rumination monitoring systems. The hypothesis was that increasing rumination time would quadratically increase milk fat yield and that an optimal goal for rumination can be established.

2.0 MATERIALS AND METHODS

2.1 Literature search

A dataset was constructed of peer reviewed papers published in English through a literature search using online manuscript retrieval databases [PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Google Scholar (http://www.scholar.google.com/), ScienceDirect (http://www.sciencedirect.com/), and Journal Dairy of Science (http://www.journalofdairyscience.org/)] using the key word searches. Approximately 600 publications were retrieved using search terms “ruminating time” and “dairy cow.” Secondly, an author search was conducted based on principle investigators who have were known to publish rumination observation data (M. ALLEN, L. ARMENTANO, K. BEAUCHEMIN, T. DeVRIES, R. GRANT, and V. KEYSERLINGK). Papers satisfying the predetermined criteria were included in the analysis.

2.2 Inclusion and Exclusion Criteria

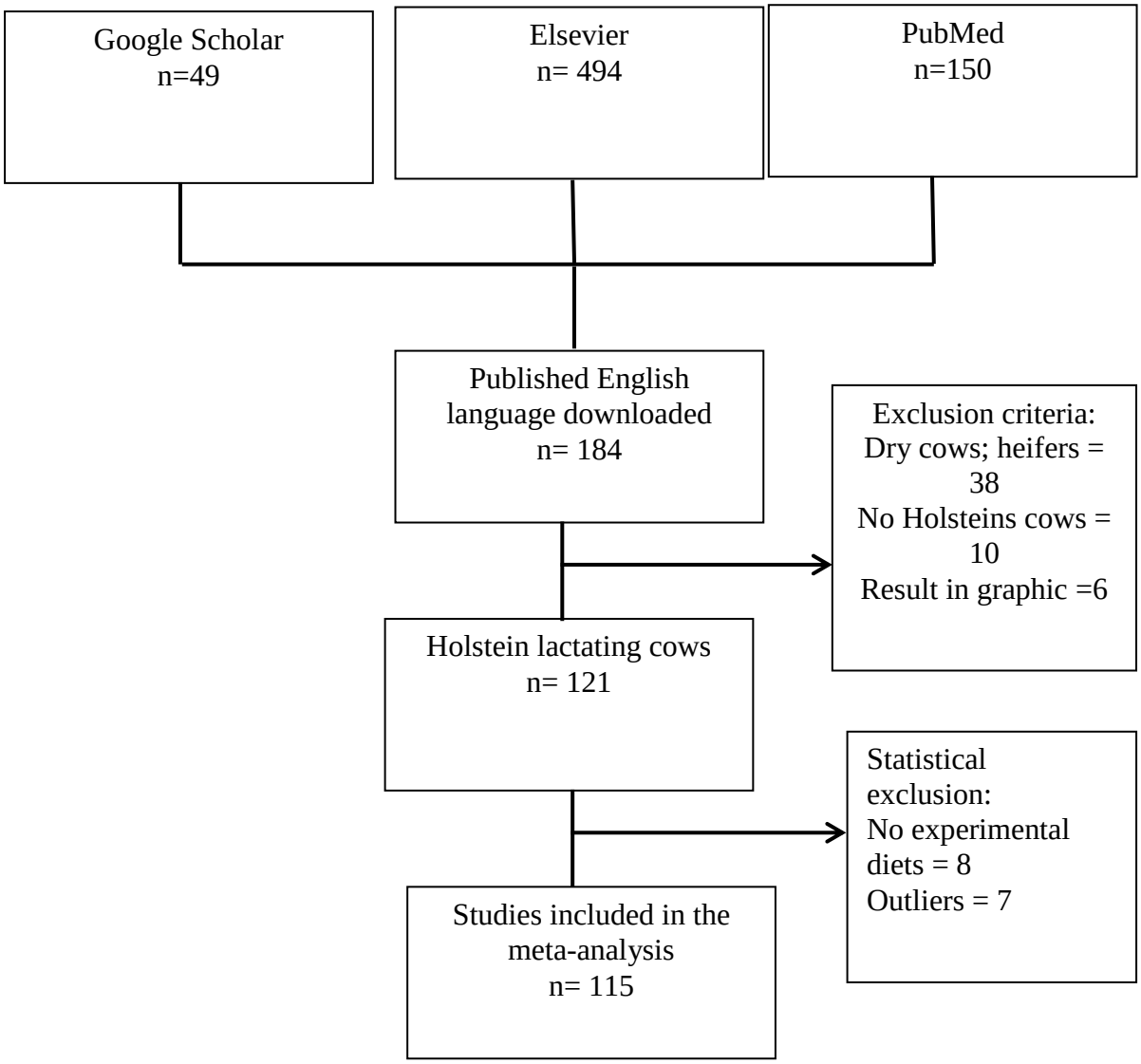
The analysis included English articles from the literature search published between 1986 and 2017. The above search resulted in 184 peer-reviewed articles investigating rumination from journals published in English. Of these, 137 investigated lactating Holstein cows, 131 reported numerical results for rumination time. Further exclusion criteria were applied based on identification of outliers during preliminary data analysis. A total of 9 papers used early lactating dairy cows, 2 papers fed very low dietary fiber (<15% NDF), 8 papers had no dietary treatment (e.g. investigated pest flies, genetic, parity) and 6 papers were excluded because of Studentized residual outside of ± 3.5 (JMP 12.0; SAS Institute). The final dataset included 115 papers.

2.3 Data Extraction

2.3.1 Eating behavior

After the aforementioned inclusion criteria, data from 115 peer-reviewed papers were entered into a spreadsheet. A total 94 peer reviewed papers reported milk yield and milk fat concentration and 101 peer reviewed papers reported the variance of rumination. Data recorded for each treatment, if reported, was diet composition (DM, OM, NDF, starch, CP, EE, FA and peNDF), intake of DM, NDF, and forage, milk yield and composition, rumination and ruminal parameters (total rumination time min/d, rumination min/kg DM intake, rumination min/kg NDF intake, rumination min/kg of forage NDF, bout/d, bout length), rumen pH, and total-tract digestibility of DM and NDF,. A total of 83 peer reviewed papers reported composition of experimental diets Total NDF intake was calculated based on DMI and diet composition for papers who did not report the data. The number of replicates and variances (SEM or SD) were extracted for total rumination time (min/d) for weighting according to (St-PIERRE, 2001 and SAUVANT et al., 2008). The complete data set is available in an Excel file (Microsoft Corp., Redmond, WA) file in appendix A1.

Figure 1. Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of rumination behavior and milk fat.



2.3.2 Feeding System

The feeding systems used to measure rumination and feeding behavior was recorded. Feeding systems were grouped in 6 categories according the following criteria:

- Visual observation (VO): Feeding behavior obtained by visual observation every 1, 5, or 10 minutes over a total of 24 or 48 h. This included manual observation of video recordings.

- Beauchemin 1989: the feeding behavior obtained by the automated system composed by a transducer attached to leather halter which basically transforms jaw movements into electrical signals described in Beauchemin et al. (1989).
- Dado 1993: Automatic feeding behavior system described by Dado and Allen (1993) that utilizes a pressure sensor in a pneumatic nosepiece attached to a halter.
- AJAWS: Other automated feeding behavior systems that measured ruminal behavior based on jaw movement including pneumatic systems attached to a cord under the jaw (DESWYSEN et al., 1987), small balloon filled with foam rubber under the jaws (BRUN et al., 1984; TAFAJ et al., 2005), leather automatic halters with a piezo disk (DeVRIES et al., 2003), automatic halters that measured mouth movements (GIRARD and LABONTE, 1993), Graze Jaw Movement Analysis Software (IGER; RUTTER et al., 1997).
- SCR: Feeding behavior obtained by a microphone based rumination-monitoring system (SCR Engineers Ltd., Netanya, Israel).
- Other: feeding behavior obtained by sensors that couldn't be grouped with in the above.

Feeding system was further simplified into two categories of visual observation (VO) and all automated observation systems (All Auto).

2.4 Statistical analyses

All analysis was performed using JMP Pro 12.1 (SAS Institute, Cary, NC). First, descriptive statistics including distribution and univariate analysis (mean, SD, ranges of values) were performed to identified outlier in the data set and characterize the data set. Second, simple regression was performed to predict the correlation with the main variables of interest. This was followed by multivariate analyses to predict rumination time. Lastly, principal components analysis was preformed using a broad selection of variables recorded and a simplified set of the variables that are commonly associated with rumination time.

3.0 RESULTS

3.1 Characterization of diets

The diet composition varied drastically between experiments and treatments as expected (Table 1). The average of diets composition was 17.4% (13.0 to 22.1%) CP, 19.9% (8.6 to 44.9%) NDF roughage, 31.9% (18.2 to 49.0%) NDF, 27.3 (16.9 to 42.3%) starch, and 3.9% (1.8 to 6.2%) EE on DM basis. The NRC (2001) recommended range for lactating cows are 25% and 44% NDF (% of DM), with a minimum of 19% NDF from forages and maximum NFC of 44% (% of DM). The averaged starch in published diets fed to lactating cows is 30.9% (13.7 to 45.2 % of DM; FIRKINS et al., 2001). Lignin was the most variable (CV=43.6) component among the diets and the iNDF was the least reported (n=15) in the collected studies. The low EE in the diets reflected the basal diet for medium producing cows.

Table 1. Description of the composition of diets in experiments included in the meta-analysis

Nutrient[1]	N[2]	Mean	Min	Max	SD	SEM	CV	Median
DM, %	261	58,5	35,4	88,70	10,5	0,65	18,0	56,80
Forage, % DM	62	46,4	25,0	67,00	10,8	1,37	23,3	45,00
Roughage NDF[3], % DM	136	19,8	7,60	44,90	5,63	0,48	28.4	18,75
NDF, % DM	287	31,9	18,2	49,00	5,01	0,30	15,7	31,60
iNDF, % DM	15	11,1	9,40	15,60	1,74	0,45	15,6	10,45
ADF, % DM	224	19,6	11,3	31,00	3,65	0,24	18,5	19,75
CP, % DM	286	17,4	13,0	22,10	1,44	0,09	8,28	17,30
Starch, % DM	152	27,3	16,9	42,30	5,96	0,48	21,8	27,25
Ether Extract, % DM	90	3,89	1,78	6,20	1,23	0,13	31,7	3,70
FA, % DM[9]	39	4,90	3,50	8,30	1,24	0,20	25,2	4,60
Lignin, % DM	47	3,62	1,05	6,40	1,58	0,23	43,6	3,70

[1]Diet composition from the experiments included in the meta-analysis.
[2]Number of treatments reporting the value.
[3]Roughage NDF includes forages and beet pulp.

3.2 Characteristics of cows, intake and production, rumen pH, and total tract digestibility

The 423 treatment means represented 1621 cows in the 115 studies (Table 2) that varied from 14 to 292 days in milk (DIM). Milk yield averaged 34.4 kg/d (14.2 to 52.1 kg/d) and milk fat averaged 3.47% (2.20 to 4.60%). Dry matter intake averaged 23.2 kg/d (15.5 to 32.6 kg/d) and the most variable nutrient intakes across the dataset were forage (4.3 to 13.2 kg/d; CV = 30.5%) and starch (3.12 to 9.40 kg/d; CV = 27.03). The mean rumen pH was 6.09 (5.3 to 7.00) in the 256 treatments reporting the observation and it was also a more homogenous variable (CV = 4.70). Total tract digestibility averaged 67.9% of DM (51.4 to 78.9) and 48.8% of NDF (23.0 to 76.0%). The average of rumination activity were 449 min/d (151 to 638 min/d) of total rumination time, 19.3 min/kg DMI (7.6 to 36.1 min/kg DMI), 62.7 min/kg NDF (24.7 to 126 min/kg NDF), 13.7 bout/d (7.8 to 17.4 bout/d) and bout length 31.1 min/d (20.0 to 48.1 min/d). The least and most rumination parameters reported in the studies was min/kg of forage NDF (n=69) and min/d (n=402) respectively.

Table 2. Description of the cows and average production parameters and rumination observations in the treatments included in the meta-analysis.

Item	N[1]	Mean	Min	Max	SD	SEM	CV	Median
# cows	423	13.49	3	156	15.56	0.76	115.35	9
Intake, kg/d								
DM	396	23.24	15.5	32.6	3.05	0.15	13.11	23.1
Forage	44	9.09	4.3	13.2	2.19	0.35	24.09	9.3
NDF	266	7.31	4.15	10.6	1.32	0.08	18.04	7.3
Starch	59	6.39	3.12	9.4	1.73	0.23	27.03	6.62
Milk yield, kg/d								
Milk	355	34.36	14.20	52.10	7.43	0.39	21.61	34.10
Fat	351	1.19	0.58	1.93	0.27	0.01	22.43	1.18
Milk Composition, %								
Fat	355	3.47	2.20	4.60	0.40	0.02	11.54	3.50
Protein	311	3.17	2.66	3.81	0.24	0.01	7.50	3.15
Rumen								
Ph	256	6.09	5.3	7.00	0.29	0.02	4.70	6.09
Rumination								
min/d	402	449	151	638	94.7	3.74	21.06	449

min/kg DMI[2]	386	19.3	7.59	36.1	4.65	0.18	24.0	19.1
min/kg NDF[3]	360	62.7	24.7	126	19.3	0.79	30.7	60.1
min/kg of forage NDF	69	110	51.6	193	30.4	3.66	27.6	111
Bout/d	121	13.7	7.8	17.4	1.96	0.14	14.3	14.3
Bout Length, min	109	31.1	20.0	48.1	7.69	0.60	24.7	32.7
Total Tract Digestibility, %								
DM	117	67.9	51.4	78.9	5.57	0.51	8.21	68.5
NDF	135	48.8	23.0	76.0	9.58	0.82	19.6	47.9

[1]N = number of variables included.
[2]DMI, calculated based on %DM of the diet.
[3]NDF calculated based on DMI and %NDF of the diet composition.

3.3 Characteristics of rumination observation systems and rumination behavior

The rumination monitoring visual observation and automated systems averaged 443 min/d (204 to 638 min/d; n= 217) and 446 min/d (151 to 446 min/d; n=190), respectively. Numerous rumination observation systems were used in the experiments. To characterize the effect of observation system they were categorized into 6 categories as described in the methods. These averaged, ajaws 447 min/d (242 to 558 min/d), beauchemin 1989 392 min/d (151 to 486 min/d), dado 1993 463 min/d (310 to 616 min/d), SCR 493 min/d (357 to 618 min/d) and other automated 314 min/d (285 to 340 min/d). Among the category ajaws (n=81) was the main automated system used to monitoring ruminal behavior, which correspond to 57.3% of the automated systems (Table 3).

Table 3. Comparison of total rumination time reported by different rumination observation methods.

System[1]	N[2]	Mean	Min	Max	SD	SEM	CV	Median
Visual Obs.	217	443	204	638	78.2	5.31	17.7	443
Automated Obs.	190	446	151	618	76.4	5.54	174.1	458
AJAWS	81	447	242	558	73.8	8.20	16.5	464
Beauchemin 1989	31	392	151	486	75.6	13.0	18.5	414
Dado 1993	46	463	310	616	62.2	9.17	13.4	474
SCR	28	496	357	618	56.5	10.7	11.4	503
Other Auto	4	314	285	340	25.6	12.8	8.16	316

[1]Visual Obs.=Feeding behavior obtained by visual and video recordings observation every 1, 5 or 10 minutes over a total of 24 or 48 h; Automated Obs.= all automated feeding behavior systems; AJAWS=

Feeding behavior obtained by automatic system consisting of a cord under the animals jaws; Beauchemin 1989= Feeding behavior obtained by the automated system described in Beauchemin et al. (1989); Dado 1993= Feeding behavior obtained by the automated system described by Dado and Allen (1993); SCR= Feeding behavior obtained by a microphone based system (SCR Engineers Ltd., Netanya, Israel); Other Auto = Feeding behavior obtained by other sensors that couldn't be grouped with the above categories.
[2]Number of treatment means in each category.

3.4 Simple regression of rumination with key variables

Simple regression was conducted to determine the relationship between rumination and key parameters including milk and milk fat yield, rumen pH, and total tract NDF digestibility.

Table 4. Simple regressions of rumination time and milk production, rumen pH, and total tract digestibility

Dependent variables		Linear			Quadratic		Model Fit	
X	Y	Int.	Slope	*P*-value	Slope	*P*-value	R^2	RMSE
Rumination (min/d)								
	Milk kg/d	11.73	0.046	<0.0001	0.00009	0.0187	0.22	8.77
	MFY, kg/d	0.31	0.002	<0.0001	-	-	0.27	0.32
	Fat %	3.18	0.001	<0.0001	8.41295E-06	0.0008	0.08	0.43
	pH	6.21	-0.0002	0.3181	-	-		
	DMI kg/d	18.12	0.012	<0.0001	-	-	0.08	3.64
	NDF intake kg/d	5.85	0.003	0.001	-	-	0.03	0.03
	NDF diet, %DM	31.89	-0.0004	0.912	-	-	4.52e-5	6.26
	TTD NDF %DM	63.63	-0.0316867	0.402	-0.0002	0.0048	0.069	11.84
Rumination (min/kg DM)								
	Milk kg/d	37.88	-0.15	0.1887	-0.036	0.0453	0.017	9.9
	MFY, kg/d	1.24	-0.0019	0.662	-0.0022	0.001	0.032	0.36
	Fat %	3.16	0.015	0.004	-0.002	0.0045	0.048	0.44
	pH	6.15	-0.0017	0.687	-	-	0.0006	0.34
	DMI kg/d	31.21	-0.39	<0.0001	-	-	-	
	NDF intake kg/d	9.83	-0.12	<0.0001	-	-	0.08	1.79
	NDF diet, %DM	28.78	0.15	0.093	-	-	0.01	6.26
	TTD NDF %DM	50.74	-0.1498	0.5107	-	-	0.00355	12.2

Rumination (min/kg NDF)							
Milk kg/d	35.8	-0.013	0.641	-	-	0.0007	10
MFY, kg/d	1.28	-0.0013	0.2196	-	-	0.004	0.37
Fat %	3.50	-0.002	0.2008	-0.0002	0.001	0.03	0.45
pH	6.08	0.0002	0.808	-	-	0.0002	0.32
DMI kg/d	27.54	-0.06	<0.0001	-	-	0.10	3.67
NDF intake kg/d	11.83	-0.069	<0.0001	-	-	0.52	1.28
NDF diet, %DM	45.79	-0.22	<0.0001	-	-	0.42	4.76
TTD NDF %DM	51.84	-0.1094	0.043	0.0104	0.003	0.108	11.96

[1]TTD NDF= NDF Total tract digestibility.

Total time spent ruminating was quadratically related to milk yield, milk fat concentration, and total tract NDF digestibility and linearly related to milk fat yield and rumen pH (Table 4). The total time spent ruminating (min/d) was linearly related to MFY, pH, DMI, NDF intake and dietary NDF (% of DM). As rumination time (min/d) increased MFY, DMI and NDF intake linearly increased 0.12 kg/d, 0.72 kg/d and 0.18 kg/d respectively per 60 minutes of rumination time. Ruminal pH and dietary NDF (% of DM) linearly decreased 0.012 and 0.024% respectively per 60 minutes of rumination time. Milk fat concentration was quadratically related to rumination (min/d), rumination (min/kg DM) and rumination (min/kg NDF).

Time spent ruminating (min/kg DM) was quadratically related to milk yield and milk fat yield and concentration. As rumination time (min/kg NDF) increased, there was a linear decrease of 0.78 kg/d of milk yield, 0.078 kg/d of milk fat yield, and 0.12 percentage points of milk fat concentration per 60 minutes increase of rumination time. Rumination time (min/kg DM) also increased quadratically with increasing milk yield and milk fat yield and concentration (All $P < 0.05$). Total rumination (min/kgNDF) was quadratically related to total tract NDF digestibility, and fat concentration. There was a linear relationship between total rumination (min/d) and milk fat yield (P <0.0001) and rumen pH and total tract NDF digestibility (P<0.0001; Table 4).

3.5 Multivariant Analysis

Multivariate regression was conducted with step-wise removal of non-significant terms to predict total rumination time. The final model for prediction of total rumination time (Table 5) included milk yield, milk fat percent, and DMI (Total rumination time (min/d) = 96.73 + 6.96 x milk + 64.10 x fat% – 4.86 x DMI). This total R^2 of the model was 0.37 and RMSE was 79.72. The prediction of rumination time increased with milk yield and milk fat concentration and decreased with increasing DMI.

Table 5. Multivariant prediction of total rumination time.

Item	Estimate	SEM	t-Ratio	*P*-Value
Intercept	96.73	60.00	1.61	0.1080
Milk, kg/d	6.96	0.887	7.84	<0.0001*
Fat, %	64.10	11.77	5.45	<0.0001*
DMI, kg/d	-4.86	1.97	-2.47	0.0141*

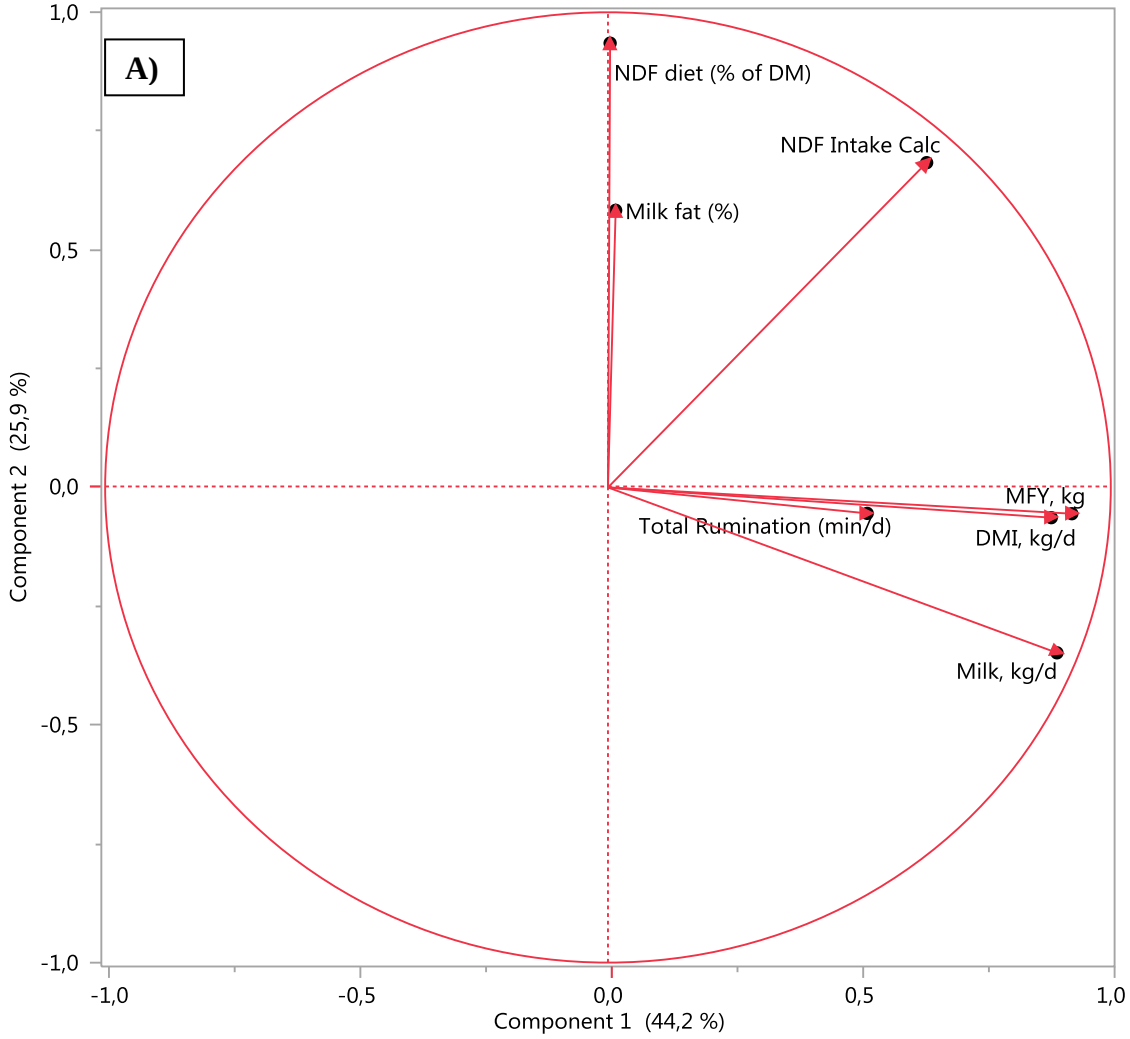
SEM= Standard error mean; *= P<0.05;

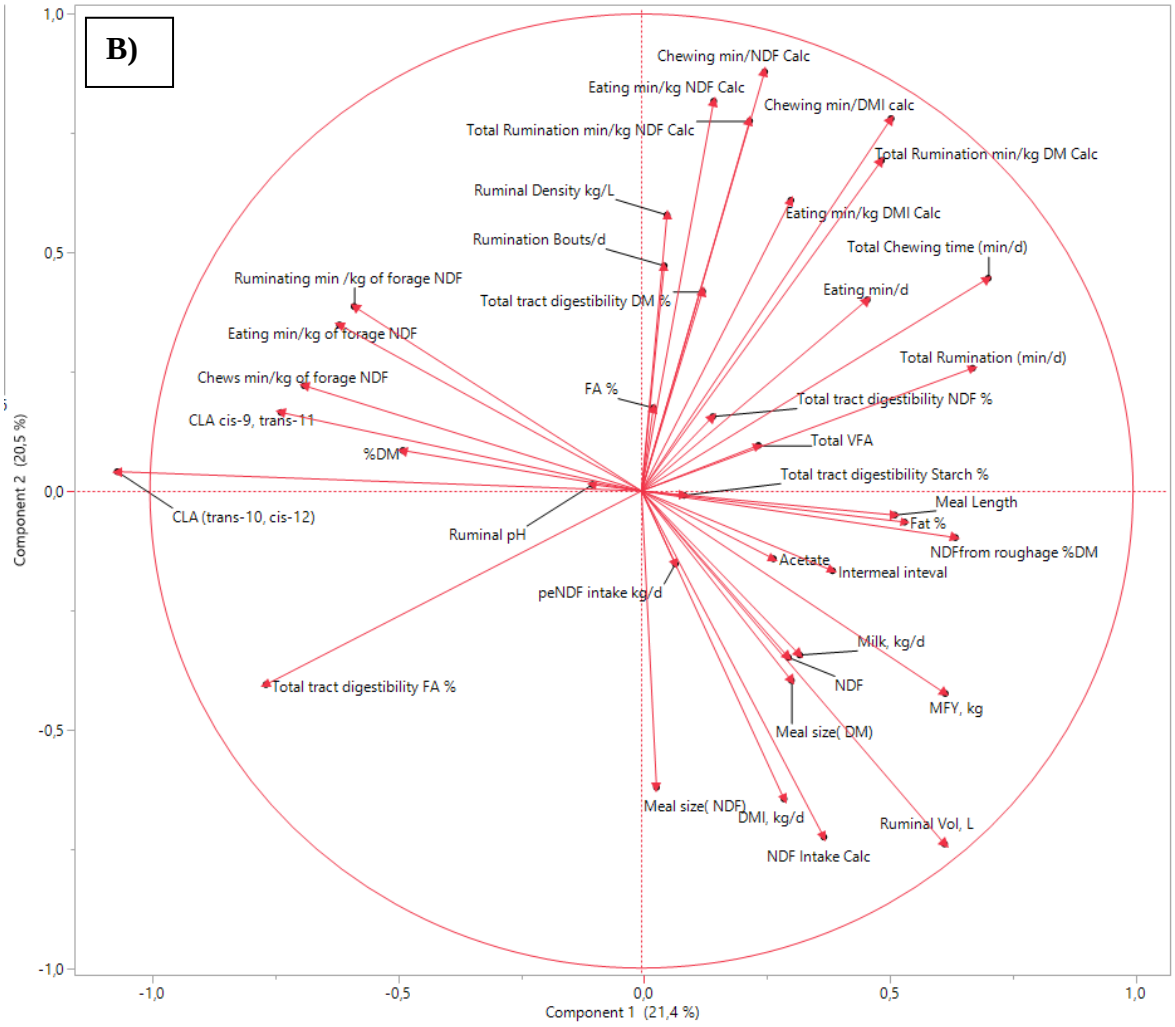
Principle component analysis was used to explore relationships between variables recorded in the study. The analysis was conducted both with a broader set of variables and a select set that was reported in a larger number of studies. The broad analysis included 38 variables and the selected set included 7 variables. In the PCA (Figure 2, A) the first principal component (PC1) and the second principal components (PC2) explained 70.1% of the total variation. Total rumination (min/d), DMI (kg/d) and MFY (kg/d) were in PC1. The most influential variable in PC2 was diet NDF concentration (% of DM) and in PC1 was milk yield (kg/d). Milk fat concentration and diet NDF concentration were highly correlated. Total rumination (min/d), DMI, milk yield and MFY are positive correlated.

In a broad analysis, the PCA explained 41.9% of the variables, which is less than in the PCA with the selected set of variables. The factors better explained by the principal components (PC) were total chewing (min/NDF calc) and ruminal volume (L) by PC2, and *trans*-10, *cis*-12 CLA by PC1 (Figure 2B). The total rumination (min/kg NDF calc), chewing (min/ kg NDF calc), and eating (min/kg NDF calc) were positive correlated and are partially explained by PC2 (Figure 2B). The variables explaining the least variation were ruminal pH by PC1 and peNDF intake (kg/d) by PC2. Milk fat *cis*-9, *trans*-11 CLA, diet DM percent, chewing per kg of forage NDF, eating time per of kg of forage NDF,

ruminating per kg of forage NDF, rumen pH, and milk fat *trans*-10, *cis*-12 CLA are highly positive correlated in PC1 (Figure 2B).

Figure 2. Principal component analysis of the simplified dataset (Panel A) and the full dataset (Panel B).





4.0 DISCUSSION

It is well recognized that changes in diet composition and management have impacts on rumen function and eating and ruminating behavior. These effects have been investigated extensively in individual experiments over the last 30 years (e.g. BEAUCHEMIN and BUCHANAN-SMITH, 1989; CROSSLEY et al., 2017; DADO and ALLEN, 1994; FUSTINI et al., 2017; RAMIREZ et al., 2016). Allen (1997) conducted a meta-analysis to investigate the effect of particle size and diet composition of rumination and chewing time, but considerable work has been published since this time. The current project identified 115 papers with 429 treatment means allowing ample power for a meta-regression. These manuscripts report varying response parameters and many interactions

could be investigated, but we have focused our effort to understanding the effect of diet on rumination time and the relationship between rumination and milk fat.

Diet composition varied drastically between experiments as expected as they represent different feeding strategies and philosophies. This variation aids meta-regression in providing a broad range to observe. Diet composition impacts rumen fermentation and function and the amount and type of nutrients absorbed by the cow (PICCIOLI-CAPPELLI et al., 2014). Diet composition has been shown to affect rumination time in individual experiments. Briefly, increased diet NDF quadratically increased rumination (BEAUCHEMIN and BUCHANAM-SMITH, 1989), decreasing dietary particle size (1.0 mm) decreased time spent ruminating (min/kg NDF intake; GRANT et al., 1990), and increasing dietary peNDF increased rumination (YANG and BEAUCHEMIN, 2006). It is generally accepted that rumination activity is stimulated by the intake of physically effective fiber (peNDF) with a particle size greater than 1.18 mm (MERTENS, 1997). Results found by Grant et al. (1990) suggest that reducing forage particle size reduces the time spent ruminating and chewing, and decreases ruminal pH and milk fat yield. The quadratic effect found when fit the simple regression of rumination time (min/d) by milk yield (kg/d), milk fat percent, and NDF total tract digestibility (Table 4), shows the rumination time have a limitation by those, which can be managed by nutrition. Dado and Allen (1996) fed silages with similar NDF, but different NDF digestibility, to lactating dairy cows and observed that DMI increased 1.0 kg/d DMI and milk yield increases 1.9 kg/d when the NDF digestibility was 12.5% higher.

Rumination time per kg of NDF intake had a negative relationship with DMI (table 4). When DMI was higher, there commonly was more fermentable feed or lower diet NDF. Furthermore, this feed remains in the rumen for less time, reducing rumination time. The maximum total tract NDF digestibility of 50.3% was at 390 min/d of rumination (best fit $y = 63.63 - 0.0316867x - 0.0002654x^2$; Table 4). Total tract NDF digestibility was a good indicator of DMI. Forages with high NDF digestibility have shorter rumen retention time, allowing greater DMI (OBA and ALLEN, 1999). This is explained because NDF generally ferments and passes from the reticulorumen slower than other dietary constituents (ALLEN, 1996). Mertens (1994) in attempt to predict filling effects and energy content of the diets, used NDF concentration as the only feed characteristic, and it was positively correlated to DMI when energy limits intake, but

negatively correlated with NDF concentration when fill limits intake. Increased in vitro NDF digestibility is associated with higher energy intake, which resulted in increased milk yield (OBA and ALLEN, 1999). Also, the increases in peNDF content increase milk fat concentration (YANG and BEAUCHEMIN, 2007).

Maximum milk fat yield of 1.24 kg/d was obtained at 19.24 min/kg DM of rumination. Low rumination time is associated with diets high in starch, which have high fermentability and caused diet induced milk fat depression similar ot the 15% depression in milk fat yield reported by Oba and Allen (2003) and decreasing the fermentability the rumination activity might be decreased by physical limitation (ALLEN, 1997).

The average of NDF and starch in the diets was 31.9% (of DM) and 27.3% (of DM) respectively. The performance parameters averaged 23.2 kg/d of DMI, 34.4 kg/d of milk yield, 1.2 kg/d of MFY and 3.5% of fat. The rumen behavior parameters averaged in 6.09 rumen pH, 449 min/d of rumination time. Despite the differences among the studies, the averages found provides a benchmark for nutritionist.

The mean of the NDF in the diets were 31.9% of DM, which is higher than the minimum NDF dietary concentration of 25% of DM (NRC, 2001). However the minimum NDF were 18.2% of DM, which can explain the variability in rumination time, once it is affect mainly by NDF dietary. In the current study, total rumination time was positively associated with dietary NDF (% of DM), and negatively affected by NDF intake. The reduction in rumination time caused by NDF intake is associated with the quality of the dietary NDF and the physical capacity of the rumen, while dietary NDF concentration is only related to feed characteristics. The NDF in the diets is considered a primary factor affecting chewing activity, and it is used as an indicator of the diet's effect on rumen health and function (YANG et al., 2001).

The multivariate model that included milk yield, milk fat concentration and DMI provided the best fit to predict rumination time. Changes in milk yield, fat, and DMI give change rumination time. An increase in milk yield by 1 kg/d increases rumination time by 6.96 minutes. When the milk fat increases 1%, the cow ruminates for 64.1 more minutes. The DMI affects rumination time negatively; an increase in 1 kg of DMI decreases rumination time by 4.86 minutes.

Ambriz-Vilchis et al. (2015) found positive correlation between rumination activity measured through rumination collars, video recordings, and visual observation.

In addition to these, there are several other methods for measuring ruminal activity. We described the monitored system in six groups and the mean of each feed system monitoring was similar between the studies. In the two main groups visual observation and automated observation with a mean of total rumination 446 minutes/d and 443 minutes/d respectively (Table 3), it is important to mention there are many differences among the studies which can affect the ruminal activity, and the aim in the currently study is a comparison between the studies and not a method validation. Rumination time varied greatly between experiments, which was expected since DMI, MY and diet composition varied greatly between experiments. Visual observation was the mostly observation method, probably because it have no cost and has been utilized for decades and can be done in small herd as described by (STANGAFERRO et al., 2016) it is a tool to previously identify if the animals have some metabolic disorders.

Principal component analysis is often used to reduce the dimensionality of data profiles containing intercorrelated variables aim elucidate mutual metabolic relationships (FIEVEZ et al., 2003). The positive correlation between DMI, milk yield and MFY highlight again the importance of DMI in animal performance. Intake is affected by many factors including diet fermentability (VOELKER and ALLEN, 2003), forage to concentrate ratio (ALLEN, 2000), and NDF digestibility (DADO and ALLEN, 1996). Reduces in DMI combined with lower energy intake result in marked reduces on milk yield. The milk fat concentration increased as dietary NDF concentration increased (Figure 2A). It is known the concentration of NDF in the ration is important to maintain the ruminal function and to maximize milk yield (ALSTRUP et al., 2016). In a meta-analysis, Allen (1997) showed the total chewing time decreased considerably as forage particle size decreased. It was also shown that the peNDF required to maintain adequate ruminal pH are positive correlated.

Chewing activity is usually a good indication of rumen health because chewing stimulates saliva secretion (MAEKAWA et al., 2002) and it can prevent ruminal dysfermentation and subacute ruminal acidosis (ZEBELI et al., 2006). In general the similar variables (i.e. total rumination (min/kg NDF calc), chewing (min/ kg NDF calc), and eating (min/kg NDF calc)) are highly positive correlated (Figure 2B).

5.0 CONCLUSIONS

Increasing rumination time (min/kg DM) quadratically increases milk fat yield. Milk fat yield was not significant by total rumination time (min/d) and rumination (min/kg NDF). The total time spent ruminating (min/d) is linearly related to MFY, pH, DMI, NDF intake and dietary NDF (% of DM).

Milk yield averaged 34.4 kg/d (14.2 to 52.1 kg/d) and milk fat averaged 3.47% (2.20 to 4.60%). Dry matter intake averaged 23.2 (15.5 to 32.6). The total rumination time averaged 449 min/d (151 to 638 min/d),

The best-fit model for prediction of total rumination time included milk yield, milk fat concentration and DMI (Total rumination time (min/d) = 96.73 + 6.96 x milk + 64.10 x fat% – 4.86 x DMI), which suggested that is the main variables that affect rumination activity.

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APPENDIX 1

List of publications describing the experiments used in the meta-analysis

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CAPÍTULO II

Effect of coconut oil on rumen biohydrogenation of linoleic acid in dairy cows

Effect of coconut oil on rumen biohydrogenation of linoleic acid in dairy cows

ABSTRACT

The objective was to evaluate the effect of coconut oil supplementation on the rate of biohydrogenation of LA using an in vivo approach. Four ruminally cannulated lactating Holstein cows were randomly assigned in a cross over design with two treatments. The control treatment was composed of a typical lactating diet balanced to be 17.3% CP, 30.5% NDF, 3.6% total FA adjusted to be lower in LA. The coconut oil treatment was composed of coconut oil infused for three days 430 g/cow per day. The bolus infusion consisted of 200 g of 70% LA safflower and 12 g of heptadecanoic acid (17:0; marker for FA passage rate) blended with the oil. At the time of the bolus the oil was thoroughly manually mixed with the digested rumen. The digested rumen was collected at -1, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 h relative to bolus infusion. The COO did not alter DMI, milk yield, milk fat concentration, milk fat yield, milk protein concentration or yield. In contrast, the COO increased the *trans*-11 C18:1 in the milk. The total FA prior and post bolus infusion was lower in the cows receiving COO ($P<0.05$). Similarly, the total FA (% DM) enrichment in the rumen was lower ($P<0.05$) in the control (2.53 to 3.76 % of DM) than in cows receiving the COO (4.40 to 6.35 % of DM). The C17:0 was higher in cows not receiving COO (0.50 % TFA) than cows receiving it (0.24 % TFA) before the bolus infusion ($P<0.01$). Similarly, cows receiving COO had lower concentration of C18:2 in prior-bolus ($P<0.01$), and enriched the rumen LA from 12.08 to 27.10 and 6.68 to 23.61 (% TFA) in the control and COO treatments, respectively. Total rumen disappearance of C17:0 did not differ between treatments ($P = 0.17$) and averaged 6.98 %/h and 7.54 %/h in the control and COO treatments, respectively. Total rumen disappearance of C18:2 ($P = 0.43$) and rate of C18:2 biohydrogenation ($P = 0.82$) did not differ between treatments and averaged 45.6%/h and 38.3 %/h, respectively. The extension of rumen biohydrogenation of C18:2 was not different between treatments, and averaged 84.8 % and 83.3% in control and COO, respectively. The cows receiving the COO treatment had higher rumen *trans*-10 C18:1 at 3 and 4 hours after the bolus infusion. Relative to the bolus infusion, cows in the treatment control had lower rumen *trans*-11 C18:1 at 3 and 4 hours, tended to have higher *cis*-9, *trans*-11 CLA at h 0.45, and had

lower *trans*-10, *cis*-12 CLA at 2 and 3 hours. The fractional rates of rumen biohydrogenation estimated by the compartmental model for the control treatment were 0.035, 0,975, 0.331 (h^{-1}) for LA-CLA, CLA-VA, VA-stearic acid, respectively. Lastly, the fractional rates of rumen BH estimated by the compartmental model for the COO treatment were 0.037, 0.512, 0.189 (h^{-1}) for LA-CLA, CLA-VA, VA-stearic acid, respectively.

Key words: bolus, CLA, fatty acid kinetic, safflower.

1.0 INTRODUCTION

Milk quality for human health can be improved by changing the profile of fatty acids (FA) in milk trough dietary manipulation. The *cis*- 9, *trans*-11 CLA is formed in the rumen through biohydrogenation (BH) and it is generally considered to be the main health-promoting CLA for human consumption (PARIZA, 2004). Increasing rumen outflow of *trans*-11 C18:1 can increase *cis*- 9, *trans*-11-CLA because *trans*-11 C18:1 serves as substrate for the synthesis of *cis*-9, *trans*-11-CLA in animal tissues (GRIINARI et al., 2000; MOESLEY et al., 2006). Dietary manipulations may also focus on decreasing ruminal synthesis of *trans*-10, *cis*-12 CLA, as this FA causes milk fat depression (MFD; BAUMGARD et al., 2000). The occurrence of MFD can be characterized by the marked increase of *trans*-10 C18:1 in milk fat (BALDIN, 2016).

Unsaturated FA (UFA) are the major FA of oilseeds present in ruminant diets. The most common UFA found in traditional diets is linoleic acid (LA). In the rumen, 70-95% of LA is hydrogenated to C18:0 (stearic acid; LOCK and BAUMAN, 2004). In the major BH pathway, LA is first isomerized to *cis*-9, *trans*-11-CLA and then reduced to *trans*-11 C18:1. If BH goes to completion, *trans*-11 C18:1 is reduced to C18:0 (KEPLER et al., 1966).

The BH pathways can be shifted by factors that modify the rumen environment or change substrate for fermentation. Shifts in pathways may results from changes in BH rates or inhibit of a specific step. Dietary ingredients that change microbial activity associated with ruminal BH can also affect CLA formation (GRIINARI and BAUMAN, 1999). Dietary manipulations have been studied for many years to try to increase milk fat and improve milk FA profile. The 2-hydroxy-4-(methylthio) butanoate (HMTBa) seems to have a role for stabilizing rumen BH in high-producing by decreasing concentration of alternate BH isomers (i.e. *trans*-10 C18:1) and increase isomers of the normal pathway (i.e. *trans*-11 C18:1; BALDIN et al., 2016). Safflower oil combined with monensin increased CLA in milk (BELL et al., 2006). These dietary changes may result from changing rumen microbial profile and/or changes in their activity rate.

Dietary ingredients attempting to manipulate rumen BH may act through changes in ruminal pH (e.g. buffers, concentrate level), microbiota (e.g. concentrate level,

HMTBa), rate of organic matter fermentability (e.g. starch level and degradability), or target specific bacteria species (e.g. monensin). Medium chain FA (MCFA) also have potential to modify the rumen microbial profile (FACIOLA et al., 2013).

Coconut oil (COO) is a natural source of MCFA. The COO is rich (75%) in MCFA with 48% lauric and 20% myristic acid (HOLLMANN et al., 2013; ORSAVOVA et al., 2015). Several studies have investigated the potential of COO in modifying the rumen microbial profile and mitigating enteric methane emissions (HRISTOV et al., 2009; HRISTOV et al., 2011;) or to increase nitrogen efficiency (FACIOLA and BRODERICK 2014; FACIOLA et al., 2013; HRISTOV et al., 2009). By changing rumen microbial profile, changes in the BH of dietary UFA may also occur. However, COO has not been used in an attempt to manipulate ruminal BH rates or pathways.

Baldin (2016) recently developed an assay to asses BH in vivo. The principle of this assay is a "perturbation tracee model" which includes enrichment of the rumen concentration pool of a targeted UFA (i.e. OA, LA, or ALA), and subsequent observation of disappearance of the UFA and formation of intermediate FA and stearic acid.

Therefore, the objective of this study was to assess the effect of COO as a source MCFA on rumen BH of LA in dairy cows using the *in vivo* assay developed by Baldin (2016). We hypothesized that COO alters BH rates, increasing its intermediates by decreasing the last two step of LA BH.

2.0 MATERIAL AND METHODS

2.1 Experimental design and treatments

All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. The experiment was conducted in April 2016 at the Pennsylvania State University Dairy Production Research and Teaching Center. Four multiparous Holstein cows fitted with ruminal cannulas were used in the experiment. All cows had passed peak lactation and averaged milk production of 38.6 kg/day (± 6.8) at the beginning of the experiment. Cows were housed individually in tie stalls with rubber mattresses and sawdust bedding and had continuous access to water. Cows were grouped and arranged in a 5-d period cross over design with two treatments: control and coconut oil [(COO), Figure 1]. Cows in the control treatment received a typical lactating diet balanced to meet NE_L and MP requirements according to NRC 2001 (Table 1). Cows in the COO treatment received the basal diet and for 3 days were infused in the rumen with 430 g/cow/d of coconut oil.

Coconut oil was provided as a pulse dose once daily immediately before morning feeding. Immediately before dosing, approximately 5 kg of rumen contents were removed to provide space for initial mixing. The oil was pulse-dosed through the rumen cannula and thoroughly mixed manually with the digesta for approximately 2 min; then the removed digesta was returned and contents were thoroughly mixed for an additional 1 min. In a relative basis, the COO contained 7.7% C8:0, 5.8% C10:0, 46.4% C12:0, 18.1% C14:0, 10.1% C16:0, 3.0% C18:0, 6.8% *cis*-9 C18:1, 1.7% C18:2 n-6, and 0.4% others. The daily dose of COO and the duration in d of COO infusion were chosen based on HRISTOV et al. (2004) to provide 200 g of C12:0 per cow/d. A 12-d washout interval was allowed between periods to prevent treatment carryover effect.

Figure 1. Schematic of experimental design. Four ruminally cannulated cows were arranged in a 5-day period cross over design (P1: period 1, P2: period 2). Pretrial measurements (d1) served as a covariate. Cows in the coconut oil treatment received for 3 days 430 g/cow per day of coconut oil. On d3 a bolus infusion assay (perturbation tracee model) was performed to assess extent, rate, and biohydrogenation pathways of linoleic acid. All cows received the control diet on d5.

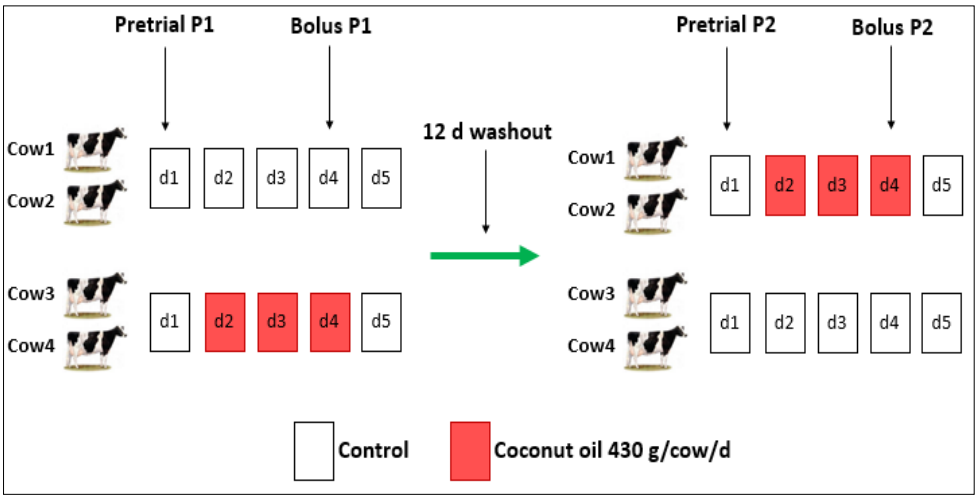


Table 1. Diet ingredients and chemical composition of the basal diet.

Ingredient	% of Diet DM
Corn silage	43.5
Alfalfa haylage	15.0
Grass hay/straw	2.7
Ground corn	3.6
Canola meal	13.8
Minerals and vitamins mix[1]	2.9
Optigen	0.4
Bakery by-product	8.2
Corn gluten meal	4.6
Molasses	5.2
Composition, % of DM	
CP	18.5
NDF	30.1
Starch	27.9
Total FA	3.4
Unsaturated FA	2.7
Mean particle size[2], mm	5.8

[1]Composition (DM basis): 11% CP; 18% NDF; 5.2% fat; 14.9% Ca; 0.35% P; 4.58% Mg; 0.41% K; 0.31% S; 357 mg/kg Cu; 1,085 mg/kg Zn; 181 mg/kg Fe; 6.67 mg/kg Se; 262,105 IU/kg vitamin A; 65,421 IU/kg vitamin D; and 1,970 IU/kg vitamin E (Cargill Animal Nutrition, Roaring Spring, PA).
[2]Analyzed using the Penn State Particle Separator. Particles remaining (% of total) in the upper sieve, middle sieve, lower sieve, and bottom pan were 4, 44, 14, and 38, respectively.

Cows were milked twice daily at 0500 and 1700 h and milk yield determined by an integrated milk meter (AfiMilk, SAE Afikim, Afikim, Israel). Milk was sampled at both milkings on experimental day 1, 3, and 4 of each period.

2.2 Bolus experiment

On experimental d 4, a bolus infusion assay (perturbation tracee model) was performed to assess extent, rate, and BH pathways of linoleic (LA). Because the perturbation tracee model requires steady state, a frequent feeding schedule was implemented with the goal of maintaining near steady state ruminal conditions. On experimental d 2, cows were fed in equal meals every 6 h; on d 3, cows were fed equal-sized meals every 2 h; and on the day of the bolus infusion (d 4), cows were fed hourly at 4.2%/h (1/24th) of expected daily dry matter intake (DMI). On d 1 and d 5 cows were fed at 110% of expected daily dry matter intake. Refusals were removed and measured once per day. Feed ingredients were sampled once per period and stored at −20°C.

The bolus infusion consisted of 200 g of 70% LA safflower (Jedwards International Inc., Braintree, MA). An odd-chain saturated FA (C17:0 as free FA, Tokyo Chemical Industry Co., Tokyo, Japan) was included in the oil bolus to serve as a marker of FA passage from the rumen. The oil mixture (200 g of LA-oil + 12 of C17:0) was placed in a water bath at 50^{o}C for 30 min to aid mixing. Immediately before dosing, approximately 20% of rumen contents were removed to provide space for initial mixing. The oil mixture was bolus-dosed through the rumen cannula and thoroughly mixed manually with the digesta for approximately 1 min; then the removed digesta was returned and contents were thoroughly mixed for an additional 4 min.

Rumen digesta was collected at -1, -0.25, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 h relative to the bolus. The first two samples (-1 and -0.25 h) were used to characterize background levels of LA and C17:0 in the rumen. At each sampling, whole digesta was collected from five different locations in the rumen (cranial dorsal, cranial ventral, central, caudal dorsal, and caudal ventral), mixed in a bucket, and a composite subsample of approximately 200 g was collected and immediately placed on dry ice. A second subsample was strained through 0.5 mm plastic mesh and pH was measured using a hand-held pH meter (model # 35631-60, Oakton Instruments, Vernon Hills, IL). The remainder

rumen content was immediately returned to the cow. Rumen samples were stored at -20 ^{o}C until analysis.

2.3 Sample analysis

One milk sample was stored at 4°C with preservative (Bronolab-WII, Advanced Instruments Inc., Norwood, MA) until analyzed for fat and protein by fourier transform infrared spectroscopy [Fossomatic 4000 Milko-Scan and 400 Fossomatic, Foss Electric, Hillerød, Denmark; Dairy One Laboratory, Ithaca, NY]. A second milk sample was immediately spun at 3000 x g for 15 min at 4^{o}C and a fat cake was stored at −20°C before analysis of FA composition as described by (RICO and HARVATINE, 2013). Briefly, lipid extraction was performed according to Hara and Radin (1978) using hexane:isopropanol. Fatty acid methyl esters were prepared by base-catalyzed transmethylation according to (CHOUINARD et al., 1999). Fatty acid methyl esters were quantified by gas chromatography using an Agilent 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a fused-silica capillary column (SP-2560, 100 m × 0.25 mm i.d. with 0.2-μm film thickness; Supelco Inc., Bellefonte, PA) and a flame ionization detector with hydrogen as the carrier gas. Initial oven temperature was 80°C, which was increased by 2°C/min to 190°C and held for 15 min. Inlet and detector temperatures were 250°C with a 100:1 split ratio. Constant gas flows were 1 mL/min for hydrogen carrier, 25 mL/min for detector hydrogen, 400 mL/min for detector airflow, and 40 mL/min for detector nitrogen plus carrier. Fatty acid peaks were identified using FAME standards (GLC 461, GLC 780, and pure *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA, NuChek Prep Inc., Elysian, MN; Bacterial Acid Methyl Ester Mix, 47080-U, Sigma-Aldrich Inc, St. Louis, MO; and GLC 110 mixture, Matreya LLC., State College, PA). Recovery of individual FA were determined using an equal weight reference standard (GLC 461; NuChek Prep Inc.). Correction factors for individual FA and calculation of milk FA yield were carried out as described by (RICO and HARVATINE, 2013).

Feed samples were analyzed for DM, CP, and starch by wet chemistry procedures according to AOAC International (2000) and for NDF and ADF according to (VAN SOEST et al., 1991). Total FA concentration and FA profile of feed samples was

determined by gas chromatography after direct methylation Sukhija and Palmquist (1988) as described by (RICO and HARVATINE, 2013). Frozen rumen samples were lyophilized for 52 h with a five-step program (-35ºC for 60 min, -10ºC for 120 min, 0ºC for 480 min, 3ºC for 1250, 30ºC for 1250; Virtis 3.5 L XL, The Virtis Co., Gardiner, NY). The dried digesta was then ground to <1mm using a spinning blade coffee grinder (Hamilton Beach, model 80335R). Fatty acids were methylated using sodium methoxide followed by methanolic HCl as described by (JENKINS, 2010). Internal standards for determination of FA concentrations were *cis*-10 C17:1 FFA and C19:0 FAME. The gas chromatography settings were identical to analysis of FA in milk except that initial oven temperature was 45ºC for 4 min, after which it was increased 13°C/min to 150°C and held for 47 min, and then increased 4°C/min to 215°C and held for 25 min. Peaks were identified using reference standards (GLC 780, 461, and 566, NuChek Prep Inc., Elysian, MN; Bacterial Acid Methyl Ester Mix, 47080-U, Sigma-Aldrich Inc, St. Louis, MO; and GLC 110 mixture, Matreya LLC., State College, PA).

2.5 Statistical analysis

Data were analyzed as a cross over design using PROC MIXED of SAS (version 9.4; SAS Institute Inc., Cary, NC). Effect of period and the two and three-way interactions between period, and time and treatment were initially tested as fixed effects but removed from all models due to lack of significance. Period was then assigned as random effect as well as sequence and cow nested within sequence. Subject was cow*treatment. Dry matter intake, milk yield, milk components, rate of dry matter intake, rumen pH, and rumen intermediate FA of C18:2 BH were analyzed as repeated measures. Fixed effects for this model were treatment, time and the interaction treatment*time. Measurements on d 1 (pre-treatment) or h -1 (pre-bolus) were used as covariate in the repeated measures model when significant. The ARH(1) or AR(1) covariance structures were used depending on model fit and the Kenward-Rogers denominator degrees of freedom adjustment was employed. The CS covariance structure was used for measurements unequally spaced in time. Preplanned contrasts tested differences between CON and COO at each time point. Rumen disappearance of C18:2 and C17:0 were modeled using the

first-order kinetic model of Ørskov and Mcdonald (1979) implemented in the nonlinear procedure of JMP Pro 12.1 (SAS Institute, Cary, NC). This model is described as a "Single Available Pool, First-Order Kinetic Decay", such as $Y = C + Pe^{(-k*t)} + \varepsilon$, where Y = concentration of FA (g/100g FA) at time t, C = pool of unavailable FA, P = pool of available FA, k = fractional rate of FA disappearance (h^{-1}); t = time (h), and ε = residual error. A pool of unavailable FA did not parameterize for C17:0. Rumen disappearance of C18:2 and C17:0 was fit for each individual cow within treatment. The fractional rate of FA disappearance (k) was then compared between treatments as a single measurement using the mixed model described above. In this analysis, the inverse of the standard error of the fit for k for each cow*treatment was used a weight factor. The rate of C17:0 disappearance was assumed to represent the rate of FA passage from the rumen. The fractional rate of C18:2 BH was calculated as the difference between total C18:2 disappearance and total C17:0 disappearance. This required the following assumptions: a) C17:0 disappeared only by passage, b) C18:2 disappeared by passage and BH, and c) the rate of passage of C17:0 and C18:2 are equal. Biohydrogenation was calculated by the difference between total rumen disappearance C18:2 – Total rumen disappearance C17:0. The Extent of biohydrogenation was calculated by the following equation: Biohydrogenation = Biohydrogenation / (Biohydrogenation + Total rumen disappearance C17:0) x 100.

Enrichment of rumen FA concentration pools, rate of C18:2 BH, and extent of C18:2 BH were also analyzed as single measurements using the mixed model described above. Data points with Studentized residuals outside of ± 3.0 were considered outliers and excluded from analysis. Significant differences between treatments were declared at $P \leq 0.05$ and tendencies at $P > 0.5 \leq 0.10$.

A muticompartimental model was also tested using SAAM II (2.3). All 18 carbons FA were analyzed with the design as describe above by the PROC MIXED of SAS. Before the analyses, the best error structure was defined based on the AIC parameters using the command REPEATED. Degrees of freedom and tests were adjusted using the Kenward-Roger option. The averaged FA for each treatment in each time point was used to determine the BH rates (Figure 10), and all observations were weighted by their respective standard error. Treatment differences were teste by Studentized t Test with unequal variances.

3.0 RESULTS

3.1 Overall performance

Treatments did not alter ($P > 0.05$ for all) DMI, milk yield, milk fat concentration, milk fat yield, milk protein concentration and yield (Table 2; Figure 2, panel A and B; Figure 3, panel A, B, C, and D respectively). Additionally, no interactions of treatment by time were observed for overall performance. On the other hand, a tendency ($P = 0.09$) was observed on milk yield and protein concentration (Table 2) Cows in the control treatment had higher milk yield on d2 and d3 during the evening milking (Figure 2, panel B). Similarly, cows in control treatment had higher milk protein concentration on d1 evening milking and on d4 morning milking ($P= 0.07$; Figure 3, panel B).

Table 2. Performance variables

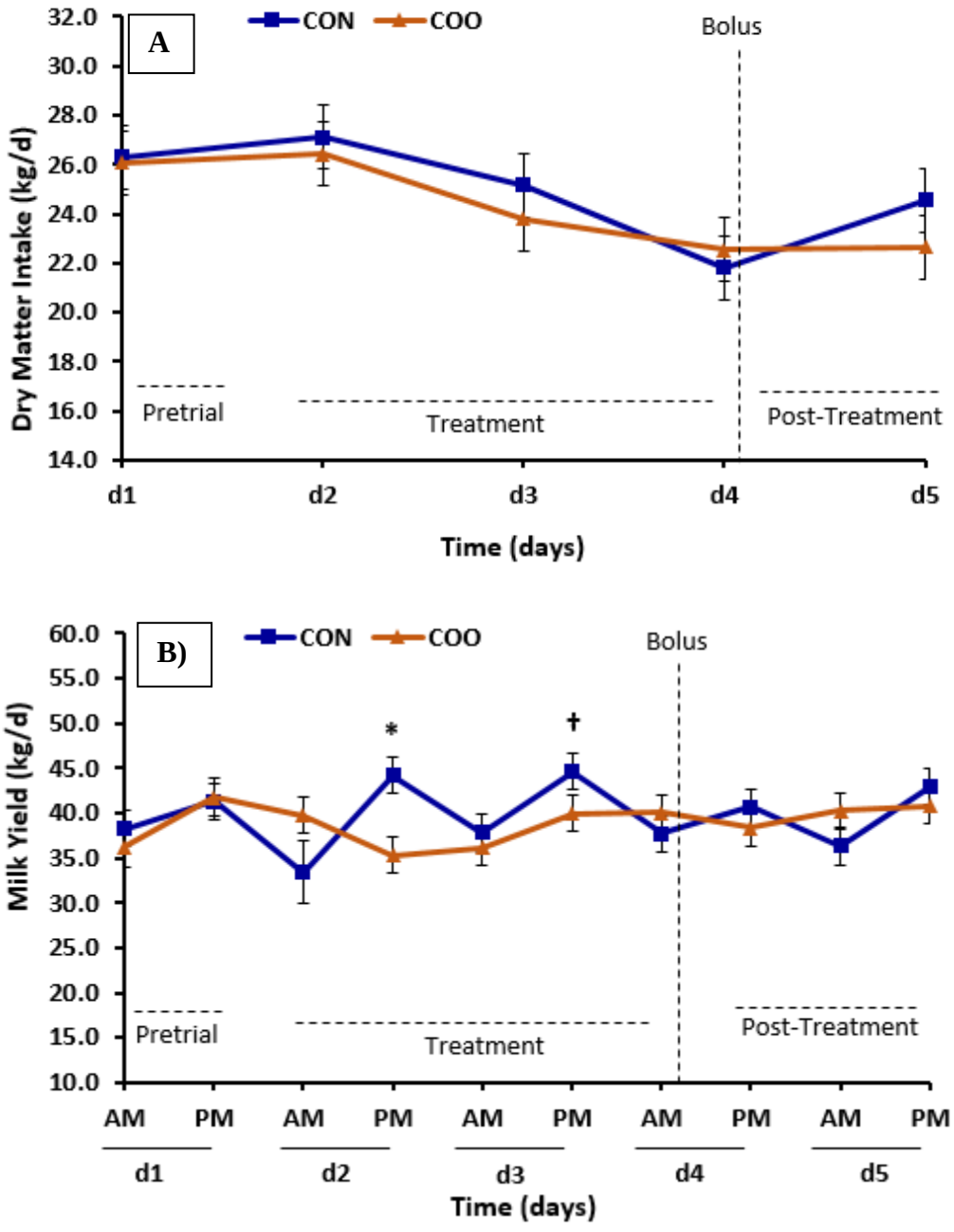
	Treatment[1]			*P-values*[2]		
	CON	COO	SEM	Trt	Time	Trt*Time
DMI, kg/d	24.3	26.3	0.77	0.55	0.02	0.67
Milk Yield, kg/d	39.8	38.8	0.84	0.16	0.19	0.09
Fat, %	3.48	3.54	0.19	0.69	0.79	0.38
Fat Yield, kg/d	1.39	1.35	0.08	0.74	0.53	0.57
Protein, %	3.13	3.11	0.13	0.21	0.70	0.07
Protein Yield, kg/d	1.21	1.19	0.08	0.66	0.37	0.39

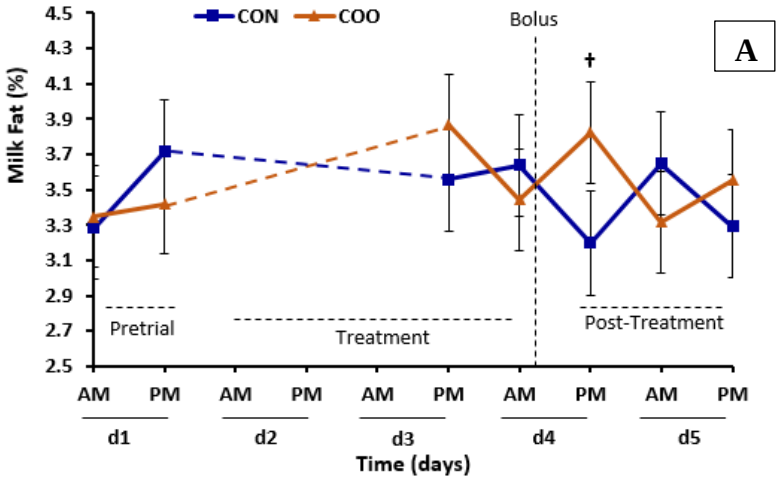
[1]Treatment was control (CON) and coconut oil (COO). CON treatment only a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused through the rumen cannula. COO 430 g/cow/d of coconut oil (COO) infused through the rumen cannula for 3 d immediately before morning feeding and a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused.
[2]Effect of treatment, experimental day, and their interaction.

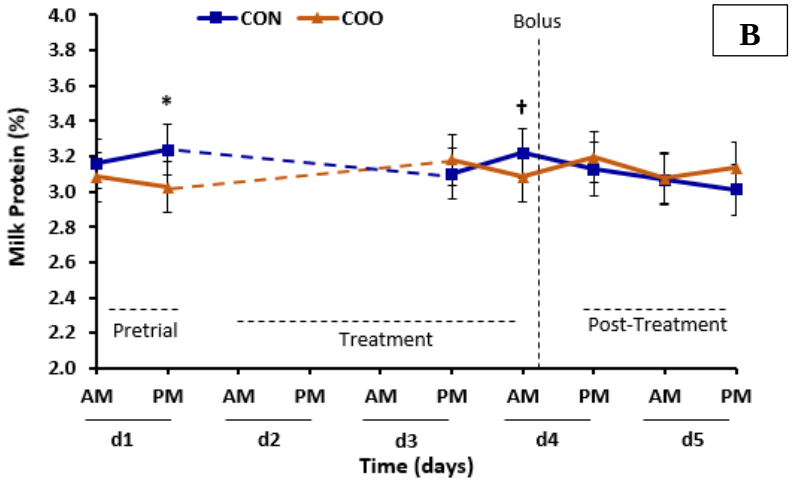
Concentration of C12:0 and C14:0 was higher in milk from cows in the COO treatment, and had a treatment by time interaction ($P<0.001$), but did not increase the total saturated fatty acids (SFA) in the milk (Table 3). Concentration of C18:0, C18:1, C18:2 and saturated/unsaturated ratio were affected by the treatments (Table 3). There was an interaction between treatment by time to C12:0, C14:0, C18:0, C18:2 and

saturated/unsaturated ratio. The COO increased the *trans*-11 C18:1 in the milk (Table 3; Figure 4, panel A and B respectively).

Figure 2. Dry matter intake (top) and milk production (bottom). Treatments were control (CON) or coconut oil (COO, 430g/cow/d). † = P < 0.10, * = P < 0.05, ** = P <0.01, *** = P < 0.001




A
CON
COO
Bolus
†
Milk Fat (%)
4.5
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
Pretrial
Treatment
Post-Treatment
AM
PM
d1
d2
d3
d4
d5
Time (days)


B
CON
COO
Bolus
*
†
Milk Protein (%)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
Pretrial
Treatment
Post-Treatment
AM
PM
d1
d2
d3
d4
d5
Time (days)

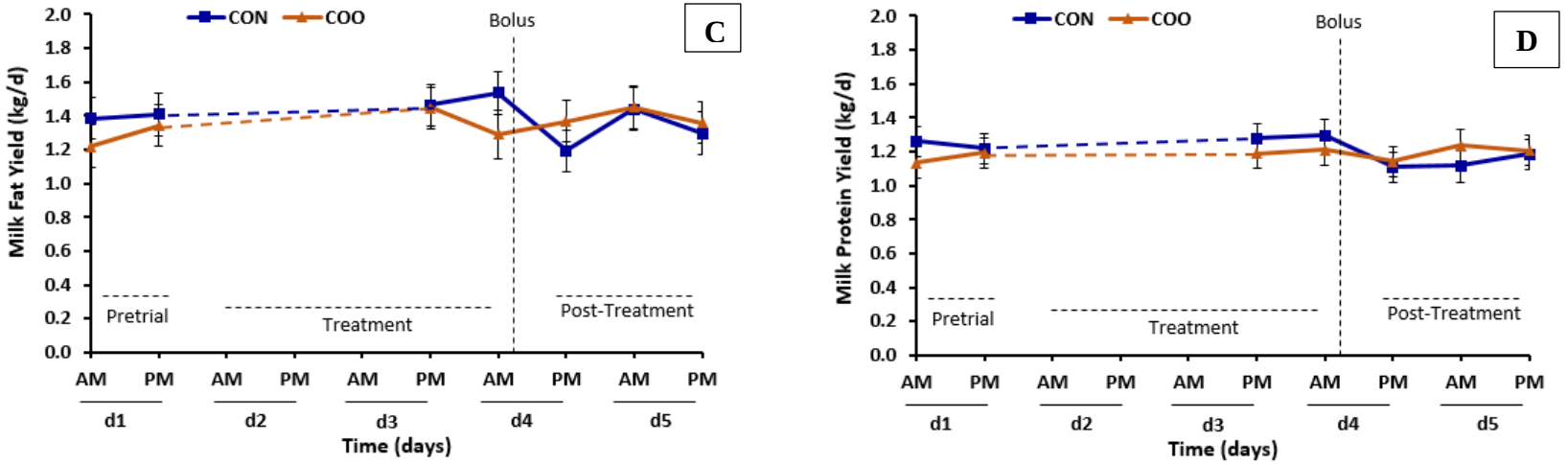


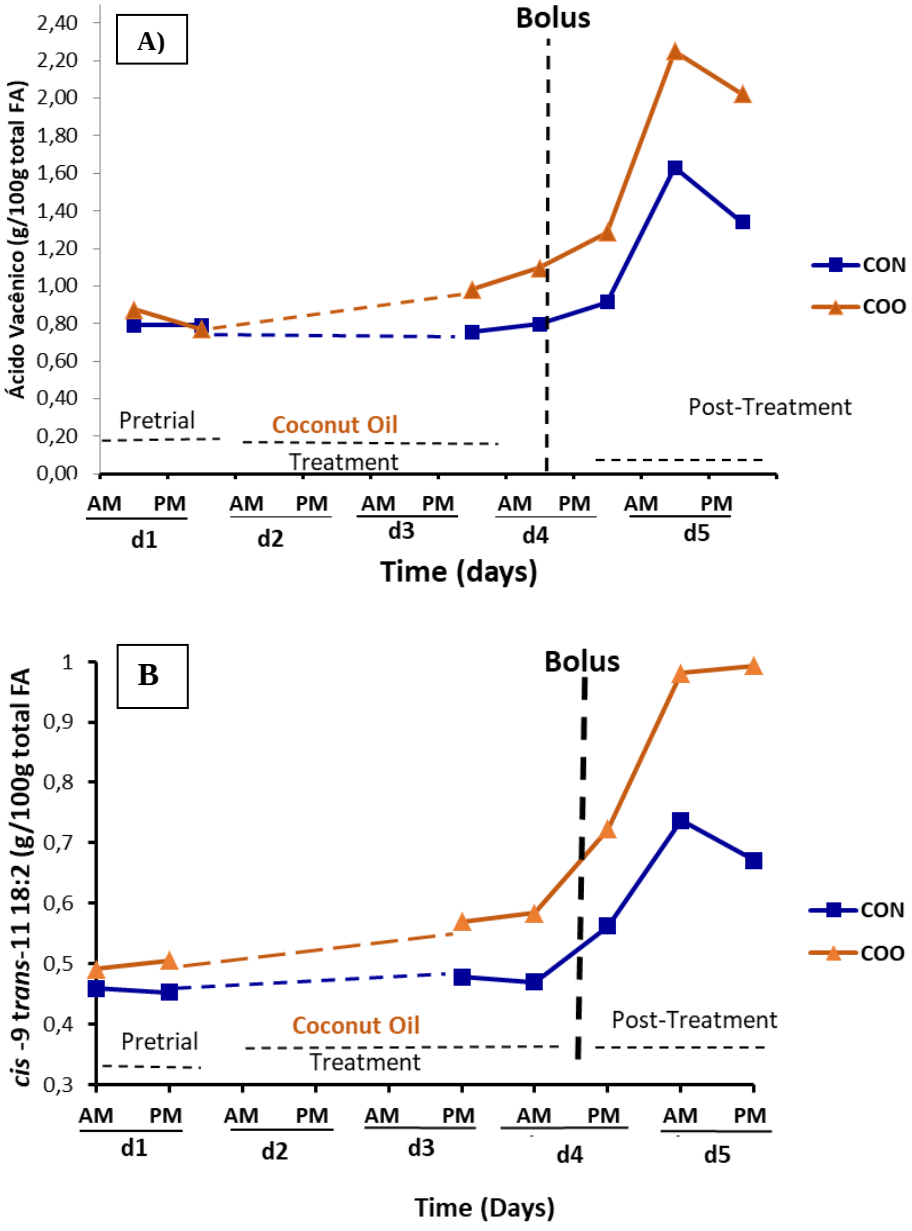
Figure 3. Concentration of fat and protein (panels A and B, respectively) in milk and yields of milk fat and protein (panels C and D, respectively). Treatments were control (CON) or coconut oil (COO, 430g/cow/d). † = P < 0.10, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Table 3. Milk fatty acid profile of dairy cows submitted of coconut oil infusion.

	Treatment[1]			*P-values2*		
FA, g/100g Total FA	CON	COO	SEM	Trt	Time	Trt*Time
C4:0	3.97	3.90	0.152	0.29	0.06	0.35
C6:0	2.28	2.15	0.056	0.04	0.20	0.25
C8:0	1.35	1.25	0.039	0.02	0.14	0.18
C10:0	3.36	3.06	0.231	<0.001	0.33	0.15
C12:0	4.03	6.46	0.254	<.0001	<.0001	<.0001
C14:0	11.63	13.18	0.139	<0.01	<0.01	<0.001
C16:0	29.25	27.41	0.860	0.15	<0.01	0.03
C18:0	9.45	8.44	0.338	0.02	0.03	<0.01
C18:1	16.81	16.01	0.502	0.02	<0.001	0.27
trans-10 C18:1	0.44	0.67	0.129	0.16	<0.01	0.18
trans-11 C18:1	1.00	1.33	0.102	<0.01	<.0001	0.23
C18:2	2.53	2.28	0.083	<0.01	<0.001	<0.001
trans-10, *cis*-12 CLA	Trace	0.003	0.001	0.17	0.167	0.16
cis-9, *trans*-11 CLA	0.54	0.692	0.083	0.12	<0.001	0.59
C18:3	0.40	0.37	0.025	0.29	<0.01	0.06
UFA	21.15	20.87	0.725	0.34	<0.01	0.10
SFA	3.09	3.07	0.108	0.62	0.01	0.15
S/U[3]	0.146	0.147	0.0002	<0.01	<.0001	<0.01
SUM[4]	87.22	87.04	0.456	0.41	<0.01	0.66

[1]Treatment was control (CON) and coconut oil (COO). CON treatment only a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused through the rumen cannula. COO 430 g/cow/d of coconut oil (COO) infused through the rumen cannula for 3 d immediately before morning feeding and a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused. [2]Effect of treatment, experimental day, and their interaction. [3]S/U= Ratio of saturated and unsaturated FA. [4]SUM= Sum of selected FA.

Figure 4. Effect of coconut oil bolus infusion on vaccenic acid (Panel A) and CLA *cis*-9 *trans*-11 (Panel B) in the milk.



3.2 Parameters during bolus assay

Overall, the rate of DMI (kg/h) was not affected by treatments and no interaction treatment by time was observed (Table 4; Figure 5, panel A). Contrarily, cows receiving COO had overall higher rumen pH than cows in the control treatment during the bolus (6.56 vs. 6.47), but no interaction treatment by time was noted (Table 4, and Figure 5B).

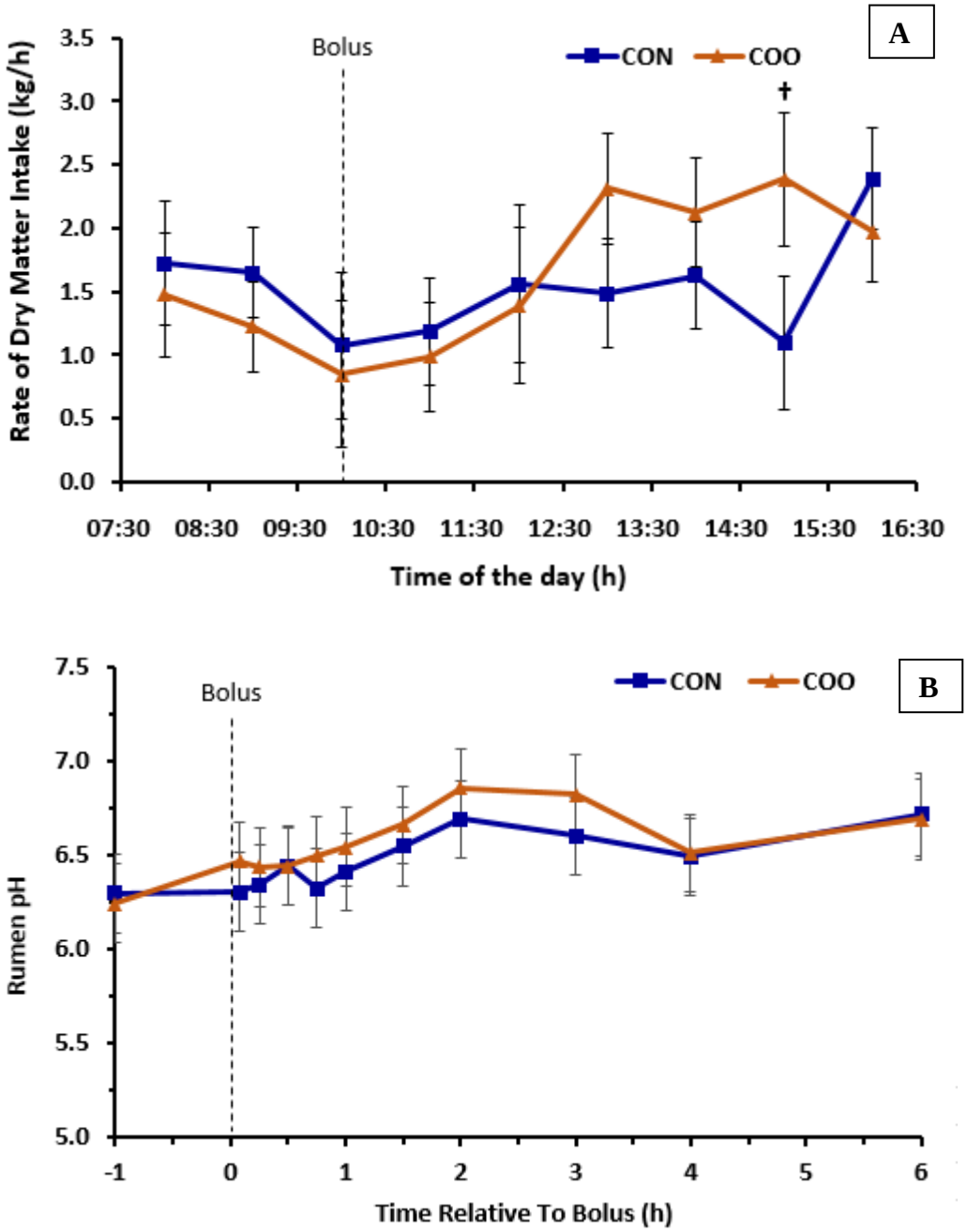
The total concentration of FA (TFA) in the rumen prior the bolus was higher in cows receiving COO than Control (4.40 vs 2.53 % TFA; $P < 0.001$; Table 4). After bolus the TFA was also higher in cows receiving COO than control (6.35 vs 3.76 % TFA; $P= 0.02$; Table 4). However, no difference in fold enrichment was observed between the treatments (Table 4).

Table 4. Rate of dry matter intake, rumen pH, and enrichment of concentration pools of total fatty acids (TFA), heptadecanoic acid (C17:0), and linoleic acid (C18:2) in the rumen following a bolus infusion assay.

	Treatment[1]			*P-values*[2]		
	CON	COO	SEM	Trt	Time	Trt*Time
Rate of DMI, kg/h	1.53	1.64	0.36	0.71	0.12	0.22
Rumen pH	6.47	6.56	0.18	0.02	<0.001	0.93
TFA, %DM						
Prior-bolus	2.53	4.40	0.22	<0.001	--	--
Post-bolus	3.76	6.35	0.80	0.02	--	--
Fold-enrichment	0.51	0.44	0.19	0.71	--	--
C17:0, %TFA						
Prior-bolus	0.50	0.24	0.02	<0.01	--	--
Post-bolus	2.25	1.88	0.44	0.47	--	--
Fold-enrichment	3.49	7.01	1.55	0.10	--	--
C18:2, %TFA						
Prior-bolus	12.08	6.68	1.06	<0.01	--	--
Post-bolus	27.10	23.61	4.12	0.45	--	--
Fold-enrichment	1.34	2.66	0.60	0.15	--	--

[1]Treatment was control (CON) and coconut oil (COO). CON treatment only a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused through the rumen cannula. COO 430 g/cow/d of coconut oil (COO) infused through the rumen cannula for 3 d immediately before morning feeding and a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused. [2]Effect of treatment, experimental day, and their interaction.

Figure 5. Rate of dry matter intake (top) and rumen pH (bottom) during bolus infusionassay (perturbation tracee model). Treatments were control (CON) or coconut oil (COO, 430g/cow/d). † = P < 0.10, * = P < 0.05, ** = P < 0.01, *** = P < 0.001



Rumen concentration of 17:0 prior the bolus was higher in control than COO cows (0.50 vs. 0.24 % TFA, *P* < 0.01; Table 4). Rumen concentration of C17:0 and C17:0

enrichment did not differ between treatments immediately after the bolus infusion (Table 4; Figure 6, panel A). Similarly, the rumen concentration of C18:2 prior the bolus was higher in control than COO (12.08 vs 6.68; % TFA; $P<0.01$). Rumen concentration of C18:2 and C17:0 enrichment did not differ between treatments immediately after the bolus infusion (Table 4; Figure 6, panel B).

Total rumen disappearance of C17:0 did not differ between treatments (P = 0.17) and averaged 6.98 %/h and 7.54 %/h in control and COO treatments respectively (Table 5; Figure 7). Similarly, total rumen disappearance of C18:2 (P = 0.43) and rate of C18:2 BH (P = 0.82) did not differ between treatments and averaged 45.6%/h and 38.3 %/h, respectively (Table 5; Figure 8, panel A and B). Extent of rumen biohydrogenation of C18:2 was not different between treatments and averaged 84.8 % and 83.3 in control and COO respectively (Table 5).

Table 5. Total rumen disappearance rate (%/h) of concentration pools of heptadecanoic acid (C17:0), and linoleic acid (C18:2) following a bolus infusion assay.

	Treatment[1]			
	CON	COO	SEM	P-value
Total rumen disappearance C18:2, %/h	45.8	45.3	6.96	0.43
Total rumen disappearance C17:0, %/h[2]	6.98	7.54	1.34	0.17
Rate of biohydrogenation C18:2, %/h[3]	38.8	37.7	7.33	0.82
Extent of rumen biohydrogenation C18:2, %[4]	84.8	83.3	4.04	0.33

[1]Treatment was control (CON) and coconut oil (COO). CON treatment only a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused through the rumen cannula. COO 430 g/cow/d of coconut oil (COO) infused through the rumen cannula for 3 d immediately before morning feeding and a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused. [2]Assumed to represent rate of fatty acid passage from the rumen. [3]Biohydrogenation = Total rumen disappearance C18:2 – Total rumen disappearance C17:0. [4]Extent of biohydrogenation = Biohydrogenation / (Biohydrogenation + Total rumen disappearance C17:0) x 100.

Cows receiving COO had higher rumen *trans*-10 C18:1 in the rumen at time 3 and 4 hours after the bolus infusion (Table 6; Figure 9, panel A). An interaction treatment by time was observed for rumen *trans*-11 C18:1, *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA ($P < 0.05$ for all; Table 6). Relative to the bolus infusion, cows in the control treatment had lower rumen *trans*-11 C18:1 at 3 and 4 hours, tended to have higher *cis*-9, *trans*-11 CLA at h 0.45, and had lower *trans*-10, *cis*-12 CLA at 2 and 3 hours (Figure 9, Panel B, C, and D, respectively).

Figure 6. Enrichment and disappearance of concentration pools of heptadecanoic acid (C17:0, top panel), and linoleic acid (C18:2, bottom panel) during the bolus infusion assay (perturbation tracee model). Treatments were control (CON) or coconut oil (COO, 430g/cow/d). Data points represent LSMEANS ± SEM (n = 4 cows/treatment) obtained with the following mixed model: FA concentration = treatment + time + treatment*time + error. Period, sequence, and cow (sequence) were considered random effects. Time was the repeated variable and cow*treatment the subject. Compound symmetry was the covariance structure used because measurements were not equally spaced in time.

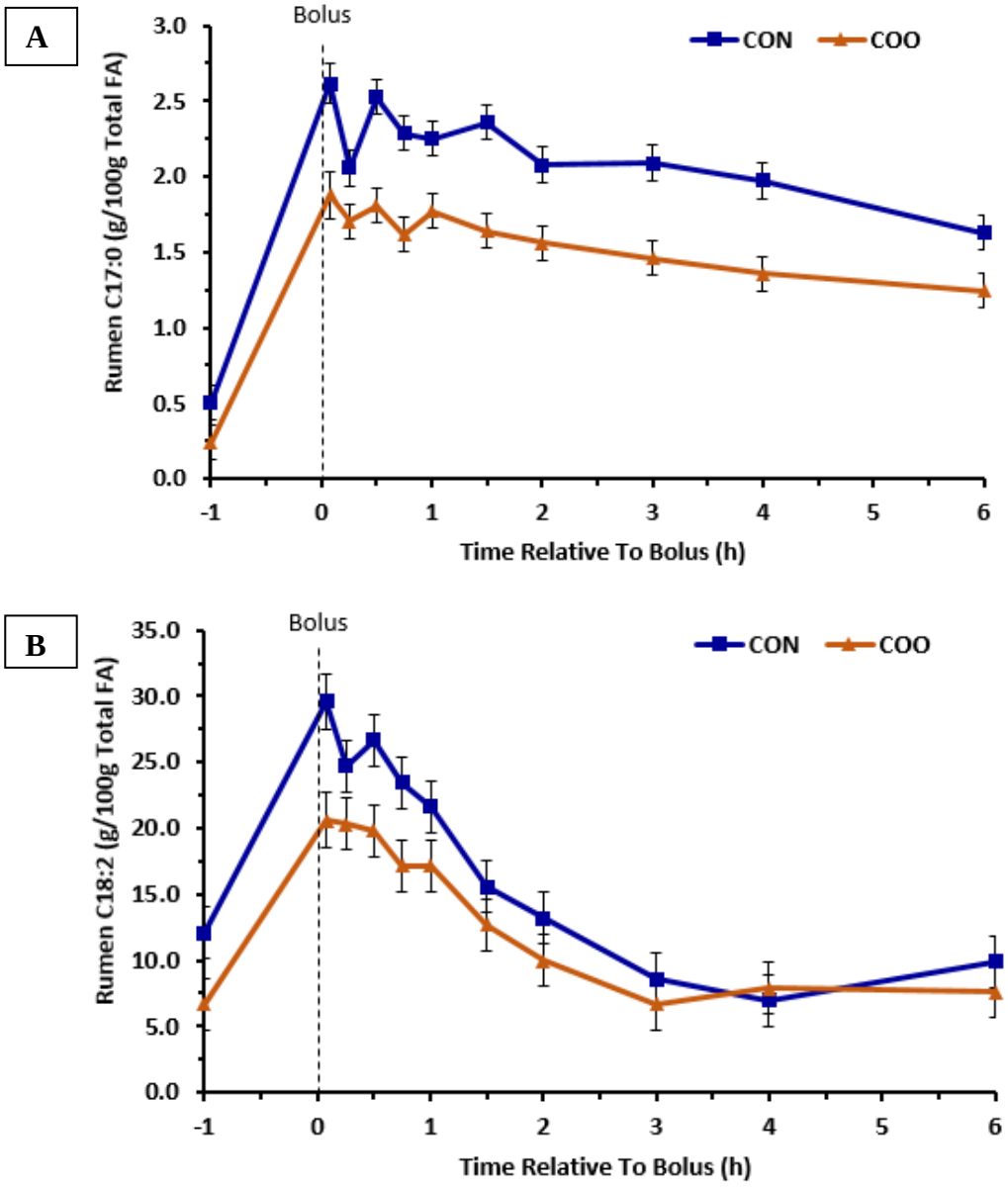


Figure 7. Total rumen disappearance of concentration pool of heptadecanoic acid (C17:0) following a bolus infusion assay (perturbation tracee model). Treatments were control (CON) or coconut oil (COO, 430g/cow/d). RMSE of fit for total rumen disappearance was 15.1 for CON and 13.1 for COO. Rate of disappearance was 6.98

%/h for CON and 7.54 %/h for COO (SEM = 1.34, *P* = 0.17). RMSE of fit for total rumen disappearance was 0.44 for CON and 0.41 for COO.

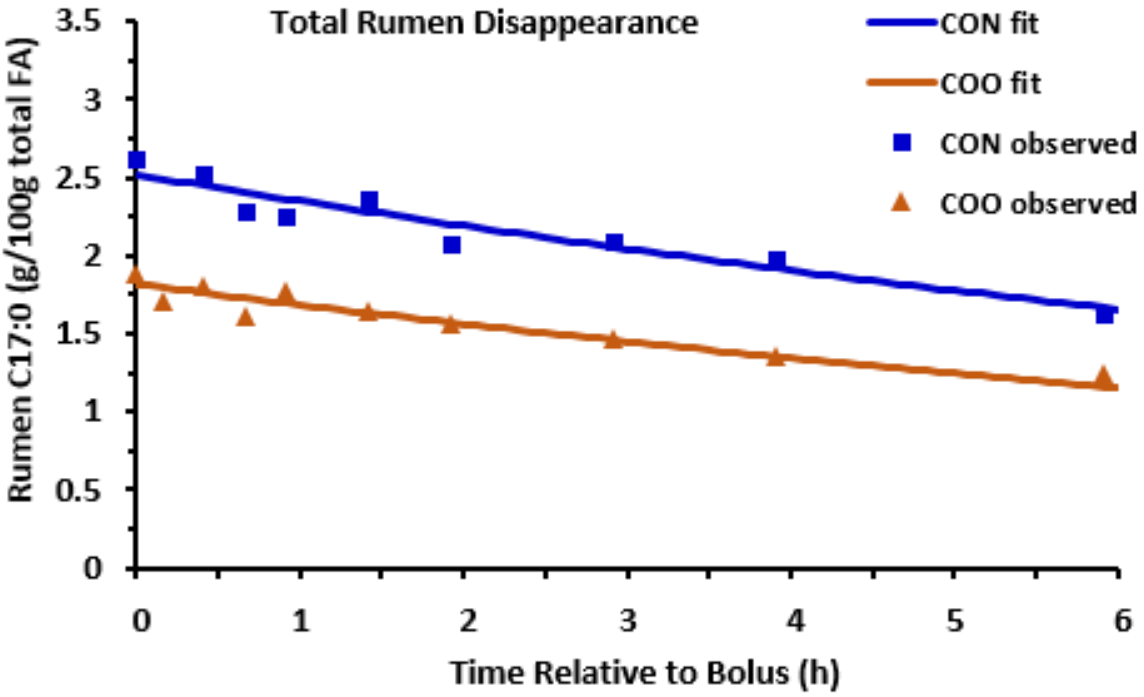


Table 6. Rumen concentration of fatty acids intermediates of linoleic acid biohydrogenation following a bolus infusion assay.

	Treatment[1]			*P-values*[2]		
	CON	COO	SEM	Trt	Time	Trt*Time
trans-10 C18:1	1.296	1.427	0.039	0.06	<0.001	0.43
trans-11 C18:1	3.632	4.016	0.191	0.23	<0.001	0.02
cis-9, *trans*-11 CLA	0.580	0.367	0.112	0.36	<0.001	0.04
trans-10, *cis*-12 CLA	0.086	0.098	0.012	0.06	<0.001	<0.01

[1]Treatment was control (CON) and coconut oil (COO). CON treatment only a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused through the rumen cannula. COO 430 g/cow/d of coconut oil (COO) infused through the rumen cannula for 3 d immediately before morning feeding and a day bolus dose of 12g of C17:0 + 200g/cow/d of safflower oil was infused.

Figure 8. Total rumen disappearance of concentration pool (top panel) and model-estimated rate of biohydrogenation (bottom panel) of linoleic acid (C18:2) following a

bolus infusion assay (perturbation tracee model). Treatments were control (CON) or coconut oil (COO, 430g/cow/d). RMSE of fit for total rumen disappearance was 15.1 for CON and 13.1 for COO.

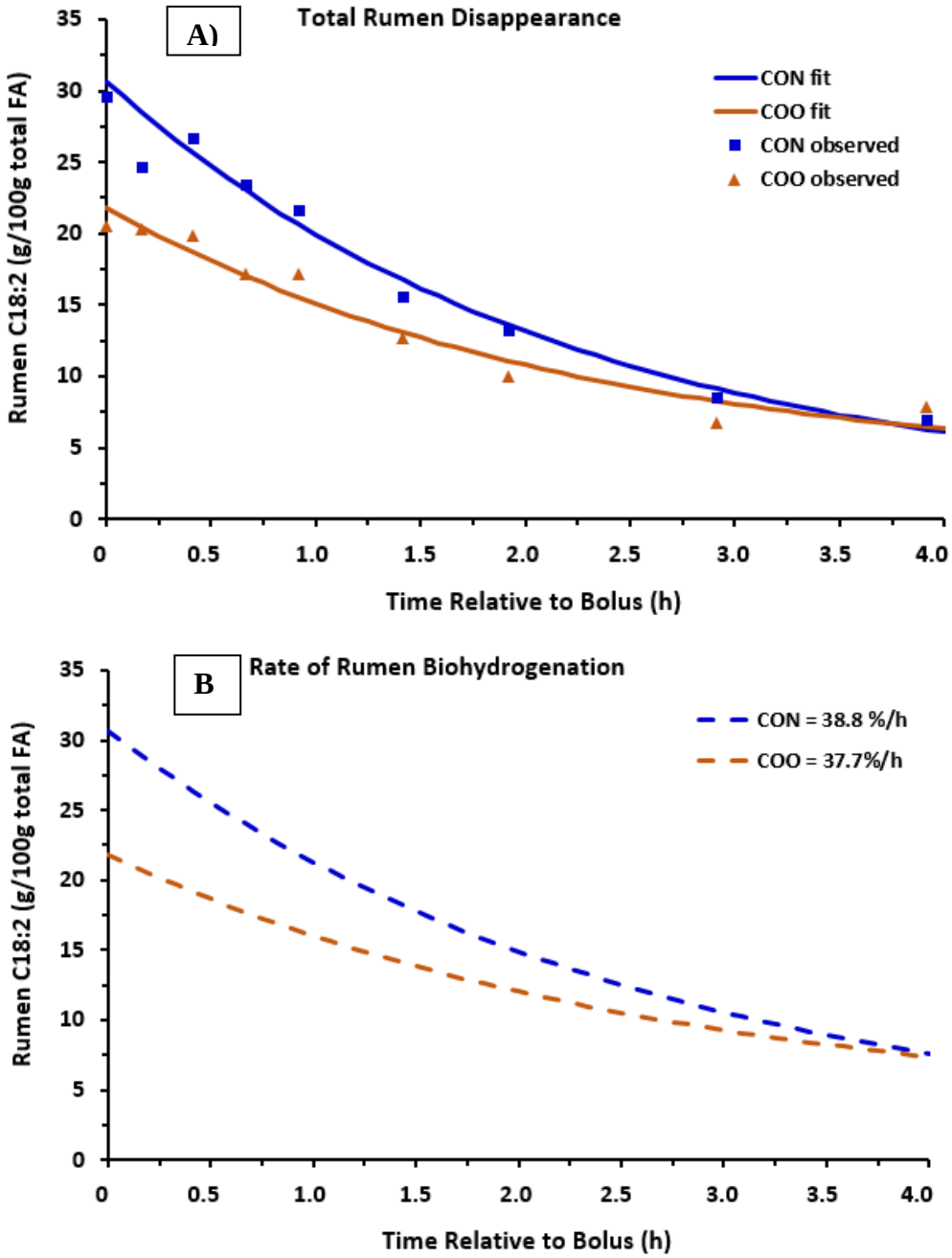


Figure 9. Rumen concentration of pool of *trans*-10 C18:1 (A), *trans*-11 C18:1 (B), *trans*-9, *cis*11 CLA (C), and *trans*-10, *cis*-12 CLA (D) following a bolus infusion assay (perturbation tracee model). Treatments were control (CON) or coconut oil (COO, 430g/cow/d). RMSE of fit for total rumen disappearance was 15.1 for CON and 13.1 for COO. † = P < 0.10, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

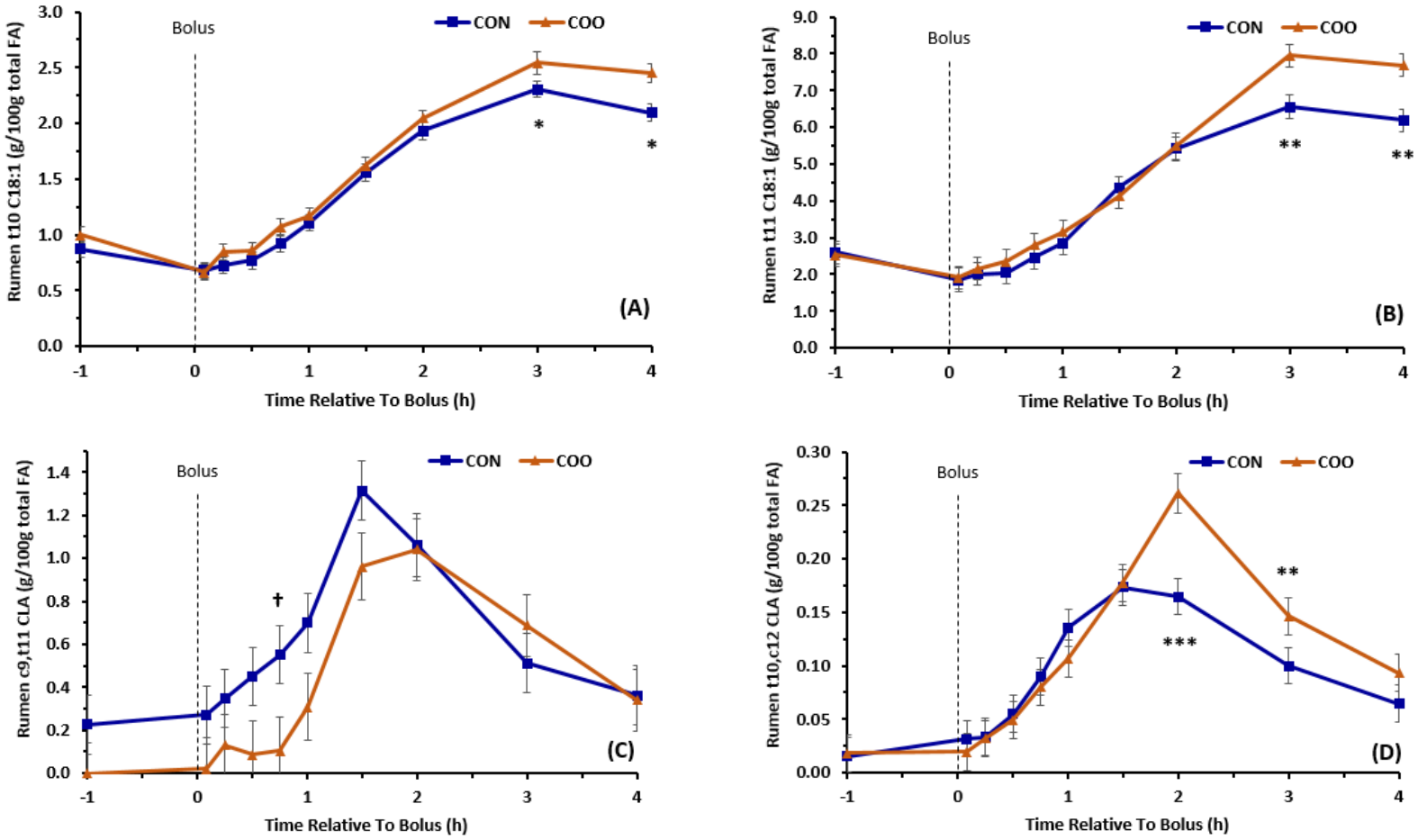
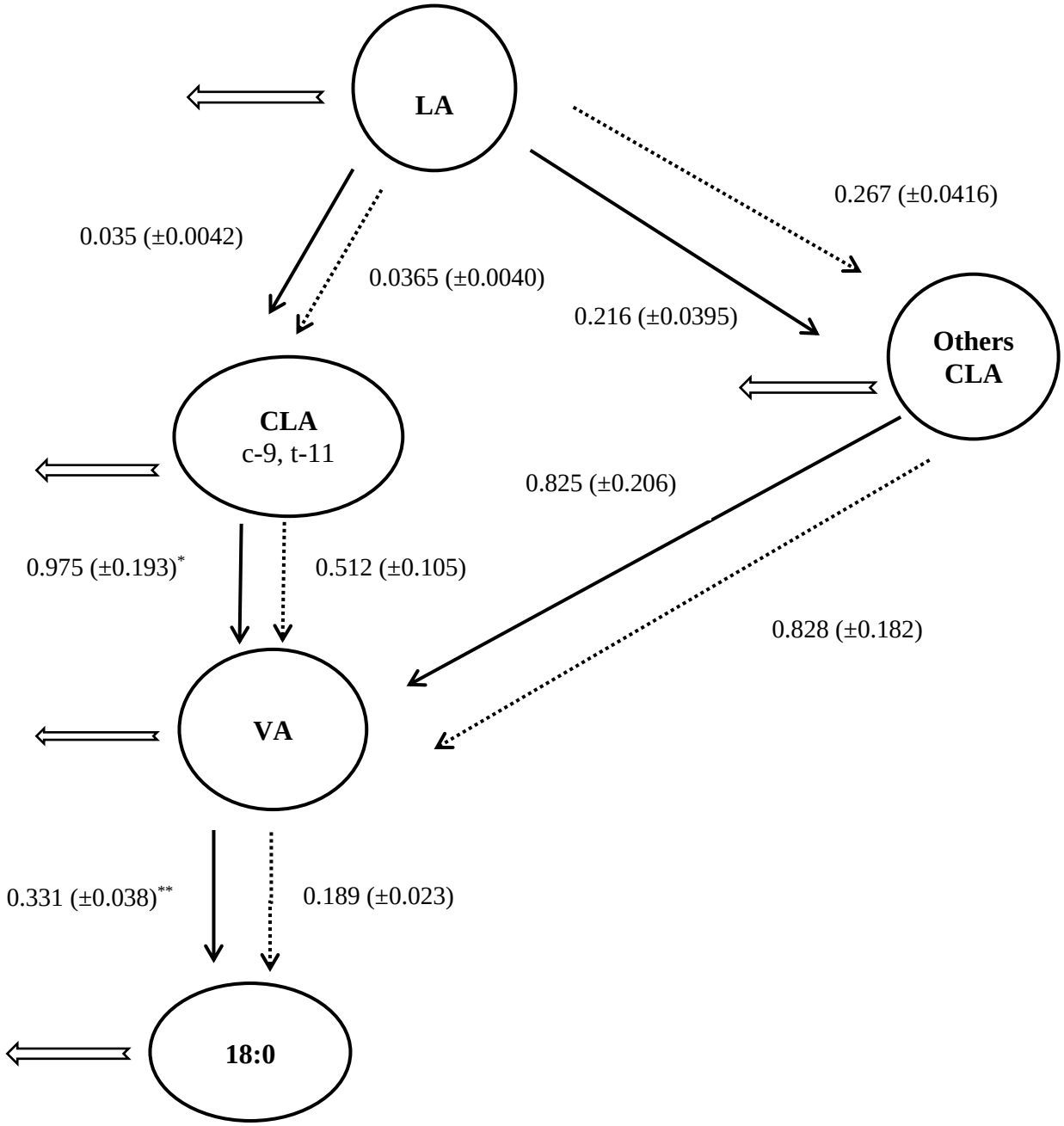


Figure 10. Multicompartmental model of in vivo ruminal biohydrogenation of linoleic acid using a perturbation tracee approach. Treatments were control (—) or coconut oil (-----); 430g/cow/d. Ovals represent the ruminal fatty acid pools (LA = linoleic acid; CLA= *cis*-9, *trans*-11 18:2; VA = *trans*-11 18:1; 18:0 = stearic acid; Other CLA = other conjugated diene intermediates from LA biohydrogentaion) and arrows represent fractional rates (h^{-1}) of transfer between pools during biohydrogenation with the standard deviation in parentheses. The ruminal passage rate of the fatty acid pools (⇒) were 6.98 and 7.54 %/ h for the control and the coconut oil treatment, respectively. * = $P < 0.05$; ** = $P < 0.10$.



A multicompartmental model was also used (Figure 10) to determined transfer rates of BH intermediates of LA. Preliminary data were used and not all FA pools were included in the model. Also, because the FA pools were in g/100 total FA (and not grams) and the amount of dietary FA consumed was not taken into consideration, the magnitude of the BH rates may not be accurate and only comparison between treatments and sensitivity to COO infusion will be interpreted. The fractional rates of rumen BH estimated by the compartmental model for the control and COO treatment were 0.035 vs 0.0365, 0.975 vs 0.512, 0.331 vs 0.189 (h^{-1}) to LA-CLA, CLA-VA, VA-stearic respectively (Figure 10). There was no difference in the BH of LA to CLA for the control and COO treatments (0.035 vs 0.0365 h^{-1}), respectively. Subsequently, the CLA converted to trans-11 C18:1 (VA; vaccenic acid) was higher in the control treament (0.975 vs 0.512 h^{-1}). The VA converted to stearic acid tended to be higher for the control treatments (0.331 vs 0.189 h^{-1}). The rates of BH for the other CLA between the control and COO treatment were 0.216 vs 0.267, 0.825 vs 0.828 (h^{-1}) to LA-Other CLA, CLA-VA, respectively.

4.0 DISCUSSION

In general, infusing COO in the rumen before feeding did not affect the cow performance. Excessive supply of MCFA to the rumen can decrease DMI. Hristov et al. (2004) provided 0 to 0.6% (as a percentage of an assumed rumen weight of 80 kg) of lauric acid to cows, infused via rumen cannula before feeding time, and found that up to 0.2% (rumen weight) lauric acid in the rumen content did not affect DMI. However, infusing 0.4% (rumen weight) of lauric acid decreased DMI, increased ruminal pH, and ruminal defaunation was observed. The authors also found that infusing 0.6% (rumen weight) of lauric acid, cows stopped eating. These authors then suggested that 0.3% of rumen weight to be the maximum amount of lauric acid that can be infused without negative effects on DMI or rumen microbes. In mature Holstein cows, this limit is approximately 240 g of lauric acid. In this study, no negative effects on DMI were

observed because the amount of COO supplied was less than the limit (200 g of lauric acid-oil), and it was supplied for a short period of time (3 days of infusion). There are some challenges when infusing more than 240 g/cow/d of lauric acid or 530 g/cow/d of COO (≈ 240 g/cow/d of lauric acid) via rumen cannula as a pulse dose. This can decrease DMI, inhibit rumen protozoa, and increase rumen pH (HRISTOV et al., 2004; HRISTOV et al., 2009; HRISTOV et al., 2011). Interestingly, the effectiveness of lauric acid or COO in inhibiting rumen protozoa when provided in 0.96% of TMR DM was not as effective as the same dose bolus infused via rumen cannula (FACIOLA et al., 2005). Based on the findings of Faciola et al. (2005), we provided COO via rumen cannula and as a pulse dose.

The hourly feeding schedule implemented on the day of the bolus appeared to be effective in stabilizing the rumen. Cows usually consumed the entire portion of the diet offered hourly, which would have contributed to the lack of difference in DMI rate (kg/h), which is expected to have decreased fluctuations in rumen DM pool size.

The rumen pH was slightly higher when cows were receiving COO. Even though it was statically different, it was biologically similar. Similarly results were shown by Hristov et al. (2009) in which COO slightly increased ruminal pH. It is important to note that rumen pH did not drop below 6.2 in both treatments, which can depress fiber digestion in the rumen (Mould et al., 1983).

Providing COO in addition to the bolus contributed to increase the total FA prior and post bolus. However, the COO plus bolus dose only increased the total amount of FA in the rumen to 6.3%, which is lower than the toxic concentration to ruminal microbes that could disrupt fermentation (JENKINS, 1993). Contrarily, baseline concentration of C17:0 and C18:2 in the rumen was lower in cows receiving COO simply due to the dilution of FA other than C12:0 and C14:0. Total rumen disappearance of C17:0 (6.6%/h) was similar to found in the assay described by Baldin (2016). Disappearance of C17:0 is expected to describe rate of FA passage from the rumen. This required the following assumptions: a) C17:0 disappeared only by passage, b) UFA disappeared by passage and BH, and c) the rate of passage of C17:0 and UFA are equal.

The extent of BH is a result of ruminal retention time, fat source, and microbial population (ALLEN, 2000). It is importantly to highlight the results of extent of rumen

biohydrogentaion of C18:2 in this study was in accordance with the average of i*n vivo* studies reported (70 to 95%; SHINGFIELD and WALLACE, 2014).

In this study, rumen concentration of *trans*-10 and t*rans*-11 C18:1 was increased by COO, and *trans*-10, *cis*-12 CLA tended to increase. Panyakaew et al. (2013) attempted to understand the effect of COO on biohydrogenation of PUFA (linseed oil + sunflower oil) *in vivo.* As a result, COO reduced the second step of the hydrogenation of C18:3 n-3, which resulted in higher accumulation of C18:2 *trans*-11, *cis*-15.

The small increase in milk yield in the time which cows did not receive COO could be due to increased energy available coming from the better digestion of the fiber portion, as COO may decrease NDF digestibility (HOLLMANN and BEEDE, 2012; HOLLMANN et al., 2013). The milk composition can be changed by manipulating the diet. Milk fat is the main component in the milk that can be changed by physiological and nutritional changes. This is mainly because fat is the most variable component of milk (PALMQUIST, 2006). Milk protein yield was higher in two days along of the study in cows that did not receive COO. Results contrary to this study were reported by (HRISTOV et al., 2011), where cows receiving lauric acid were more efficient to use dietary nutrients for milk protein. On the other hand, dietary COO positively changed milk protein yield when cows were adapted to COO (HOLLMANN et al., 2013).

The increases in C12:0 and C14:0 was expected in milk of cows receiving COO because it has around 60% of both in the FA profile. Hristov et al. (2011) noted that providing 240g of lauric acid to lactating dairy cows increased C12:0 and C14:0 in milk as well. The increase of MCFA in milk and concerns to human health have been discussed by (HRISTOV et al., 2009). However, studies have shown, despite of the major contribution of milk and dairy products to saturated FA in the human diet, that the intake of milk fat is inversely associated with heart diseases and inversely associated with obesity risk (CHOWDHURY et al., 2014; GERMAN et al., 2009; KRATZ et al., 2012; PATTERSON et al., 2013). Lauric acid showed to increase the HDL, and consequently decreased the ratio of total to HDL cholesterol (MENSINK et al., 2003).

Concentrations in milk of *trans*-18:1 and *trans*-10, *cis*-12 CLA were increased by lauric acid and that indicated shifts in ruminal BH pathways, and caused MFD (HRISTOV et al., 2011). Shigfield and Griinari (2007) reported an increase in *cis*-9, *trans*-11 CLA in 1.1% of milk fat when lauric acid was provide. The increases of *trans*-

11 C18:1 acid and *cis*-9, *trans*-11 CLA in the milk of cows receiving COO in this study shows the potential of COO to shift ruminal biohydrogenation and suggest changes in ruminal microbiota.

The complete BH of LA process is divided in two steps performed for two bacteria groups: the first step is performed by the "Group A" and it reduces C18:2 to C18:1 being their major end product. The second step is performed by the "Group B", bacteria utilize C18:1 as one of the main substrates with C18:0 being the major end product (KEMP et al., 1984; POLAN et al., 1964).

The PUFA are toxic to rumen bacteria, and they inhibit its growth (HARFOOT, 1981) because they disrupt the cell membrane. Lately Maia et al. (2010) suggested, contrarily, that the toxicity may be mediated via a metabolic effect by decreasing ATP pool 2/3 (in the culture of Butyrivibrio fibrisolvens). That happens mainly by decreases in acyl CoA pools by >96% when LA is added to the culture of growing bacteria.

Butyrivibrio fibrisolvens, identified by Polan et al. (1964), has high biohydrogenating activity in LA to *trans*-11 C18:1 as the final product. Subsenquetly, *trans*-11 C18:1 are hydrogenated to C18:0 by *Butyrivibrio proteoclasticus* (McKNAIN et al., 2010). High levels of LA inhibit the BH of *trans*-11 C18:1 (POLAN et al., 1964). LA incubated with mixed ruminal bacteria results in the accumulation of *trans*-18:1 (KEENEY, 1970). It suggests a reduction in *Butyrivibrio proteoclasticus* activity that performed the second step of BH, converting *trans*-11 C18:1 to C18:0.

Studies with MCFA have shown it to have bacterial inhibitory activity as an effective role to suppress ruminal methanogenesis (DOHME et al., 2001; HRISTOV et al., 2004). This inhibitory activity seems to be because of the dissociation of the MCFA, because of their hydrophilic-lipophilic balance (GOEL et al., 2009). In generally acids penetrate the phospholipidic bacteria membrane in the undissociated form (MALOY et al., 1981). Subsequently, inside the cell, the dissociated acid due to change the protons balance resulting in higher celular pH (alkaline) and lowering the pH cytoplasm, suppressing cytoplasmatic enzymes and nutrient transport systems and decreasing energy availability, leading to cell death (FREESE et al., 1973).

Goel et al. (2009), incubating 20 and 30 mg of a MCFA (C10:0) plus 10 mg of sunflower oil as LA source, and as result it completely inhibited the production of C18:0,

resulting in an accumulation of biohydrogenation intermediates, mainly C18:1 *trans*-10 and *trans*-11.

Analysis of the data using compartmental modeling showed thee CLA converted to *trans*-11 C18:1 was 47.5% lower in cows receiving COO. Probably because MCFA has similar toxic effect than UFA in the bacterias that performed the second step of the BH, and promote similar effect in the BH.Subsenquetly, the *trans*-11 C18:1 hydrogenated to stearic acid tended to decrease 42.9% in the COO treatment, which seems similar with the reduction in the CLA hydrogenated to *trans*-11 C18:1, and should suggest the COO was more effective to decrease the CLA biohidrogenation to *trans*-11 C18:1. The decreases in the total BH, resulted in higher *trans*-11 C18:1 in the milk of cows receiving COO.

The main bacteria that perform this step is *Butyrivibrio fibrisolvens*, and gram positive bacteria (Stewart, 1997). Ruminal biohydrogenation is modified via differential toxicity to ruminal bacteria (MAIA et al., 2010). The above mentioned researches when added linoleic acid to *Butyrivibrio fibrisolvens* notices the increases in the bacteria, and corrobored with the toxicity theory. The C8:0 and C10:0 when compared to oleic acid were more toxic to ruminal rabbit bacteria (MAROUNEK et al., 2002).

The multicompartmental model developed was sensitive to detect changes in the BH process. However the concentration of ruminal fatty acids from BH estimated by the compartmental model were different to those estimated by the single available pool, first-order kinetic approach. Accordingly, the model has limitations and needed to be improved.

Additionally, further studies are necessary to investigate the COO effects to manipulate ruminal biohydrogenation. In the first we recommended in the next study use a marker to solid phase and evaluate the ruminal microbiology. In the second the effect of providing a PUFA source and COO on performance and milk fatty acid profile, more days of study would be better to know how the COO and PUFA interact in a long term metabolism.

5.0 CONCLUSION

Coconut oil infusion as a pulse dose corresponding to approximately 200g/cow/d of lauric acid did not affect DMI, milk yield and milk fat. However, it increased the percentage of *trans*-11 C18:1 in milk.

The perturbation tracee approach associated to a multicompatmental model was able to detect differences on BH rates of individual fatty acids.

Coconut oil infusion as a pulse dose corresponding to approximately 200g/cow/d of lauric acid decreases BH rates of CLA and VA, similarly to UFA. Because coconut oil has higher content of MCFA, we concluded that MCFA impair ruminal BH similarly to UFA. The coconut oil infusion as a pulse dose corresponding to approximately 200g/cow/d of lauric acid increased the concentration of total fatty acids in the rumen pior and post-bolus; but it didn't affect the C18:2 biohydrogenation.

Future research should be done to validate and challenge the sensitivity to the perturbation tracee approach regarding dietary changes that are known to alter ruminal biohydrogentaion.

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CAPÍTULO III

Palm cake in substitution to sunflower cake in lamb diets

Palm cake in substitution to Sunflower cake in Lamb diets

ABSTRACT

The objective of this study was to evaluate the substitution of dietary sunflower cake by palm cake on nutrients intake, digestibility, blood parameters, nitrogen balance and synthesis of microbial protein of feedlot lambs. Twenty, non-castrated lambs 23.81 kg (± 4.3) were individually penned and used in a completely randomized design. The animals were fed 40% Tifton hay plus 60% concentrate, with 5% of supplemental FA (% DM). The treatment diets consisted of the contribution of each fat source to the total FA supplementation, as follow: 1) 100% of supplemental FA from sunflower cake; 2) 66% from sunflower cake and 33% from palm cake; 3) 33% from sunflower cake and 66% from palm cake; and 4) 100% of supplemental FA from palm cake. It lasted 10 days. The feces, feed samples and leftovers were collected during five days by each day/animal. Blood samples were taken on the 6th d at 0, 3, 6, and 9 h after the morning feeding. Urine spot samples were taken in the 10th at 4 hours after the morning feeding. The Palm cake inclusion cubically affected ($P \leq 0.05$) all the nutrients intake. Similarly the digestibility of OM and TDN was cubically ($P \leq 0.05$) affected by the palm cake inclusion. The CP and NFC digestibility linearly decreased ($P < 0.01$) when sunflower cake was replaced by palm cake. The nitrogen intake, absorbed and retained were cubically affected ($P \leq 0.05$). The replacement of sunflower cake by palm cake linearly increased NEFA blood concentration. In conclusion palm cake in complete substitution to sunflower cake decreases the energy and protein intake.

Key words: by-products, digestibility, sheep

1.0 INTRODUCTION

Byproducts of biodiesel production may be used as dietary ingredients for feedlot animals. Several researchers have studied the effects of sunflower cake as an alternative protein source in ruminants diets (ALVES et al., 2016; OLIVEIRA et al., 2015; QWELE et al., 2013); it has high levels of protein (> 20%; OLIVEIRA et al., 2007), fat (> 20%; MOURA et al., 2015), and TDN (> 79%; GOES et al., 2015). Palm cake has high fiber (61% NDF), crude protein content similar to sunflower cake (15-20%), and ether extract (10%; VALADARES FILHO et al., 2015). Both byproducts, sunflower and palm cake, have different fatty acids (FA) profiles, this can be attributed to fatty acid profile in the oil. The sunflower oil contains 60% of polyunsaturated fatty acid (PUFA) and the palm oil contains 67% of medium chain fatty acid (MCFA; ORSAVOVA et al., 2015). The PUFA and MCFA are toxic for gram-positive bacteria and mainly cellulolytic bacteria. They can negatively impact nutrient digestibility and, therefore, blood metabolites and energy mobilization. Nevertheless, FA from oilseeds may be rumen-protected because of the hulls (DOHME-MEIER and BEE, 2012).

Sunflower cake is widely used as a dietary ingredient in ruminant diets and for the high nutritional value to feed, it has also been used in poultry diets, while palm cake due to its high fiber content the use is mainly in ruminants feeds. Santana Filho et al. (2016) evaluating palm cake in substitution to soybean meal found as result that palm cake can be included at up to 21% (w/w) in cattle feed without compromising the physicochemical, sensory and acceptance characteristics of the meat. Sunflower cake can be used to goat diets up 16% (DM basis) without affect the meat quality (OLIVEIRA et al., 2015). Few studies evaluate use of cake in animals metabolism, it is important to understand the nutritional characteristics based in animal performance and metabolism, and to identify the feed nutritional limitations. A better understanding of the feed in animal metabolism is important to decisions about which feed include, based also in it availability.

Based on that, the objective of this study is to test the hypothesis that the suflower cake can be replaced for palm cake without affect intake, digestibility, blood parameters and microbial nitrogen efficiency in growing lambs.

2.0 MATERIAL AND METHODS

This study was conducted at the Experimental Farm of the Federal University of Bahia, Brazil. All procedures were approved by the Animal Care Committee at the same institution.

2.1 Experimental design and treatments

Twenty, non-castrated Dorper × Santa Ines lambs (8 months old), with an average initial body weight (BW) of 23.81 kg (± 4.3) were used in a completely randomized design, with five replicates and four treatments. The lambs were housed individually in feedlot cage (1.00 x 1.00 m) and feed twice daily (07h00 and 16h00). Water and feed were supplied *ad libitum*, ensuring 10% refusal of the diet. The animals were receiving the experimental diets for 35 days when this study was conduct for 10 days, with four days of adaptation to the canvas bags followed by six days of sample collection.

Table 1. Chemical composition of dietary ingredients

Item ($g.kg^{-1}$ DM)	Ingredients				
	Corn	Soybean meal	Sunflower cake	Palm cake	Hay
DM[1] (as fed basis)	886	883	889	958	918
Organic matter	977	934	939	986	929
Mineral matter	20.9	65.7	60.7	14.4	71.0
Crude protein	51.4	502	249	107	125
Ether extract	64.3	17.4	185	172	18.7
NFC[2]	688	318	181	21.0	6.00
NDF[3]	175	96.6	324	762	686
ADF[4]	72.2	80.1	274	489	359

[1] DM= Dry matter; [2]NFC= Non fiber carbohydrate; [3]NDF= Neutral detergent fiber; [4]ADF= Acid detergent fiber.

Diets were composed of 40:60 roughage to concentrate ratio, with tifton (Cynodon spp.) hay as the only forage source. All experimental diets were formulated to be isonitrogen and isoenergetic and to meet the requirement for gain of 200 g/day, according to the NRC (2007). The tifton hay was chopped using a hay shredder with a 6.35 mm sieve (TF-150, Laboremous, Brazil). The concentrate contained ground corn, soybean

meal and a mineral and vitamin nucleus (Table 1). The treatments consisted of four diets, all with 5% of supplemental FA (% DM), as follow: 1) 100% of supplemental FA from sunflower cake; 2) 66% from sunflower cake and 33% from palm cake; 3) 33% from sunflower cake and 66% from palm cake; and 4) 100% of supplemental FA from palm cake (Table 2).

Table 2. Ingredients and chemical composition of experimental diets.

Item (g.kg^{-1} DM)	Treatments[1]			
	0	33	66	100
Palm cake	0.00	74.0	154	235
Mineral	10.0	10.0	10.0	10.0
Soybeal meal	33.0	48.0	65.0	82.0
corn grain ground	331	310	287	265
Urea + Ammonium sulfate	8.00	8.00	8.00	8.00
Hay tifton	400	400	400	400
Sunflower cake	218	150	76.0	0.00
Total	100	100	100	100
Chemical composition (g.kg^{-1} DM)				
DM (g.kg^{-1} fresh material)	926	926	926	925
Organic matter	947	947	950	949
Ash	53.0	53.4	50.3	50.8
Crude protein	166	165	165	167
Ether extract	49.1	49.9	49.6	45.3
apNDF[2]	294	332	354	381
Non fiber carbohydrate	438	400	381	356
Lignin	34.6	57.0	44.5	60.0
NDICP, g/kg CP[3]	182	218	209	251
ADICP, g/kg CP[4]	76.0	86.0	81.0	94.0

[1]Percentagem of palm cake in substitution to sunflower cake added to diets to supplement 5% (DM basis) of fatty acids; [2]apNDF= neutral detergent fiber corrected for ash and protein; [3]NDICP= neutral detergent insoluble CP; [4]ADICP= acid detergent insoluble CP.

2.2 Sampling procedure

The daily intake during the whole experiment was recorded to evaluate the digestibility and nutrient intake. Daily composite of feed samples and orts was collected from each animal/day, and stored at -10ºC for analyses. The feces were collected during 5 days by the total collection method with the aid of appropriate canvas bags attached to the animals using nylon strips. Samples were mixed and a composite sample was taken

and dried at 55 °C for 72 h and stored for further analysis. Feed samples and orts were then thawed, weighed, and dried in a forced air oven at 55 °C for 72 h, ground (Willye TE-680, TECNAL, Brazil) through 1-mm sieve, homogenized and stored for further analysis.

Blood samples were taken using test tubes with anticoagulant (EDTA) on the 6th d at 0, 3, 6, and 9 h after the morning feeding. They were immediately centrifuged at 3000 rpm for 15 minutes, and then plasma samples were withdrawn, placed in micro tubes and frozen at -15 ºC for analysis of urea nitrogen (mg/dL), creatinine (mg/L), albumin (g/dL), nonesterified fatty acids (NEFA; mmol/L), triglycerides (mg/L), glycerol (mmol/L), *beta-hydroxybutyrate* (BHBA; mmol/L).

Urine spot samples (10mL) were taken on the 10th day, 4 hours after the morning feeding, with bottles containing 40 mL of 0.036N sulfuric acid and stored at -15 °C for analysis of total nitrogen, urea, allantoin (AL), xanthine, hypoxanthine, uric acid (UA) and creatinine.

2.3 Laboratory analyses

Feed, dietary ingredients, feces and leftovers samples were analyzed according to AOAC (2010), for dry matter (DM; method 934.01), ash (method 942.05), nitrogen (method 2001.11) and ether extract (EE; method 920.39). The neutral detergent fiber (NDF) were determined using TNT bags (5cm x 5cm), maintaining an average of 14 mg DM per cm^{2} of tissue and 100 ml neutral detergent.g^{-1} with thermostable alpha amylase without the use of sodium sulfite, and corrected for ash residue (MERTENS, 2002) and the residual nitrogen compounds (LICITRA et al., 1996). Lignin was obtained by treatment of ADF residue with 72% sulfuric acid (VAN SOEST et al., 1991).

Non fibrous carbohydrate (NFC) was calculated as: NFC (%) = 100 - (%CP + %NDF + %Ash + %EE. The levels of NFC corrected for ash and protein were calculated, as proposed by Hall (2000), with adaptations: NFC = 100 - [(CP - CP urea derivative + urea in the diet) + NDFfom + EE + ashes]. The intake of total digestible nutrients (TDN) were calculated as described by Sniffen et al. (1992) using the equation ITDN = (ICP − CPf) + 2.25 (IEE − EEf) + ITC − TCf), where ICP, IEE, and ITC represent the intake of

CP, EE, and total carbohydrate (FDNfom+NFCcp), respectively; and CPf, EEf, and TCf refer to the excretion of CP, EE, and total carbohydrate in the feces, respectively. The concentrations of TDN were obtained from the following equation: TDN (%) = intake of TDN/intake of DM × 100. The apparent digestibility coefficient (CAD) of DM, OM, CP, EE, NDF, NFC, and TDN were calculated using the following equation: CAD (%) = [(nutrient intake (g) - nutrient excretion (g)) / nutrient intake (g)] x 100.

Analyses of plasma urea and creatinine were performed by enzymatic colorimetric assay, with commercial kits (Urea #27, Creatinine # 35 Labtest Diagnostica SA, Lagoa da Santa, Minas Gerais, Brazil). The NEFA, BHBA and glycerol were analyzed by colorimetric method using commercial kits (FA115, RB1007, GY105, Randox Laboratories, USA).

2.4 Purine derivatives and estimation of microbial protein

Creatinine was used as a marker to estimate urinary volume, considering that the mean excretion of creatinine per lamb is 23.1 mg/BW (FAICHNEY, 1995). The urinary volume (UV) was estimated by UV (L) = BW * 23.1 mg/kg/creatinine in the urine (mg/L). Purine derivatives (PD) in urine include uric acid, allantoin, xanthine + hypoxanthine. Urinary uric acid was determined by commercial kit (#140, Labtest Diagnostica SA, Lagoa da Santa, Minas Gerais, Brazil). The allantoin, xanthine and hypoxanthine concentrations in urine and estimation of microbial N were performed based on colorimetric method as described by Chen and Gomes (1992). The total purine derivatives (TP) excretion was calculated by the sum of hypoxanthine, xanthine, acid uric and allantoin in the urine and expressed as mmol/d. The absorbed purines (AP) were estimated based on the total amount of purine derivatives excreted in sheep (PE_{sheep}), according to Chen and Gomes (1992): $PE_{sheep} = 0.84\ AP + (0.150\ MBW^{0.75} * e^{-0.25AP})$, where PE_{sheep}= Purine derivatives excreted in sheep (mmol/d), AP= purines absorbed (mmol/d), MBW= *metabolic body-weight (kg).*

The supply of microbial nitrogen (MN) was estimated as: MN ($g.day^{-1}$) = (70 * AP) / (0.83 * 0.116 * 1000) = 0.727 x AP; *assuming that the digestibility of microbial*

purine is 0.83, the N content of purines is 70 mg N/mmol and the purine-N:total-N ratio in mixed rumen microbes is 11.6:100 (CHEN and GOMES, 1992). The efficiency of microbial N synthesis was expressed as grams of microbial N per kilogram of digestible OM fermented in the rumen (DOMR; calculated as total tract digestible OM × 0.65, according to the (ARC, 1984)).

2.5. Nitrogen balance

The urea nitrogen in feed, dietary ingredients, feces, leftovers, and urine samples were analyzed for Kjeldahl via method 2001.11 (AOAC, 2010). The nitrogen balance (NB) was obtained by the difference between the total amount of nitrogen ingested and the total nitrogen excreted in feces (N-feces) and urine (N-urine):

$$NB\ (g.day^{-1}) = N_{intake} - (N_{feces} + N_{urinary}).$$

2.6. Statistical analysis

The data were analyzed using the PROC MIXED of SAS in a completed randomized design. Heterogeneity of variance was tested by the command REPEATED and used when significant. Polynomial contrasts were used to test the linear, quadratic and cubic effects of substitution of sunflower cake by palm cake on all parameters. Initial body weight and DMI before the beginning of the experiment were tested as covariables and used when significant. The temporal effect of the diets on plasma parameters after feeding was analyzed in a completed randomized design with repeated measure in time. Before the analyses, the best error structure (variance-covariance matrix) was defined based on the AIC parameter using the command REPEATED. Degrees of freedom and tests were adjusted using the Kenward-Roger option. The time effect was decomposed in three orthogonal polynomial contrasts: linear, quadratic and cubic. Significance was declared at $P \leq 0.05$ and trend at $0.05 > P \leq 0.10$.

3.0 RESULTS

3.1 Intake, digestibility and plasma metabolites

Feeding palm cake in replacement to sunflower cake cubically affected (P<0.05) all the nutrients intake (Table 3). Similarly the digestibility of OM and TDN were cubically afected ($P \leq 0.05$) with palm cake inclusion in the diets (Table 3). The CP and NFC digestibility linearly decreased (P<0.01) when sunflower cake was replaced by palm cake (Table 3).

Table 3. Least square means of nutrient intakes (g/d) and digestibility (g/kg DM) of feedlot lambs fed palm cake in substitution by sunflower cake

Item	Treatments[1]				SEM[3]	*P*-value[2]		
	0	33	66	100		L	Q	C
Intake (g/d)								
Dry matter	1373	948	1144	870	80.7	<0.01	0.36	<0.01
Organic matter	1302	898	1087	827	76.4	<0.01	0.36	<0.01
Crude protein	213	159	192	148	14.1	0.05	0.75	0.02
Ether extract	65.0	49.6	58.9	41.8	3.86	<0.01	0.84	0.01
apNDF[4]	386	301	385	310	28.5	0.28	0.88	0.02
Non fiber carbohydrate	570	388	451	327	34.4	<0.01	0.46	<0.01
TDN[5]	1055	651	836	598	68.4	<0.01	0.24	<0.01
Digestibility (g/kg)								
Dry matter	709	657	693	650	23.9	0.20	0.85	0.14
Organic matter	745	665	704	665	20.3	0.04	0.32	0.04
Crude protein	780	731	746	698	17.1	<0.01	0.96	0.11
Ether extract	907	885	926	881	24.8	0.75	0.65	0.19
apNDF	570	555	609	567	32.4	0.77	0.69	0.26
Non fiber carbohydrate	802	693	737	712	24.1	0.05	0.11	0.06
TDN[5]	764	688	729	685	19.5	0.04	0.41	0.03

[1]Percentagem of palm cake in substitution to sunflower cake ratios were added to diets to supplement 5% of fatty acids (DM basis). [2]Probabilities of orthogonal contrasts for testing linear (L), quadratic (Q) and cubic (C) effects of the treatments (Treat), time and the treatment by time interaction (Treat x Time). [3]SEM= Standard error of the mean.[4]apNDF= neutral detergent fiber corrected for ash and protein.[5]TDN= total digestible nutrients.

The palm cake, in substitution to sunflower cake, had no treatment effect ($P>0.05$) on albumin, urea, triglycerides, creatinine, BHBA and glycerol (Table 4). Moreover, NEFA (mmol/L) increased ($P \leq 0.05$) with the replacement to sunflower cake by palm

cake (Table 4). The plasma metabolites showed a quadratic response for albumin, urea, triglycerides and glycerol concentrations over time, while creatinine and BHBA showed a quadratic tendency and NEFA, a linear tendency over time (P>0.05<0.10, Table 4). There were treatment by time interactions for glycerol and NEFA (Table 4). Similarly triglycerides showed a tendency (P= 0.09) to interaction treatment by time (Table 4).

Table 4. Least square means of plasm metabolites of feedlot lambs fed with palm cake in substitution by sunflower cake.

Item	Treatments[1]				SEM[3]	*P*[2]					
						Treat			Time		Treat x
	0	33	66	100		L	Q	C	L	Q	Time
Albumin, g/dL	3.24	3.20	3.52	3.26	0.158	0.61	0.49	0.20	0.90	<0.01	0.15
Urea, mg/dL	39.31	43.55	48.96	47.11	3.70	0.10	0.42	0.71	0.09	<0.01	0.16
TG[4], mg/L	18.35	18.53	16.16	17.05	2.77	0.62	0.89	0.64	<0.01	<0.01	0.09
Creatinine, mg/L	2.40	3.02	2.95	2.85	0.282	0.31	0.22	0.60	0.97	0.09	0.51
BHBA[5], mmol/L	0.37	0.43	0.42	0.45	0.057	0.34	0.77	0.67	0.31	0.06	0.20
Glycerol µmol/L	90.42	90.43	90.65	90.49	0.456	0.82	0.86	0.78	0.41	<0.01	0.02
NEFA[6], mmol/L	1.01	1.15	1.18	1.31	0.067	<0.01	0.99	0.46	0.06	0.90	0.01

[1]Percentagem of palm cake in substitution to sunflower cake ratios were added to diets to supplement 5% of fatty acids (DM basis). [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects of the treatments (Treat), time and the treatment by time interaction (Treat x Time). [3]SEM= Standard error of the mean. [4]TG = triglycerides; [5]BHBA= *Beta-hydroxybutyrate.* [6]NEFA= Nonesterified fatty acids.

Figure 1. Temporal change of plasm NEFA (mmol/L) of feedlot lambs fed with 0, 33, 66 e 100% of palm cake in substitution to sunflower cake; * = Significant ($P \leq 0.05$) for the treatment by time interaction.

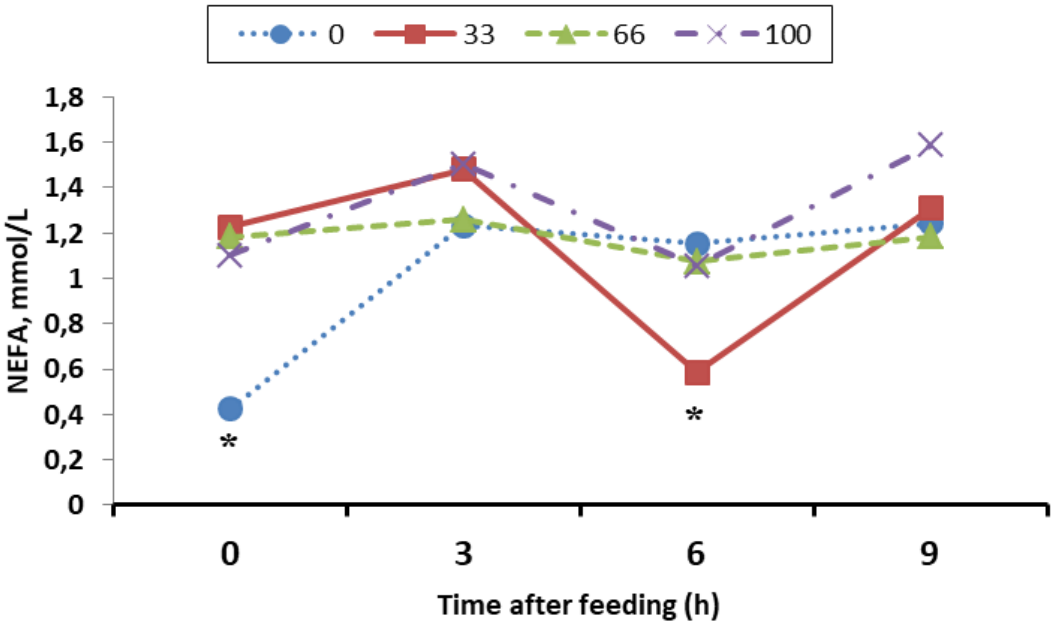


Figure 2. Temporal change of plasm glycerol (µmol/L) of feedlot lambs fed with 0, 33, 66 e 100% of palm cake in substitution to sunflower cake; * = Significant ($P \leq 0.05$) for the treatment by time interaction.

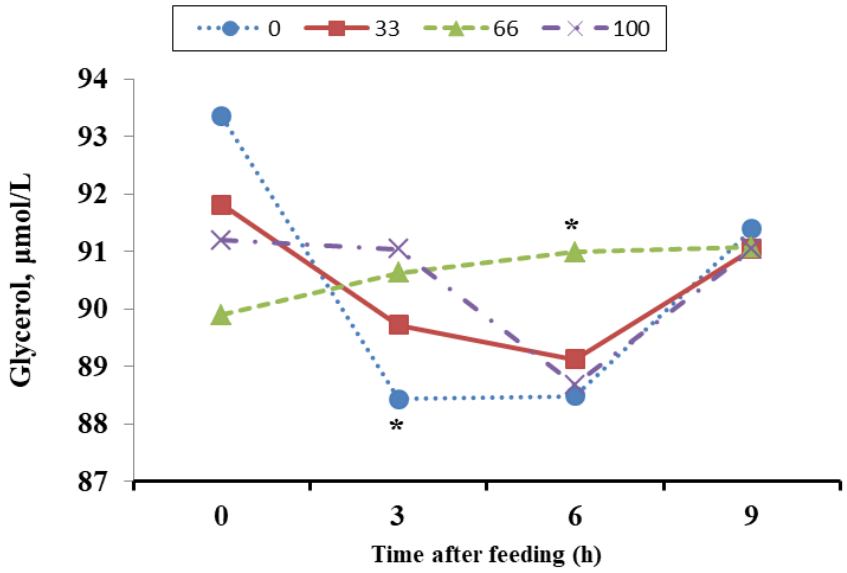


Figure 3. Average temporal change (0, 3, 6 and 9 hours after morning feeding) of plasm albumin (g/dL) of feedlot lambs fed with palm cake in substitution by sunflower cake.

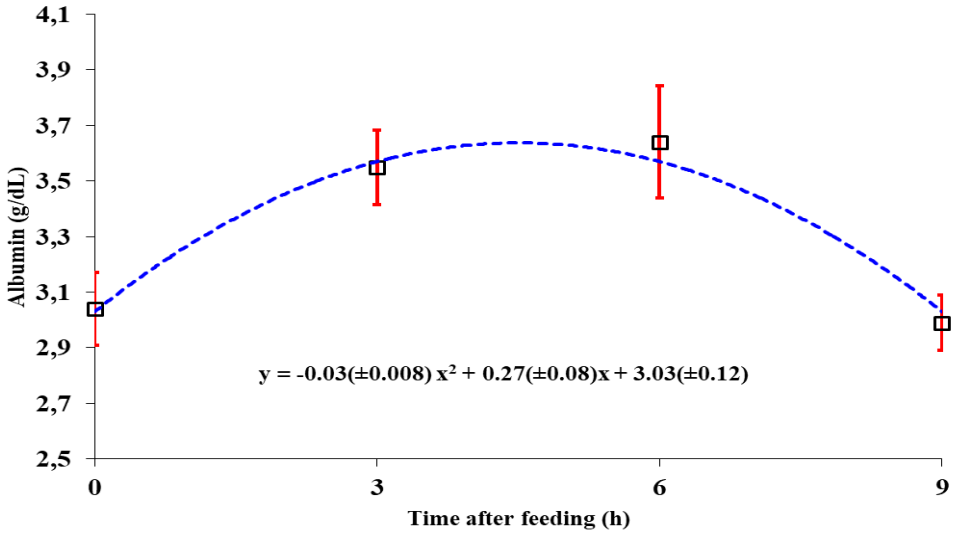


Figure 4. Average temporal change (0, 3, 6 and 9 hours after morning feeding) of plasm triglycerides (mg/L) of feedlot lambs fed with palm cake in substitution by sunflower cake.

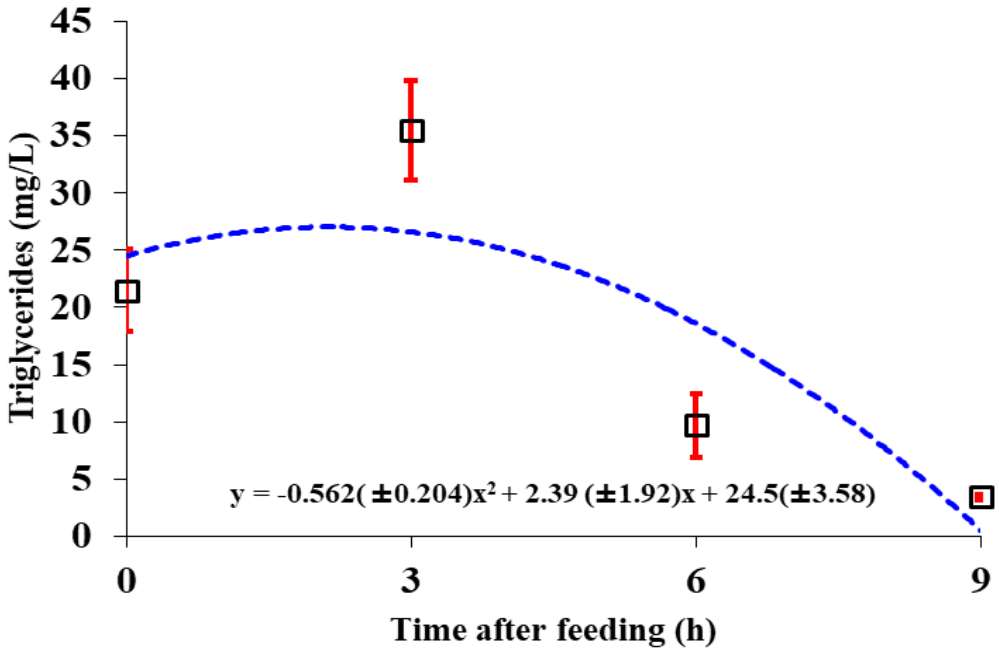
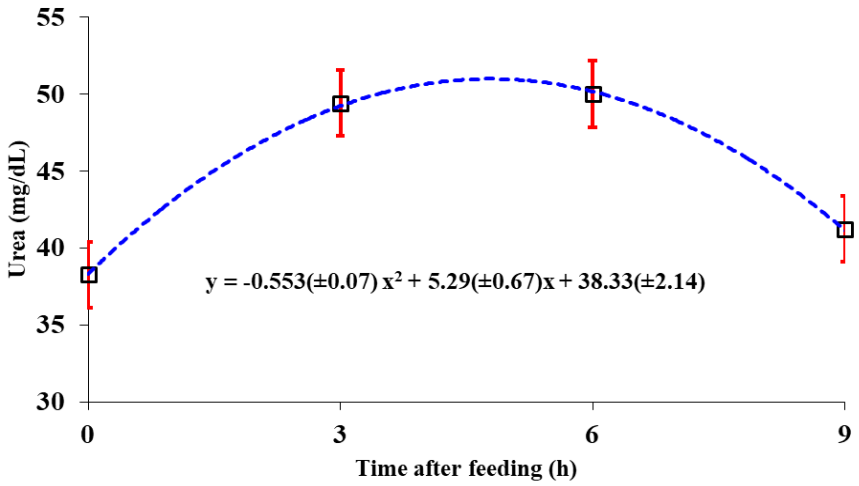


Figure 5. Average temporal change (0, 3, 6 and 9 hours after morning feeding) of plasm urea (mg/dL) of feedlot lambs fed with palm cake in substitution by sunflower cake.



3.2 Nitrogen balance and microbial protein

The nitrogen intake, absorbed and retained were cubically affected (P<0.01) when palm cake was included in the diets (Table 5). The microbial protein synthesis and N microbial efficiency per kg of digestible OM fermented in the rumen (MEDOMR) have no difference among the treatments (P> 0.05; Table 5).

Table 5. Least square means of nitrogen balance and microbial protein of feedlot lambs fed with palm cake in substitution by sunflower cake.

Item	Treatments[1]				SEM[2]	P[3]		
	0	33	66	100		L	Q	C

N intake (g/day)	37.00	22.87	30.78	23.70	1.75	<0.01	0.06	<0.001
N excretion (g/day)								
Fecal	7.47	6.82	7.82	7.16	0.61	0.98	0.99	0.23
Urinary	7.60	7.94	7.52	6.38	1.84	0.63	0.69	0.99
N absorbed (g/day)	29.52	18.60	23.00	16.54	1.85	<0.01	0.25	<0.01
N retention								
g/day	21.95	10.66	15.46	10.18	1.45	<0.01	0.11	<0.01
% of intake[4]	60.22	41.32	50.22	35.65	4.86	0.01	0.66	0.02
Microbial Protein								
Microbial Protein (g/day)	37.84	35.21	31.02	35.88	2.77	0.43	0.19	0.40
ME[4] (g Nmic/kg of DOMR)	11.98	13.08	10.88	13.43	1.18	0.70	0.55	0.14

[1]Percentagem of Palm cake in substitution to sunflower cake ratios were added to diets to supplement 5% of fatty acids (DM basis). [2]SEM = standard error of the mean. [3]Probability of linear (L) and quadratic (Q) effect of palm cake level. [4]ME= N microbial efficiency per kg of digestible OM fermented in the rumen.
[4]Some of data of N retention of intake was excluded for be outlier.

4.0 DISCUSSION

4.1 Intake, digestibility and, plasma metabolites

Fatty acids supplied in diets are commonly associated with DMI reduction. Fat can reduce fiber digestion in the rumen (PALMQUIST and JENKINS, 1980) and the feed will keep more time in the rumen, limiting the intake by physical capacity (ALLEN, 2000). Additionally, FA is a potential stimulator of CCK release, which cause the satiety effect (LIDDLE, 1997). Lastly, fat increases the diet energy density, which decreases DMI. Rahman et al. (2013) feeding goats with 32% of palm kernel cake found no differences on total DMI and NDF intake. Contrarily with that, in this experiment, we found differences in DMI and NDF intake with increases of palm cake in substitution to sunflower cake. The palm cake fatty acid profile is rich in MCFA (up to 45%). The lower molecular weight of MCFA results in faster action of pancreatic lipase in the small

intestine, and consequently, increases the rate of digestion of MCFA (BACH and BABAYAN, 1982). The increase in EE intake increases its digestibility as a function of the dilution of the endogenous EE and by the intestinal digestibility of the FA that is inversely related to the melting point of the FA (NRC, 2001). This suggests that it is possible that increasing EE intake would, increase EE digestibility, but the EE digestibility observed in this experiment did not show differences among diets.

The nutrients intake were affected by the diets, which might be explained for the decrease in DMI in 345 g/d, corresponding to a 36% change between the diets having 0 and 100% palm cake. The EE, NFC and TDN intake when sunflower cake was totally replaced by palm cake showed decreases by 35%, 42% and 43%, respectively. The reduction of the energy intake also should be explained by small decrease (20%) in corn grain when sunflower cake was totally replaced by palm cake in the diets. The NFC in the diets compositions decreased in approximately 28% when sunflower cake was totally replaced by palm cake (Table 2). The NFC is a source of energy and is rapidly fermentable in the rumen, and the decrease in NFC intake might be due to the decrease in the diet NFC and TDN intake, as result of a decrease in the EE intake and NFC. The NEFA concentrations in plasma increased 29.7% when sunflower cake was totally replaced by palm cake. These results suggest that fat mobilization increased, while the energy provided by the diet decreased when feeding only palm cake.

Palm cake is rich in NDF (76%), which may have limited intake and fermentation, slowing ruminal passage of NDF (VAN SOEST et al., 1994) when compared to diet with no inclusion of palm cake. The NDF has a greater filling effect over time than non-fibrous feed components (ALLEN, 1996). This is a result of greater NDF content in feedstuffs, which increase the time spent in the rumen, thereby allowing more time for bacterial colonization (RUSSELL et al., 2009).

The changes in nutrient intake can affect the digestibility of the diets when the intake decreases to a certain limit. The increases in palm cake in the diets did not affect the OM intake but affect OM digestibility, which is more attributed to the feed characteristics than nutrient intake. There was a small decrease of 10.9% of OM digestibility when sunflower was totally replaced by palm cake. Even though the sunflower cake used in this study has four times (60.7% DM) more mineral content than

palm cake (14.4% DM) and the mineral content in the diet composition was similar among the diets, the replacement did not promote differences in OM digestibility. Addiotinally the OM intake decreased in 28.3% when sunflower cake was totally replaced by palm cake in the diets.

The total substitution of sunflower cake by palm cake in the diets increased soybean meal inclusion in the diets by 15.0, 32.0 and 49.0 g/kg of DM in the 33, 66 and 100% of palm cake in the diets, which has more soluble protein than sunflower cake. Therefore, sunflower cake decreased in the diets from 218, 150, 76.0 and 0 g/kg of DM in the 0, 33, 66 and 100% diets, respectively. The impact of decreases in sunflower cake (218 g in the total diet) seems higher than the small increases in soybean meal (49 g in the total diet) in protein digestibility when sunflower cake was completely replaced by palm cake. Silva et al. (2014) evaluated the fractions of CP in sunflower cake and palm cake. Sunflower cake contained 29.3% CP (% of DM), and the fractions A= 20.1%: B1+B2 = 70.8%; B3= 3.94% and C= 5.2%, respectively. Palm cake contained 16.2% CP (% of DM), and the fractions A= 15.7%; B1+B2 = 62.7%; B3= 13.0%; C= 8.6%. Noticing that the A + B1 fractions indicate a higher supply of non-protein nitrogen and degradable peptides in the rumen. The sunflower cake provided higher levels of these fractions, which suggest sunflower cake protein is more digestible than palm cake protein.

These results show that palm cake protein is less digestible, and they suggest that even though the intake of CP was the same in the current results, the difference found in the CP digestibility might be attributed to the differences in the digestible fractions of sunflower cake and palm cake. Furthermore, increasing palm cake in the diets may have changed the digestible protein availability. Indeed, increasing levels of palm cake to 100% of substitution increased the dietary concentration of NDICP in 69.0 g/kg of CP. The increases in NDICP were 3.02, 3.60, 3.45, and 4.19 g/kg of DM of the diet composition and the difference between the CP and NDICP were 13.6, 12.9, 13.0 and 12.5 g/kg of DM for treatments 0, 33, 66 and 100%, respectively. The higher NDICP in the 100% of palm cake that correspondeded in the diet composition to 37.9% NDICP higher than the diet with only sunflower cake, which can reduce the rumen degradable protein. All those changes would have accounted for the small decrease in CP digestibility of 9.9% for the total substitution of sunflower cake.

The urea concentration increased by 19.8% in the plasma. The reduction of CP digestibility should reduce the rumen degradable protein, resulting in higher N recycling (MARINI and VAN AMBURGH, 2003). Moreover, the concentration of urea in the plasma is lower and the NFC in the diet composition is higher when sunflower cake was totally replaced by palm cake. Increased dietary NFC is often observed with lower ruminal pH (WEIMER, 1992). When the intraruminal pH is lowered, the permeability of the rumen wall for ammonia is depressed (BODEKER et al., 1990). Also, the lower energy and carbon availability for the utilization of ammonia to synthesize microbial protein with the NFC intake reduceds. Higher amounts of dietary rumen fermentable carbohydrate are associated with increased sequestration of NH3-N into microbial protein (HRISTOV et al., 2005).

The value for blood albumin are in accordance with reference values (2.4 to 3.9 g/dL; KANEKO et al., 1997). The urea and triglycerides concentrations in the plasma (33.3 to 38.7 mg/dL and 13.6 to 18.8 mg/dL, respectively) were similar to those found by MALEKKHAHI et al. (2014). However, the urea results are 12.5% higher than the maximum reference values (17.12 to 42.8 mg/dL), according to (KANEKO et al., 1997), which can be attributed to biological and feeding differences. Once released into blood, urea is excreted in urine, or it re-enters the digestive tract by diffusion into saliva or directly across the gut wall (HUNTINGTON and ARCHIBEQUE, 1999). The nitrogen excreted in feces and urine was the same among treatments, and that might indicates that the urea was being recycled.

The BHBA, glycerol, and NEFA are indicators of fat mobilization and energy status. The BHBA is an indicator of energy status when there is a relatively high glucose demand (RUSSEL and WRIGHT, 1983). Plasma glycerol is an indicator of fat mobilization (CHAVES et al., 2008). It is a potent glycogenic compound, and is released from the adipose tissue along with NEFA during fasting, ketosis, or other periods of body fat mobilization (STEINBERG and VAUGHAN, 1965). BERGMAN et al. (1968), in a study with glycerol metabolism in ewes, found that approximately one-half of glycerol (37-51%) was converted to glucose and about one-third was oxidized to CO_2. The biological implications of small increase in NEFA are not clear (MAHJOUBI et al., 2014). However, this is usually observed in pregnant ewe and cows, when there are higher FA

mobilization (DRACKLEY, 1999; PETTERSON et al., 1994; VERNON, 1981). The fact of glycerol and NEFA had a treatment by time interaction shows that changes in energetic status after feeding was a result of differences in the diets to supply energy and attend daily energy requirements. Because both NEFA and glycerol in blood can be accounted for by the same metabolic pathway, it seems that NEFA concentration is a more reliable marker for moderate changes in energy status in ruminants. Nevertheless, NEFA concentration increases could be a result of increases in albumin concentration in the blood, given that the animals were in the growing phase.

4.2 Nitrogen balance and microbial protein

Dietary supply of rumen degradable protein must be adequate to optimize microbial protein synthesis. It is well established that ruminal NH_3 availability has a major importance on microbial growth (NRC, 2001). The diets provided enough energy for microbial protein synthesis, and the amount of protein synthesized was not significantly different across diets ($P > 0.05$)

The EE intake was higher in the treatment with only sunflower cake than palm cake (5.3% DMI and 4.8% of DMI, respectively). Ruminants are relatively intolerant to high concentrations of fat. Feed intake usually decreases when fat content of the cattle diets exceeds 6% DM (PALMQUIST and JENKINS, 1980). Sheep have higher protozoal abundances than in cattle (PRASAD and PRADHAN, 1990) and dietary fat inhibits the growth of protozoa (PATRA and YU, 2013), which may result in sheep be more tolerant to fat than cattle. Dietary PUFA were found to be much more toxic to gram-positive bacteria than saturated fatty acids (MAIA et al., 2010). This could explain the microbial protein synthesis being similar in the treatment with sunflower cake (rich in PUFA) compared to palm cake, even though it has greater availability of carbohydrate, energy and protein.

The palm cake in the diets decreased linearly the N retained (N excreted / N ingested) by 63%. Although there was a reduction, it should be noted that positive values for N indicate that the supplied N met the requirements of animals fed with palm cake in substitution to sunflower cake. The N intake and absorbed tended to decrease 28.9% and

36.9%, and the N excreted did not differed when sunflower cake was totally replaced by sunflower cake, which possibly contributed to decreases in N retained. Bohnert et al. (2002) shows when CP degradability decreases, the fecal and urinary N also decrease. This is an adaptation of ruminants to recycle N in attempt to maintain homeostasis. Contrarily, in this study, it did not happen. Additionally, Van Soest (1994) suggested a low intake of N leads to reduced excretion of urea in the urine to maintain serum urea pool, which is under physiological homeostatic control. The replacement of sunflower cake by palm cake decreases the protein digestibility, while it increases the urea serum concentration. A better balance between energy and nitrogen in sunflower cake could explain a better nitrogen utilization by rumen. However, no differences were found in microbial protein synthesis.

5.0 CONCLUSIONS

The replacement of sunflower cake by palm cake cubically affected all the nutrients intake and TDN.

The replacement of sunflower cake by palm cake decreases CP and NFC digestibility.

The results presented here suggest that sunflower cake is a better energy and protein source than palm cake.

Further studies would evaluate the performance and ruminal parameters when sunflower cake are replaced totally by palm cake.

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CONSIDERAÇÕES FINAIS

A ação dos ácidos graxos no metabolismo animal é complexa devido às diferentes propriedades químicas apresentadas e a forma física em que é incluído nas dietas. O potencial de toxidade dos ácidos graxos insaturados está bem estabelecido, embora ainda se discuta o mecanismo de ação. Devido aos AGCM apresentarem propriedades químicas similares aos ácidos graxos insaturados, especula-se que possam atuar de forma semelhante sobre os microrganismos ruminais.

A inclusão da torta de dendê, embora tenha causado diminuição no consumo dos componentes energéticos da dieta aumentando a concentração de NEFA sanguíneo, pode substituir a torta de girassol. Porém, algumas considerações devem ser feitas: deve-se considerar sua disponibilidade e considerar que animais que demandam mais energia, como animais que estão em crescimento, possam ter um menor desempenho em comparação com a torta de girassol.

A inclusão de óleo de coco em dietas de vacas de leite mostrou potencial para diminuir a BH do ácido linoleicom, aumentando C18:1 *trans*-11 no leite); e também para aumentar o CLA *cis*-9, *trans*-11. A infusão de COO como uma única dose (aproximadamente 200 g/vaca/d de ácido láurico) diminuiu as taxas de BH do CLA para C18:1 *trans*-11. Como o óleo de coco tem alta concentração de AGCM, podemos sugerir que os MCFA atuam sobre a BH de modo similar aos ácidos graxos insaturados.

Em dois dos trabalhos realizados utilizamos fontes de AGCM pelo potencial antimicrobiano que apresenta. Embora os AGCM atuem reduzindo a atividade dos microrganismos fibroliticos em ambos os experimentos, o fornecimento de fontes de AGCM não reduziu o CMS. No primeiro estudo, possivelmente porque os ácidos graxos estavam na forma esterificada, tornando a liberação mais lenta e menos prejudicial aos microrganismos. Já no segundo experimento, o fornecimento de COO (200 g/vaca/d de ácido láurico) foi baseado no trabalho de Hristov et al. (2004), que mostrou que níveis acima de 240 g de ácido láurico diminuem o CMS.

Foram conduzidos vários experimentos ao longo dos anos na tentativa de melhorar a produção de gordura do leite, que está relacionado à mudança para uma rota alternativa da BH do ácido linoleico, na qual se converte o ácido linoleico à CLA *cis*-10, *trans*-12. A meta-análise permitiu agrupar esses trabalhos para um melhor entendimento da relação da ruminação e da produção de gordura do leite. A princípio, também tínhamos como

objetivo avaliar a relação do trans-10, que é proveniente da BH do CLA *cis*-10, *trans*-12 e indicativo de MFD. No entanto, poucos trabalhos reportaram dados desses ácidos graxos, não sendo possível obter este resultado através da regressão simples. Foi desenvolvido também um modelo multivariado, no qual o melhor modelo desenvolvido para predizer o tempo total de ruminação incluiu produção de leite, concentração de gordura no leite e CMS. Sucintamente, quando ocorrerem mudanças nestas variáveis, também ocorrerão mudanças no tempo que o animal gasta ruminando. O CMS foi a variável que apresentou relação negativa com o tempo de ruminação, sendo que um aumento no CMS de 1kg diminui o tempo de ruminação em 4,86 minutos. Essa diminuição pode estar relacionando à capacidade física do rúmen e também a fermetabilidade da dieta. Como esperávamos, a relação da produção de gordura do leite e tempo que o animal gasta ruminando é quadrática.

Está bem estabelecida a relação da dieta com o pH ruminal e a BH de AG. As respostas que tivemos nesses experimentos corroboram com isso. No segundo experimento, em que se forneceu óleo de coco, resultou no aumento do pH ruminal. Adicionalmente, no terceiro experimento, o pH ruminal apresentou relação linear negativa com o tempo de ruminação e alta correção com concentração de MS da dieta, e CLA *cis*-9, *trans*-11 e *trans*-10, *cis*-12.

No entanto algumas respostas são contraditórias nos experimentos:

1- No experimento com torta de dendê e girassol, esperava que ocorresse uma redução linear, e isto não ocorreu, provavelmente por algum fator de resposta do metabolismo animal dentro dos tratamentos intermediários.
2- Embora o fornecimento de óleo de coco tenha aumentado o C18:1 *trans*-11 no leite, não aumentou o CLA *cis*-9, *trans*-11, o que era esperado. O modelo desenvolvido, embora tenha mostrado sensível para detectar mudanças, mostrou limitações, sendo necessário melhorar o mesmo.
3- As limitações de se fazer uma meta-análise com um grande número de estudos e variáveis acarreta na diminuição do coeficiente de determinação. As respostas obtidas explicam somente uma fração limitada da gordura do leite, pois existem fatores ligados a genética e variação biológica do animal.

Além do mais, um modelo matemático ainda que bem desenvolvido possui erros.