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EFICIÊNCIA DE TRANSFERÊNCIA DO ISÔMERO CLA *TRANS*-10, *CIS*-12 DA DIETA PARA O LEITE DE OVELHAS E CABRAS

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Dissertação apresentada ao Programa de Pós Graduação em Ciência Animal, da Universidade do Estado de Santa Catarina, como requisito parcial à obtenção do título de Mestre em Ciência Animal, área de concentração em Produção Animal.
Orientador: Prof. Dr. Dimas Estrasulas de Oliveira.
Co-orientador: Prof. Dr. Cláudio Vaz Di Mambro Ribeiro.

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Lages, 19 de fevereiro de 2021.

Dedico com todo meu amor e

gratidão aos meus pais.

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“The task is to live your life in such a way that you must wish to live it again - for you will anyway […] Only make sure you come to know what gives you the highest feeling, and then spare no means. Eternity is at stake!”

(Friedrich Nietzsche)

RESUMO

Em cenários de depressão de gordura no leite (DGL) induzida pelo ácido linoleico conjugado (CLA), cabras são menos responsivas comparadas às ovelhas quando recebem as mesmas doses do isômero *trans*-10, *cis*-12 na dieta. Isso pode ser atribuído a diferenças na eficiência de transferência desse ácido graxo da dieta para o leite, entre as espécies. O objetivo deste trabalho foi avaliar a eficiência de transferência do CLA *trans*-10, *cis*-12 da dieta para o leite de ovelhas e cabras. Foram utilizados dados de cinco trabalhos previamente publicados que avaliaram a resposta dos animais consumindo o mesmo suplemento lipídico contendo CLA nas doses 0, 4,48, 8,97 e 13,45 g/d para cabras e 0, 2,99, 5,98 e 8,97 g/d para ovelhas. Cento e seis observações de cabras e sessenta e sete de ovelhas foram analisadas por regressão linear simples para determinar a eficiência de transferência em cada espécie. Análises de covariância foram realizadas para comparar as duas equações de regressão e detectar diferenças no teor de gordura e perfil de ácidos graxos do leite entre as espécies, em função da dose de CLA da dieta e no perfil dos ácidos graxos do leite nas doses 0 e 8,97 g/d de CLA. A secreção de CLA no leite aumentou com o aumento da dose desse ácido graxo na dieta de ovelhas (P<0,05) e cabras (P<0,05) e não diferiu entre as espécies (P>0,05). Entretanto, a DGL em ovelhas foi 69,1% maior que em cabras assim como a mudança no perfil de ácidos graxos no leite foi maior para essa espécie (P<0,05). Os resultados sugerem que a eficiência de transferência do CLA *trans*-10, *cis*-12 da dieta para o leite é a mesma para cabras e ovelhas (1.5%), embora as espécies apresentem níveis distintos de DGL.

Palavras-chave: Síntese de gordura; depressão de gordura no leite; suplemento lipídico.

ABSTRACT

In milk fat depression (MFD) scenarios induced by conjugated linoleic acid (CLA), goats are less responsible to the same doses of *trans*-10, *cis*-12 isomer in the diet compared to ewes. This can be attributed to different transfer efficiency of this fatty acid from diet to milk fat, between species. The objective of this study was to evaluate the transfer efficiency of *trans*-10, *cis*-12 CLA from diet to milk fat in ewes and goats. Data from five previously published studies that evaluated the animal responses to the same CLA lipid supplement in doses of 4.48, 8.97 and 13.45 (g/d) in goats and 2.99, 5.98 and 8.97 (g/d) in ewes. One hundred and six observations of goats and sixty-seven of ewes were analyzed by simple linear regression to determine the transfer efficiency in each specie. Covariance analysis were performed to compare both regression equations and detect differences in the milk fat content as a function of the CLA dose in diet and milk fatty acid profile in the doses 0 and 8.97 (g/d) of CLA, between species. The CLA secretion in milk increased as the doses of this fatty acid increased in diet of ewes ($P<0.05$) and goats ($P<0.05$) and did not differ between species ($P>0.05$). However, the MFD in ewes was 69.1% higher than in goats as well as the milk fatty acids profile were higher to this specie ($P<0.05$). The results suggest the *trans*-10, *cis*-12 CLA transfer efficiency from diet to milk is the same for goats and ewes (1.5%), although these species show different levels of milk fat depression.

Keywords: Fat synthesis; milk fat depression; lipid supplement.

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LISTA DE ABREVIATURAS E SIGLAS

ACACAα	Acetil-CoA carboxilase alfa / Acetyl-CoA carboxylase alpha
AG	Ácidos graxos
AGCC	Ácidos graxos de cadeia curta
AGCM	Ácidos graxos de cadeia média
AGCL	Ácidos graxos de cadeia longa
BHBA	Beta-hidroxibutirato
CLA	Ácido linoleico conjugado/ Conjugated linoleic acid
DGL	Depressão de Gordura no Leite
DIM	Days in milk
FA	Fatty acids
FASN	Ácido graxo sintase/ Fatty acid synthase
LCFA	Long-chain fatty acids
LPL	Lipoproteina lipase
MCFA	Medium-chain fatty acids
MFD	Milk fat depression
mRNA	messenger ribonucleic acid
R^2	R-square/ coefficient of determination
RMSE	Root of the mean square error
SCC	Somatic cell count
SCD	Estearoil-CoA dessaturase
SCFA	Short-chain fatty acids
SREBP	Proteína ligadora do elemento regulatório de esterol
VLDL	Lipoproteínas de muito baixa densidade

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1 INTRODUÇÃO

A síndrome de depressão de gordura no leite (DGL) é causada, pelo menos em parte, pelo isômero *trans*-10*, cis*-12 do ácido linoleico conjugado (CLA) que, quando fornecido na dieta dos animais ou formado a partir da biohidrogenação parcial do ácido linoleico (C18:2 *cis*-9, *cis*-12) sob certas condições ruminais, age na glândula mamária reduzindo a expressão dos genes que codificam as enzimas lipogênicas (BAUMGARD et al., 2002). Embora em alguns casos o *trans*-10, *cis*-12 tenha causado efeitos adversos em vacas e ovelhas leiteiras (BELL; KENNELLY, 2003; OLIVEIRA et al., 2012a), o mesmo pode ser utilizado como estratégia nutricional quando se almeja utilizar a suplementação lipídica para induzir a DGL sem comprometer o desempenho dos animais, podendo incrementar a produção de leite (MEDEIROS et al., 2010) e/ou melhorar o escore de condição corporal de fêmeas lactantes em balanço energético negativo (BALDIN et al., 2014).

Em pequenos ruminantes, ao utilizar o CLA *trans*-10, *cis*-12 para criar um cenário de DGL se tem observado a ocorrência de diferentes níveis de redução no teor de gordura para uma mesma dose de CLA *trans*-10, *cis*-12 na dieta. Essa resposta pode ser atribuída a diferentes taxas na eficiência de transferência do ácido graxo da dieta para a gordura do leite (SHINGFIELD; ROUEL; CHILLIARD, 2009a). Entretanto, estudos que tenham investigado de forma direta ou indireta a susceptibilidade de cabras a essa alteração metabólica e comparando-as às ovelhas, são escassos.

De Veth et al. (2004) mensuraram em vacas a eficiência de transferência de doses crescentes de CLA *trans*-10, *cis*-12 por infusões abomasais (g/d) e sua secreção no leite (g/d), chegando a um valor de 22%. Mesmo fazendo adequações de cálculo para ajustar a forma de fornecimento, a equação desenvolvida pelos referidos autores pode não se aplicar a animais que receberam a suplementação lipídica na dieta de forma desprotegida da biohidrogenação ruminal, visto que as bactérias ruminais podem hidrogenar o CLA *trans*-10, *cis*-12 reduzindo a proporção que alcança o intestino e, consequentemente, o que chegaria na glândula mamária. Dessa forma, o objetivo do presente trabalho foi, através do desenvolvimento de equações de regressão linear, determinar a eficiência de transferência de CLA *trans*-10, *cis*-12 da dieta para o leite de cabras e ovelhas, utilizando dados de trabalhos previamente publicados, nos quais as mesmas doses de CLA *trans*-10, *cis*-12 desprotegido de

biohidrogenação ruminal foram fornecidas para as duas espécies e comparar, através das equações, a eficiência de transferência entre cabras e ovelhas.

2 REVISÃO BIBLIOGRÁFICA

A gordura além de ser o principal constituinte energético do leite, é o componente capaz de sofrer maior variação e influencia diretamente no processamento e nas características físicas e organolépticas dos produtos lácteos (PALMQUIST et al., 1993). Considerando as crescentes exigências do mercado consumidor para a qualidade do produto final, bem como o pagamento da indústria ao produtor por sólidos totais, desenvolver estratégias que possibilitem a manipulação da composição do leite e compreender os fatores que regulam sua secreção, tornaram-se fundamentais para potencializar a lucratividade no setor leiteiro (SHINGFIELD; GRIINARI, 2007). Assim, a dieta é o fator ambiental mais influente na composição do leite e, dessa forma, alterações no perfil de ácidos graxos através de estratégias nutricionais são factíveis (PALMQUIST, 2006).

2.1 METABOLISMO RUMINAL DE ÁCIDOS GRAXOS E A SÍNTESE DE GORDURA NO LEITE

A fração lipídica das dietas de ruminantes alimentados com forragens e concentrados está na forma de galactolipídeos, fosfolipídeos e triglicerídeos, respectivamente, e tem em sua composição principalmente os ácidos graxos insaturados: linolênico (C18:3), oleico (C18:1) e linoleico (C18:2) (WOOD et al., 1963). Estes, podem ser saturados em até 80% a ácido esteárico (C18:0) (WHITE; KEMP; DAWSON, 1970) pelos processos de lipólise e biohidrogenação dependendo das condições ruminais, da natureza e quantidade do substrato ingerido. Alterações nesses fatores resultam em variações no perfil de ácidos graxos (AG's) presentes na carne e leite (FUENTES et al., 2011; LOURENÇO; RAMOS-MORALES; WALLACE, 2010).

A gordura do leite em ruminantes deriva de duas fontes: os ácidos graxos de cadeia longa (AGCL; C>16), denominados pré-formados, oriundos dos quilomícrons e lipoproteínas de muito baixa densidade (VLDL) absorvidos do intestino delgado ou da mobilização das reservas corporais e; dos substratos do metabolismo ruminal, acetato e beta-hidroxibutirato (BHBA), utilizados como fonte de carbono para a produção de ácidos graxos de cadeia curta (AGCC; C<16) e média (AGCM; C16) em um processo denominado síntese *de novo* que é regulado por um complexo de genes lipogênicos e fatores de transcrição na glândula mamária (BAUMAN et al., 2008).

Aproximadamente 50% dos ácidos graxos presentes no leite são obtidos pela síntese *de novo*, 40-45% são provenientes da dieta e menos de 10% da mobilização das reservas corporais (PALMQUIST; MATTOS, 1978). Esses percentuais podem variar de acordo com o balanço energético em que o animal se encontra (GROSS et al., 2011; PALMQUIST; BEAULIEU; BARBANO, 1993), o estágio de lactação e o tipo de dieta ofertada que, através de alterações no metabolismo, pode desencadear síndromes como é o caso da Depressão de Gordura no Leite (DGL) (BAUMAN; GRIINARI, 2003).

2.2 SÍNDROME DE DEPRESSÃO DE GORDURA NO LEITE EM RUMINANTES

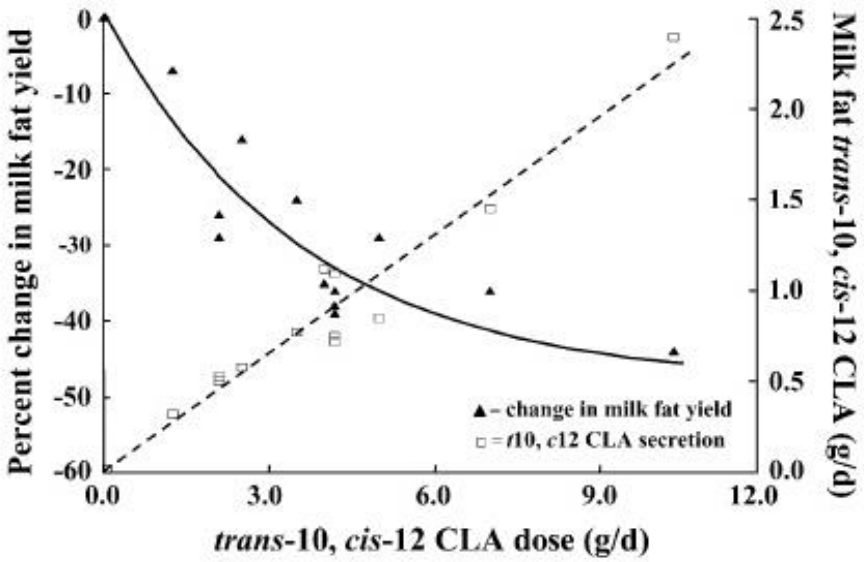
A DGL é caracterizada por uma redução na concentração e/ou produção de gordura, sem ocorrer mudanças nos demais constituintes do leite. A sua causa está relacionada a presença de ácidos graxos "*trans*", oriundos da biohidrogenação parcial dos ácidos graxos presentes na dieta (BAUMAN; GRIINARI, 2003). O isômero *trans*-10*, cis*-12 do ácido linoleico (18:2 *cis*-9, *cis*-12), denominado CLA (do inglês, Conjugated Linoleic Acid), é o ácido graxo indubitavelmente envolvido na DGL (BAUMGARD et al., 2000) e aparece em condições específicas de diminuição do pH ruminal, oriundas de dietas contendo altas proporções de concentrados rapidamente fermentáveis e/ou baixo teor de fibra ou ainda, por suplementação com lipídeos ricos em ácidos graxos insaturados (C18:2 *cis*-9, *cis*-12) (BAUMAN; GRIINARI, 2003).

O CLA *trans*-10, *cis*-12 tem efeito sobre a expressão de genes que codificam as enzimas lipogênicas na glândula mamária e alguns fatores de transcrição como da família de proteína ligadora ao elemento regulatório de esterol 1 (SREBP1) (BAUMGARD et al., 2002; EBERLÉ et al., 2004). Embora os mecanismos moleculares não estejam completamente compreendidos, sabe-se que a presença de CLA *trans*-10, *cis*-12 na célula epitelial mamária reduz a produção de gordura em até 50% e, destes, 70% é por inibição da síntese *de novo* (HARVATINE; BAUMAN, 2006). Além disso, doses muito elevadas desse isômero (45 g/d), podem reduzir a produção e teor de lactose, causar aumento de células somáticas, cloro e sódio no leite e levar a involução mamária (BELL; KENNELLY, 2003). Entretanto, quando fornecido em doses reduzidas, esse isômero pode ser utilizado na dieta de ruminantes como uma estratégia nutricional, aumentando a produção de leite e a concentração de proteína (LOCK et al., 2006; MEDEIROS et al., 2010) ou melhorando o balanço energético (BALDIN et al., 2014) quando a energia "economizada" na síntese de gordura no leite

é redirecionada para os tecidos de reserva (HARVATINE; PERFIELD; BAUMAN, 2009).

O CLA *trans*-10, *cis*-12 inibe a síntese da gordura do leite de uma forma dependente da dose em vacas (SHINGFIELD; GRIINARI, 2007). De Veth et al. (2004) observaram uma relação linear com uma média de eficiência de transferência do CLA do abomaso para o leite de 22%, além de uma relação curvilínea entre o percentual de redução na produção de gordura do leite e a quantidade de CLA na dieta (Figura 1).

Figura 1 - Relação entre infusão abomasal de doses de CLA trans-10, cis-12 e 1) a mudança na produção de gordura do leite ($y=-48,26+49,03exp^{-0,2792x}$; $R^2=0,86$) e 2) secreção de CLA *trans*-10, *cis*-12 na gordura do leite ($y=0,2175x+0,0111$; $R^2=0,94$).

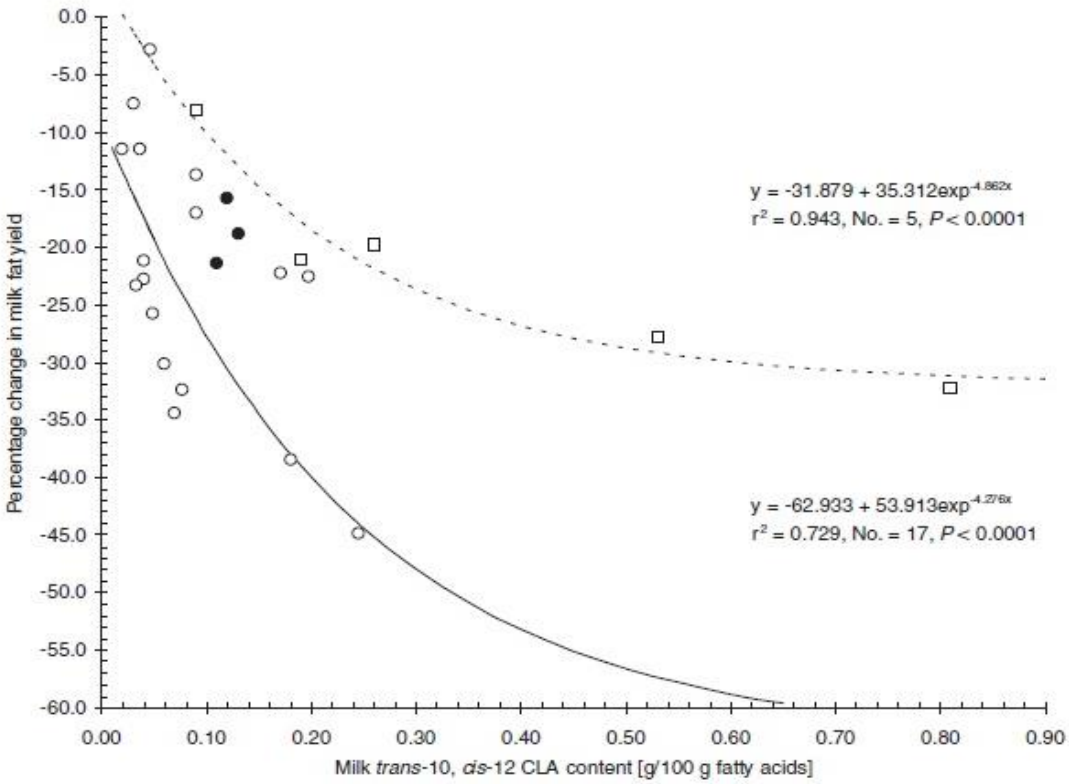


Fonte: Adaptado de Bauman et al., 2008 e De Veth et al.; 2004.

Quando se observa os efeitos da adição de CLA *trans*-10, *cis*-12 na dieta ou infundido no abomaso, nota-se diferentes respostas de indução a DGL (BAUMGARD et al., 2002; OLIVEIRA et al., 2012; BALDIN et al., 2013; FERNANDES et al., 2014). Os pequenos ruminantes são menos susceptíveis às alterações no perfil de ácidos graxos (SHINGFIELD et al., 2010a), mesmo recebendo doses, com base no peso metabólico, superiores de CLA *trans*-10 *cis*-12 que culminam em DGL em bovinos (LOCK et al., 2008). Shingfield et al. (2010) demonstraram essa diferença ao compararem a diminuição na produção de gordura do leite com a quantidade de CLA *trans*-10*, cis*-12 presente no leite (g/100g de ácidos graxos) em trabalhos realizados

com cabras, ovelhas e vacas onde, para uma mesma quantidade do ácido graxo no leite houve reduções na produção de gordura no leite distintas entre as espécies (Figura 2).

Figura 2 - Relação entre a percentagem de redução na produção de gordura do leite contendo CLA *trans*-10, *cis*-12, em resposta a suplementação de CLA com proteção a degradação ruminal em vacas (○), cabras (□) e ovelhas (●).



Fonte: Adaptado de Shingfield et al.,2010.

2.3 CLA *TRANS*-10, *CIS*-12 EM PEQUENOS RUMINANTES

Ao comparar respostas metabólicas de trabalhos realizados com cabras (BALDIN et al., 2013a, BALDIN et al., 2014; FERNANDES et al., 2014) e ovelhas (BALDIN et al., 2013b; OLIVEIRA et al., 2012a) em cenários de DGL induzidos por uma mesma dose de CLA *trans*-10, *cis*-12, obtém-se diferentes resultados na DGL.

Nos trabalhos citados anteriormente, o suplemento lipídico utilizado foi o mesmo e continha em sua composição cerca de 60% de CLA total na forma de ésteres metílicos, destes, 29,9% eram o isômero *trans*-10, *cis*-12. As escolhas das doses testadas foram baseadas em Oliveira et al., (2012) que estimaram uma

biohidrogenação ruminal de 95% e, considerando que para causar depressão de gordura no leite em torno de 25%, deveria chegar ao abomaso ~ 0,4 g/d desse isômero (LOCK et al., 2006), estabeleceram a dose de 30 g/d do suplemento de CLA como ideal, ou seja, 8,97 g/d de CLA *trans*-10*, cis*-12. Além do suplemento lipídico, a formulação das dietas, manejos e tratamentos assemelharam-se, bem como a utilização de sais de cálcio de ácidos graxos (Megalac-E) como suplemento lipídico dos grupos Controle.

Baldin et al. (2013b) testaram doses crescentes de CLA *trans*-10, *cis*-12 (2,99, 5,98 e 8,97 g/d) em ovelhas e obtiveram reduções de 6,5, 14,2 e 25,5% no teor de gordura e 9,1, 12,9 e 22,5% na produção de gordura dos respectivos tratamentos. Oliveira et al. (2012) forneceram 8,97 g/d de CLA *trans*-10, *cis*-12 para ovelhas lactantes e observaram, além de reduções na produção (38,0%) e teor (31,3%) de gordura, reduções na produção de leite, conteúdo e produção de lactose e proteína, aumento da contagem de células somáticas (CCS) e ainda, uma completa parada da secreção de leite em uma ovelha durante o tratamento, o que foi explicado pelos autores como uma possível involução mamária e/ou apoptose das células epiteliais mamárias causada pelo que consideraram uma alta dose do isômero.

Em cabras, Baldin et al., (2013a) forneceram a mesma dose de 8,97 g/d de CLA *trans*-10, *cis*-12 e obtiveram reduções no teor e produção de gordura de 19,9 e 17,9%, respectivamente, não alterando os demais componentes do leite. Quanto à depressão na secreção de gordura no leite, Fernandes et al., (2014) testaram doses crescentes de CLA *trans*-10, *cis*-12 (4,48, 8,97 e 13,45 g/d) em cabras lactantes e encontraram reduções de 8,1, 26,1 e 32,7%, e mesmo em sua dose mais alta não observaram efeitos deletérios em cabras como ocorreu nas ovelhas (OLIVEIRA et al., 2012a). Tal discrepância pode ser atribuída, pelo menos em parte, a diferenças na eficiência de transferência do CLA *trans*-10, *cis*-12 da dieta para o leite.

2.4 EFICIÊNCIA DE TRANSFERÊNCIA

A eficiência de transferência na nutrição animal refere-se à efetividade em teor que determinado nutriente exógeno é transferido até seu local de ação e/ou excreção, considerando o processo de metabolização da espécie animal, o tipo de nutriente e a forma de oferta. Em ruminantes, um dos fatores que afetam o processo de transferência de um nutriente da dieta no organismo do animal, é o metabolismo ruminal. O tipo de alimento ofertado altera a estrutura e a função da microbiota, o que

influi diretamente na quantidade e formato com que esses nutrientes são disponibilizados para a absorção intestinal (NOBLE, 1981). Do mesmo modo, suplementos lipídicos dietéticos ofertados de forma desprotegida da biohidrogenação ruminal, têm taxas de transferência menores quando comparados a suplementos protegidos. Nesse sentido, mensurar a eficiência de transferência de determinados ácidos graxos envolvidos na DGL, ofertados na dieta e suscetíveis a biohidrogenação ruminal, pode auxiliar no entendimento das variações no perfil e produção de gordura do leite, bem como nas respostas divergentes entre as espécies.

Apesar de não descrita totalmente pela literatura, sugere-se que a eficiência de transferência lipídica varia entre ruminantes por diferenças nas taxas de passagem ruminal dos ácidos graxos presentes na dieta, na absorção intestinal e/ou na captação dos mesmos pela glândula mamária e, em casos de DGL, uma menor sensibilidade dos genes lipogênicos em cabras (CHILLIARD et al., 2003; SHINGFIELD et al., 2010). Além disso, diferentes pesos metabólicos podem influenciar na magnitude do efeito do ácido graxo na expressão gênica, como demostrado por Oliveira et al. (2018), onde ovelhas de maior peso metabólico apresentaram maior expressão dos genes lipogênicos e fatores de transcrição na glândula mamária, quando submetidas a uma mesma dose de CLA *trans*-10, *cis*-12 na dieta.

A possibilidade de processos de biohidrogenação distintos entre as espécies ruminantes são sustentadas por observações de diferenças entre as populações e composições microbianas (HENDERSON et al., 2015), nas atividades de enzimas ruminais e degradação da matéria seca (MS) (MOON et al., 2010) e atuação de diferentes grupos bacterianos na saturação de AG no rúmen (TORAL et al., 2016). Enquanto estudos comparando as espécies a nível celular, demonstraram particularidades na expressão de genes e fatores de transcrição envolvidos na regulação lipídica e lipogênica na glândula mamária e tecido adiposo de ruminantes (BERNARD; LEROUX; CHILLIARD, 2008; BONNET et al., 1998).

Fougère e Bernard (2019) encontraram diferenças na abundância de RNAm de 14 dos 21 genes envolvidos no metabolismo mamário de cabras e vacas submetidas a três tratamentos com diferentes dietas lipídicas. Esses resultados corroboram com estudos prévios e sugerem que uma maior expressão do gene codificador da ácido graxo sintase (FASN) e a ação da enzima em cabras comparado a vacas, explicaria a maior concentração na soma de ácidos graxos de cadeia curta (AGCC) no leite proveniente de uma maior síntese *de novo* nessa espécie (BERNARD; TORAL;

CHILLIARD, 2017; CHILLIARD et al., 2003; TORAL et al., 2013). Tsiplakou et al. (2011) reforçaram essas especificidades de cada espécie comparando a expressão gênica em tecido adiposo subcutâneo de cabras e ovelhas sob tratamentos dietéticos contendo CLA encapsulado na dieta e encontraram maiores expressões dos genes Acetil-CoA carboxilase alfa (ACACAα) e FASN em cabras, enquanto lipoproteína lipase (LPL) e estearoil-CoA dessaturase (SCD) foram mais expressos em ovelhas.

Através da equação de De Veth et al. (2004) para a eficiência de transferência do CLA das infusões abomasais para o leite em vacas lactantes, Baldin et al. (2013b), Baldin et al. (2014) e Fernandes et al., (2014) encontraram valores de 1,68, 1,8 e 1,17% para cabras, respectivamente, e Baldin et al. (2013a) de 1,43% para ovelhas. Porém, ao considerar que o ácido graxo fornecido na dieta dos pequenos ruminantes era desprotegido e sofreu biohidrogenação ruminal parcial, chegando em quantidade desconhecida no abomaso, essa equação utilizada pode não refletir o que aconteceria em pequenos ruminantes.

3 ARTIGO – TRANSFER EFFICIENCY OF *TRANS*-10, *CIS-12* CLA FROM DIET TO MILK IN GOATS AND EWES

ABSTRACT: In a CLA-induced milk fat depression (MFD) scenario, small ruminants have been showed different responses to MFD syndrome according to the same amount fed of *trans*-10, *cis*-12 CLA. Goats are less susceptible to this metabolic alteration compared to ewes and this can be attributed to different transfer efficiency of this fatty acid between species. The objective was to evaluate the transfer efficiency of *trans*-10, *cis*-12 CLA isomer from diet to milk in goats and ewes. Data from five previously published studies evaluating animal responses under MFD induced by doses of *trans*-10, *cis*-12 CLA of 4.48, 8.97 and 13.45 (g/d) in goats and 2.99, 5.98 and 8.97 (g/d) in ewes fed the same CLA lipid supplement, were used. One hundred and six observations of dairy goats and sixty-seven of dairy ewes were analyzed by simple linear regression to determine the diet to milk *trans*-10, *cis*-12 CLA transfer in each specie. The two species regressions were compared by a covariance analysis as well as the difference in the milk fat (%) on the last day of treatment as a function of the amount fed of *trans*-10, *cis*-12 CLA by the diet (g/d). The regressions showed that the secretion of *trans*-10, *cis*-12 CLA in milk fat increased as the amount of this fatty acid increased in the diet of goats ($P<0.05$) and ewes ($P<0.05$) and, there was no difference in *trans*-10, *cis*-12 CLA secretion in milk fat between species ($P>0.05$). The comparison of milk fat content on the last day of treatment indicated that in ewes the MFD was 69.1% higher than goats. In summary, the transfer efficiency of the *trans*-10, *cis*-12 CLA isomer from diet to milk is 1.5% for goats and ewes, although these species show different levels of milk fat depression.

KEYWORDS: Fat synthesis; milk fat depression; small ruminants.

3.1 INTRODUCTION

The *trans*-10, *cis*-12 isomer of the conjugated linoleic acid (CLA) is associated with milk fat depression (MFD) syndrome because it downregulates the expression of genes encoding lipogenic enzymes in the mammary gland (BAUMGARD et al., 2002). The use of *trans*-10, *cis*-12 CLA lipid supplementation as a nutritional strategy to induce MFD without compromising the performance of the animals has been shown in cows (MEDEIROS et al., 2010) and sows (SANDRI et al., 2020); and also to improve the energy balance of lactating goats (BALDIN et al., 2014). Depending on the dose

and form that the isomer is provided to animals, the CLA reduces the secretion of milk fatty acids (FA), particularly those resulting from the *de novo* pathway, and increases the concentration of unsaturated long chain FA (BALDIN et al., 2013a; FERNANDES et al., 2014).

The transfer efficiency of 22% has been reported in cows by abomasal infusions (De Veth et al., 2004). The equation developed by the authors may not apply to animals receiving rumen unprotected CLA supplements, since ruminal biohydrogenation would reduce the amount absorbed by the small intestine.

In a CLA-induced MFD scenario, small ruminants showed different responses to MFD for the same dose of CLA. This response can be attributed to different rates of FA transfer efficiency from the diet to milk or to a different mammary gland sensitivity between species (SHINGFIELD; ROUEL; CHILLIARD, 2009a). However, there is limited information whether goats are less susceptible to CLA-induced MFD when compared to ewes. The objective of this study was to determine the *trans*-10, *cis*-12 CLA transfer efficiency from diet to milk in goats and ewes using data from previous published studies.

3.2 MATERIAL AND METHODS

3.2.1 Data description

The data used were previously published by studies that evaluated the supplementation of *trans*-10, *cis*-12 CLA to goats (BALDIN et al., 2013a, BALDIN et al., 2014; FERNANDES et al., 2014) and ewes (BALDIN et al., 2013b; OLIVEIRA et al., 2012). The criteria adopted for the use of these data were the intake of the same lipid supplement in the five experiments, the similarity in the management of the animals and the evaluation of the effects on milk fat yield and FA profile, milk yield and other milk components under CLA-induced MFD scenarios in goats and ewes. Raw data were used and the analyzes were separated by species. A summary of the experiments descriptions is shown in Table 1.

Table 1 – Experiments description and criteria adopted for using the data.

		GOATS	EWES
Studies		Baldin et al. (2013a); Baldin et al. (2014); Fernandes et al. (2014);	Baldin et al. (2013b); Oliveira et al. (2012);
Animals	Multiparous	22	24
	Primiparous	24	29
Observations		106	67
Breed		Toggenburg	Lacaune
Body weight (kg)*		45.5 ± 7.9	52.3 ± 5.1
DIM[a*]		105.0 ± 26	55.0 ± 10
Milk yield (kg/d)*		2.3 ± 0.5	1.7 ± 0.3
Diet		Corn silage and concentrate composed of ground corn and soybean meal	Pasture and concentrate composed of ground corn and soybean meal
Design		Latin square and *cross over*	Random design and *cross over*
Intake (g/d)[b]		0, 4.48, 8.97 and 13.45	0, 2.99, 5.98 and 8.97

*Mean ± standard deviation.
[a] DIM: days in milk.
[b] Intake of *trans*-10, *cis*-12 CLA through the diet (g/d).

Except for Oliveira et al. (2012), the experiments used calcium salts of FA (Megalac-E, Church & Dwight, Nova Ponte, MG, Brazil) as a lipid supplement in the control treatment. The animals supplemented with CLA received the same rumen unprotected CLA methyl ester supplement (Luta CLA 60®, BASF AG, São Paulo, SP, Brazil) and both FA lipid supplements profiles are reported by Baldin et al. (2013a). The treatments were mixed into the concentrate and provided daily. The intake was chosen considering that the CLA supplement contained 29.9% of *trans*-10, *cis*-12 18:2 and the maximum ruminal biohydrogenation of this isomer was 95% (JENKINS et al., 2008). Thereby, the intake of CLA was calculated to deliver to the rumen 4.48, 8.97 and 13.45 (g/d) for goats and 2.99, 5.98 and 8.97 (g/d) for ewes.

3.2.2 Calculations

Data from milk yield, milk fat content and CLA percentage were used to calculate CLA secretion from each observation using the following equation: Sec = MY * FC * 0.98 * 0.95 * P, where Sec is milk CLA secreted per day (g/d); MY is milk yield (g/d); FC is milk fat (%), and P is milk CLA percentage (g/100g). It was assumed that milk fat is composed by 98% triacylglycerols which contain 95% fatty acids (JENSEN, 2002).

The data for *trans*-10, *cis*-12 CLA naturally present in the Control treatment (without CLA) were discounted as a way to adjust the values in milk fat secretion to mitigate the effect of possible different ruminal environments caused by individual feed intake and/or diet composition ingested.

3.2.3 Statistical analysis

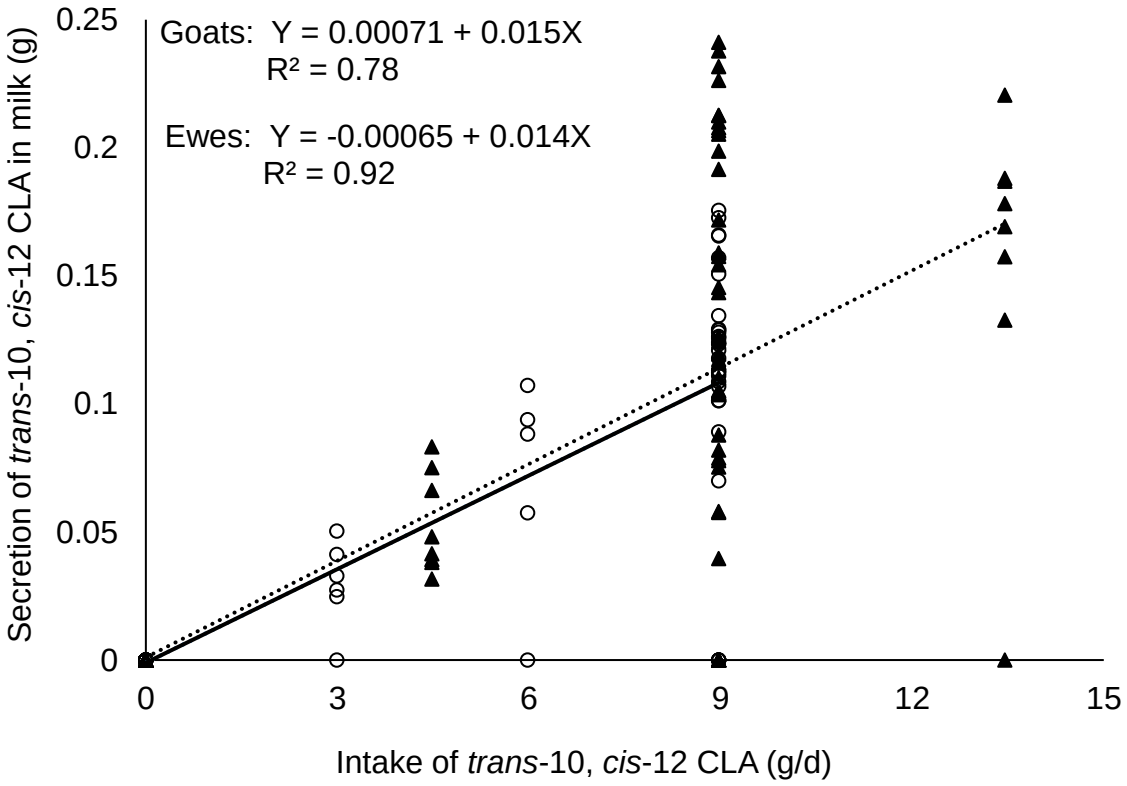
The data were analyzed by the statistical program SAS University Edition (SAS Institute Inc., 2017). The CLA transfer efficiency for goats and ewes was analyzed by simple linear regression using the PROC REG command with the following equation: **Y = a + bX**, where **Y** represents g/d of CLA secreted in milk; **a** is the intercept, **b** is the slope (transfer efficiency) and **X** is the CLA intake (g/d) in the diet. The same procedure was used to analyze the transfer efficiency of the amount of CLA estimated from the abomasum (g/d) to milk (g). The studentized residuals outside the range ± 2.8 were considered outliers and removed from the analyses. The R^2 (coefficient of determination) and RMSE (root of the mean square error) were considered as indicative of model adequacy. Also, the PROC MODEL was used to verify the homogeneity of variance, residual normality and error independence, through the White, Shapiro Wilk and Durbin Watson tests, respectively.

The PROC GLM was used to detect differences in the CLA transfer efficiency between goats and ewes. The species was used as a fixed effect in the linear model described above to analyze the differences between species in the milk fat (%) on the last day of treatment as a function of dietary CLA intake (g/d) and as a function of the amount of CLA estimated at the abomasum (g/d). The same procedure was used to detect differences for short (SCFA), medium (MCFA) and long (LCFA) chains FA in milk fat at intake 0 and 8.97 g/d of CLA. Effect of species, intake and their interaction were considered when $P<0.05$.

3.3 RESULTS

The secretion of CLA in milk increased as the intake of this FA increased in diets of goats and ewes. The linear equations for transfer efficiency of CLA from diet to milk had a R^2 of 0.92 and 0.78 and RMSE of 0.018 and 0.038 for ewes and goats, respectively. The transfer efficiency for both species was 1.5%. Nine and six outliers were found and removed from the goat and ewe dataset, respectively. The intercepts for both species did not differ from zero (P>0.05; Figure 3).

Figure 3 - Effect of intake of *trans*-10, *cis*-12 CLA (g/d) and its secretion in milk (g) for ewes (○; continuous line) and goats (▲; dotted line). The slope and intercept did not differ between species (P>0.05).



Similarly, the secretion of CLA in milk increased as the amount of CLA estimated at the abomasum increased for both species. The linear equations had a R^2 of 0.92 and 0.75 and RMSE of 0.018 and 0.043 for ewes and goats, respectively. Five outliers for ewes and six for goats were found and removed from the analysis. There was no difference (P>0.05) for intercepts and slopes between species (Table 2). However, when the regressions were compared to the equation for cows (Y = 0.2175X + 0.0111;

De Veth, 2004), where X represent the estimated amount of CLA in the abomasum, the slopes of ewes and goats were, respectively, 30 and 44% higher than the slope of cows.

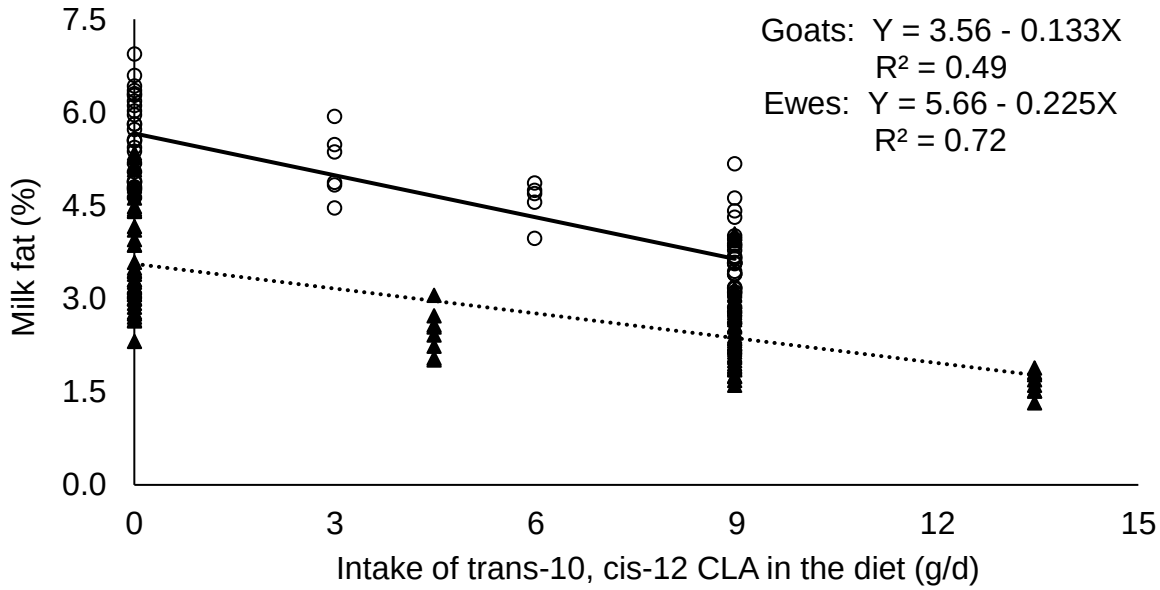
Table 2 – Transfer efficiency equations of the estimated amount of *trans*-10, *cis*-12 CLA in the abomasum to milk.

	Y[a]	X[b]	Intercept	Slope	Adj R-Sq	RMSE	*P*[c]
Ewes	CLA in milk	CLA in abomasum	-0.00065	0.2829	0.92	0.018	<0.0001
Goats	CLA in milk	CLA in abomasum	0.00114	0.3134	0.75	0.043	<0.0001

[a] Dependent variable;
[b] Independent variable;
[c] Model Significance (p<0.05).

Nevertheless, the milk fat depression was different between species. When the milk fat content was regressed on the intake of *trans*-10, *cis*-12 CLA fed in the diet (X), ewes showed an intercept 58.9% higher than goats. In addition, the difference between slopes (P<0.05) indicates that ewes depressed milk fat content 69.1% more than goats per unit of CLA intake (Figure 4). The RMSE were 0.58 and 0.66 for ewes and goats, respectively.

Figure 4 - Effect between intake of trans-10, cis-12 CLA (g/d) and the fat content in milk (%) for ewes (○; continuous line) and goats (▲; dotted line). Slope and intercept were different between species (P<0.05).



When comparing the milk FA chain lengths at intake 0 and 8.97 g/d of CLA/day in ewes and goats, there was a decrease in the concentration of FA with less than 16 carbons (<C16) by 21.2 and 13.9%, and an increase of FA with more than 16 carbons (>C16) by 21.9% and 16.4%, respectively. Only goats had a 9.7% decrease in the concentration of C16 (Table 3).

Table 3 - Milk fatty acids grouped by chain length at intake 0 and 8.97 g/d of CLA in ewes and goats.

	EWES				**GOATS**			
	0 g/d	8.97 g/d	SEM[1]	*P*[2]	0 g/d	8.97 g/d	SEM[1]	*P*[2]
<C16	34	26.8	0.9	<0.0001	30.8	26.5	0.6	<0.0001
C16	25.6	24	0.9	0.1074	26.8	24.2	0.6	0.0002
>C16	32.3	39.4	0.9	<0.0001	41.5	48.3	0.6	<0.0001

[1]SEM = standard error of the mean.
[2]Probability of significant effects due to interaction between profile and intake.
<C16= fatty acids with less than 16 carbons.
C16= fatty acids with 16 carbons.
>C16= fatty acids with more than 16 carbons.

3.4 DISCUSSION

The range of milk fat content is different between species. Goats usually synthesize less fat than ewes, resulting in different intercepts (Figure 4). The highest fat content with no CLA supplementation observed for ewes and goats were 5.6 and 3.5%, respectively. Ewes had 26.3% less fat content that was expected for this specie (~7.6% of fat; JANDAL, 1996) with no CLA supplementation. The *trans*-10, *cis*-12 CLA was present in the control treatments, including the experiment by Oliveira et al. (2012), which did not receive lipid supplementation. This can be explained by the amount of concentrate fed to ewes in this study, around 1.2 kg of dry matter (DM)/day. The presence of linoleic FA in the lipid fraction of the concentrate diet, composed by ground corn and soybean meal, coupled with a possible low pasture intake (not reported), enabled an alternative route in the ruminal biohydrogenation that synthesizes the *trans*-10, *cis*-12 CLA isomer, causing a non-CLA induced MFD (BAUMAN; GRIINARI, 2003a). Goats did not present milk fat depression with the Control treatments since the

amount of concentrate in the diet was lower, around 1.0 kg of DM/day, and the roughage fraction was corn silage supplied in a controlled manner.

Although the range of fat content was less than expected, ewes presented a reduction in fat content 69.1% higher than goats (Figure 4). This result shows that, for the same dose of CLA, ewes had a greater milk fat depression. In agreement, Lock et al. (2006) showed reductions in milk fat content of 23% and fat yield of 16% in ewes fed lipid-encapsulated containing 2.4 g/d of *trans*-10, *cis*-12 CLA. Similarly, Sinclair et al. (2007) reported reductions on the percentage (23%) and yield (20%) of fat, and decreases in milk concentrations of lactose and protein. In addition, Oliveira et al. (2012) reported that one of the ewes stopped completely the milk secretion during the trial, which was explained as a mammary involution and/or an apoptosis of mammary epithelial cells due to a high dose (8.97 g/d) of rumen-unprotected *trans*-10, *cis*-12 CLA fed to animals. In contrast, Lock et al. (2008) found in goats decreases in the milk fat yield of 8 and 21% and milk fat content of 5 and 18% for lipid-encapsulated treatments containing 3 and 6 g/d of *trans*-10, *cis*-12 CLA, respectively. Whereas Shingfield et al. (2009) reported that goats receiving doses of 7.47, 14.9 and 22.4 g/d of *trans*-10, *cis*-12 CLA in the form of calcium salts decreased milk fat yield by 19.8, 27.9 and 32.3% and milk fat content by 16.2, 22.7 and 29.4% while changes in lactose, protein and milk yield were not detected. These literature findings reinforce that there are differences among ruminants regarding propensity to MFD since the goats even receiving higher doses of CLA did not present changes in other milk components as ewes did.

Particularities in ruminal biohydrogenation, intestinal absorption and sensitivity of the mammary gland are pointed out as possible explanations for the different responses to MFD among species (BERNARD et al., 2009; SHINGFIELD et al., 2009; TORAL et al., 2016). Estimating the amount of CLA that reached the abomasum from the intake of this FA in diets of goats and ewes allowed a comparison with De Veth et al. (2004) equation for abomasal infusion of CLA in cows. To the same amount of CLA in the abomasum of the three species, goats and ewes transferred 44 and 30% more of this isomer to milk than cows but had the same transfer efficiency between them (Table 2). Cows are known to have greater sensitivity and distinct metabolism behavior under induced-MFD scenarios compared to small ruminants (SHINGFIELD et al., 2010). However, the similarity also observed in the transfer efficiency of CLA from diet to milk in goats and ewes (Figure 3) suggests that there are no differences in the

transfer of the digestive tract from mammary gland of FA between these two species. In addition, the CLA concentration naturally present in the control group were discounted from the other treatments to mitigate, at least in part, the effects of individual feed intake and the composition of the ingested diet. This ensured that the results were only from the effect of CLA doses of the treatments and suggested that there is no difference in CLA biohydrogenation and intestinal absorption between small ruminants. Thus, we hypothesized that goats and ewes differ in the susceptibility of the downregulation of *de novo* FA synthesis by CLA, but not in the amount of FA that reach the mammary glands.

Studies comparing the expression of genes involved in the fat synthesis in the mammary gland and adipose tissue of ruminants suggest that there are distinctions in the regulation of lipolytic and lipogenic mechanisms between species (BONNET et al., 1998; CHILLIARD et al., 2007; SHINGFIELD et al., 2010a). Bernard et al. (2017) showed differences in abundance of fifteen mRNA genes and in 4 enzyme activities involved in lipid metabolism in the mammary gland between goats and cows. Fougère and Bernard (2019) identified differences in the mRNA abundance for fourteen of the twenty-one genes involved in the mammary gland metabolism of these same species submitted to three treatments with different lipid diets. In agreement, Chilliard et al. (2003) found a higher concentration of SCFA and MCFA in milk of goats fed lipid supplement compared to cows, and suggested particularities between species in the FA elongation process. In our study, ewes had a higher concentration of SCFA (36.9% of total FA) at the dose 0 g/d of *trans*-10, *cis*-12 CLA while goats had higher concentration of LCFA (41.9%). However, at the dose of 8.97 g/d of *trans*-10, *cis*-12 CLA ewes had a higher concentration of LCFA (43.7%) comparing to SCFA (29.7%) while goats maintained a higher concentration of LCFA (48.8%) compared to SCFA (26.8%; Table 3). These more pronounced changes in ewes may be associated with the range that the higher milk fat production of this species allows, or with differences between species in the FA that make up the triglyceride. Furthermore, goat genes may be more resistant to the effects of CLA. An *in vitro* cultured mammary slices demonstrated that the goat mammary tissue, unlike cow is not sensitive to the anti-lipogenic effect of *trans*-10, *cis*-12 CLA, and that this FA can also promote lipogenesis in high concentrations (BERNARD et al., 2013).

Although we had not measured gene expression in our studies and research comparing goats and ewes are lacking, studies with cows and goats lead us to suggest

that the differences regarding the CLA effect on MFD between small ruminants may also be related to the lower sensitivity of the mammary gland of goats compared to ewes. Furthermore, we cannot exclude the possible effects on MFD from other FA originated from the biohydrogenation of CLA supplement fed and also from the diet such as linoleic acid (KADEGOWDA; PIPEROVA; ERDMAN, 2008; SHINGFIELD et al., 2010).

3.5 CONCLUSION

The transfer efficiency of *trans*-10, *cis*-12 CLA from diet to milk is 1.5% for both, goats and ewes. However, the effect of CLA on MFD is more pronounced (69.1% higher) in ewes than goats.

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