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ENTEROLACTONA E ÁCIDOS GRAXOS POLI-INSATURADOS NO LEITE DE VACAS ALIMENTADAS COM CASCA E ÓLEO DE LINHAÇA

Autor: Ricardo Kazama
Orientadora: Prof[a]. Dra. Lúcia Maria Zeoula
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Estado do Paraná
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"Tese apresentada, como parte das exigências para obtenção do título de DOUTOR EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá – Área de Concentração Produção Animal".

MARINGÁ
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“Educar e educar-se na prática da liberdade não é estender algo desde a “sede do saber” até a “sede da ignorância” para “salvar”, com este saber, os que habitam nesta.

Ao contrário, educar e educar-se na prática da liberdade é tarefa daqueles que sabem que pouco sabem – por isto sabem que sabem algo e podem assim chegar a saber mais – em diálogo com aqueles que, quase sempre, pensam que nada sabem, para que estes, transformando seu pensar que nada sabem em saber que pouco sabem, possam igualmente saber mais.”

Paulo Freire

A

Deus...

Meu guia, fonte da vida, sabedoria, saúde, amor e paz.

Aos

Meus pais, Tameo e Hiroko...

Primeiros professores de minha vida, responsáveis por ensinar o amor, justiça, honestidade, solidariedade e perseverança.

Aos

Meus irmãos Mauro e Edson...

Meus primeiros amigos que deram proteção, amor, amizade, incentivo e apoio até os dias de hoje.

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Minha esposa Daniele...

Minha fonte de alegria, vida, amor, amizade, apoio e compreensão. Minha família.

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Vida...

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Em março de 2006 iniciou no doutorado em Produção Animal no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá.

Em março de 2007 a fevereiro de 2008 foi contemplado com bolsa de doutorado-sanduíche do CNPq para estágio no Dairy and Swine Research and Development Centre – Agriculture and Agri-Food Canada, na cidade de Sherbrooke, província de Québec, Canadá, na qual participou de projetos na área de qualidade do leite.

No dia 24 de abril de 2009, submeteu-se à banca para a defesa da Tese e foi aprovado para receber a titulação de Doutor em Produção Animal pelo Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá.

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ABREVIAÇÕES

ABO/ABO	flax oil and flax hulls infused in the abomasum
ABO/RUM	flax oil infused in the abomasum and flax hulls administered in the rumen
ADF	acid detergent fiber
BCS	body condition score
BW	body weight
CLA	conjugated linoleic acid
CP	crude protein
CON	ration without flax hulls
DIM	days in milk
DM	dry matter
DMI	dry matter intake
ED	enterodiol
EE	ether extract
EL	enterolactone
FA	fatty acids
FCM	Fat-corrected milk
FHU	ration with flax hulls
LA	linoleic acid
LCFA	long-chain fatty acids
LNA	alpha-linolenic acid
MCFA	medium-chain fatty acids
MUFA	monounsaturated fatty acids
NDF	neutral detergent fiber

PUFA	polyunsaturated fatty acids
RUM/ABO	flax oil administered in the rumen and flax hulls infused in the abomasum
RUM/RUM	flax oil and hulls administered in the rumen
SCFA	short-chain fatty acids
SDG	secoisolariciresinol diglicoside
SECO	secoisolariciresinol
SFA	saturated fatty acids
VFA	volatile fatty acids

RESUMO

Três estudos foram conduzidos para avaliar a transferência de lignanas (enterolactona; EL) e ácidos graxos poli-insaturados (PUFA) da dieta para o leite de vacas alimentadas com casca e/ou óleo de linhaça. Além disso, objetivou também determinar o papel do rúmen no metabolismo das lignanas. Em não ruminantes, as lignanas são metabolizadas pela microbiota intestinal porém não há informação do metabolismo de lignanas em ruminantes. O primeiro estudo avaliou o papel da microbiota ruminal e os efeitos do óleo de linhaça no metabolismo "in vivo" das lignanas da casca de linhaça e a concentração de EL nos fluidos biológicos. Utilizou-se quatro vacas da raça Holandesa canuladas no rúmen, com peso corporal médio de 575 kg e 92 dias de lactação, distribuídas em delineamento experimental em quadrado latino (4 × 4) com 4 períodos de 21 dias cada. Os tratamentos foram: 1) óleo de linhaça e casca de linhaça administrados no rúmen (RUM/RUM); 2) óleo de linhaça e casca de linhaça infundidos no abomaso (ABO/ABO); 3) óleo de linhaça administrado no rúmen e casca de linhaça infundida no abomaso (RUM/ABO); e 4) óleo de linhaça infundido no abomaso e casca de linhaça administrada no rúmen (ABO/RUM). O local de administração do óleo de linhaça e da casca de linhaça não interferiu na concentração ruminal de EL. Concentrações de EL na urina, plasma e no leite foram mais elevados em vacas que receberam casca de linhaça no rúmen que àquelas que receberam casca de linhaça no abomaso. Sendo assim, observa-se neste estudo que a microbiota ruminal desempenha importante papel no metabolismo de lignanas da linhaça. O segundo estudo objetivou avaliar os efeitos do local de administração (rúmen e/ou abomaso) da casca e/ou do óleo de linhaça sobre a ingestão, digestibilidade total, produção e composição do leite, bem como a composição em ácidos graxos do leite de vacas. Os tratamentos foram: 1) óleo de linhaça e casca de linhaça administrados no rúmen (RUM/RUM); 2)

óleo de linhaça e casca de linhaça infundidos no abomaso (ABO/ABO); 3) óleo de linhaça administrado no rúmen e casca de linhaça infundida no abomaso (RUM/ABO); e 4) óleo de linhaça infundido no abomaso e casca de linhaça administrada no rúmen (ABO/RUM). A IMS foi diferente entre os tratamentos. A digestibilidade total da MS foi menor para os tratamentos RUM/RUM e RUM/ABO e, a digestibilidade total da FDN e FDA foram menores no tratamento RUM/RUM. A produção de leite corrigida para 4% de gordura diminuiu quando os produtos da linhaça foram adicionados ao rúmen, porém, a composição do leite foi similar entre os tratamentos. A concentração de ácidos graxos de cadeia curta (SCFA) no tratamento RUM/RUM reduziu 26% em relação aos demais tratamentos. O tratamento RUM/RUM apresentou a maior concentração em ácidos graxos octadecenoico. Concentração de *cis*9,*trans*11-CLA na gordura do leite foi maior quando houve administração de óleo de linhaça no rúmen que no abomaso. O tratamento RUM/RUM aumentou a razão n-6:n-3 na gordura do leite quando comparado com os demais tratamentos, a razão apresentou-se abaixo de 4:1, na qual é considerada ideal para beneficiar a saúde humana. O terceiro estudo objetivou avaliar a concentração de EL e PUFA no leite de vacas e avaliar o efeito da presença do óleo de linhaça no intestino delgado sobre o metabolismo de lignanas. Foram utilizadas seis vacas multíparas da raça Holandesa, com peso corporal médio de 650 kg e 95 dias de lactação, distribuídas em quadrado latino (6 × 6) em esquema fatorial 2 × 3. Os seis tratamentos consistiram de duas diferentes dietas (CON *vs* FHU) e três níveis de óleo de linhaça infundidos no abomaso (0, 250 e 500 g/d), com seis períodos de 21 dias. A ração FHU e crescente quantidade de óleo de linhaça infundido no abomaso diminuíram a IMS pelas vacas. A ração FHU aumentou a digestibilidade aparente da MS, PB e EE, provavelmente pela substituição parcial do grão de milho quebrado e do farelo de soja pela casca de linhaça. A ração FHU diminuiu a produção de leite, e, consequentemente a de proteína, gordura, sólidos totais e lactose do leite (kg/d). Vacas alimentadas com casca de linhaça na dieta apresentaram menor concentração de SCFA, MCFA, SFA e n-6, e maior concentração de LCFA, MUFA, PUFA e n-3 na gordura do leite, reduzindo desta forma, a razão n-6:n-3 na gordura do leite das vacas. Concentração de EL no plasma, leite e urina foram maiores em vacas alimentadas com a ração FHU. Óleo de linhaça infundido no abomaso não mostrou efeito na concentração de EL no leite de vacas.

Palavras-chave: lignanas, ômega-3, produção de leite, qualidade do leite

ABSTRACT

Three studies were conducted to evaluate the transfer of lignans (enterolactona; EL) and polyunsaturated fatty acids (PUFA) from the diet into milk of lactating dairy cows fed with flax oil and/or hulls. Moreover, another objective was to determine the role of rumen on lignan metabolism. In non-ruminants, lignans are metabolized by intestinal microbiota but there was no information on the metabolism of lignans in ruminants. The first study evaluated the role of ruminal microbiota and the effects of flax oil on *in vivo* metabolism of flax hull lignans and EL concentrations in biological fluids. Four ruminal cannulated Holstein cows with 575 kg of BW and 92 DIM were used in a 4 × 4 Latin square design with four 21-d periods. Treatments were: 1) flax oil and flax hulls administered in the rumen (RUM/RUM); 2) flax oil and flax hulls infused in the abomasum (ABO/ABO); 3) flax oil administered in the rumen and flax hulls infused in the abomasum (RUM/ABO); and 4) flax oil infused in the abomasum and flax hulls administered in the rumen (ABO/RUM). The site of administration of flax oil and flax hulls did not affect ruminal concentrations of EL. Concentrations of EL in urine, plasma and milk were higher in cows that received flax hulls in the rumen compared to those that received flax hulls in the abomasum. Therefore, this study showed that ruminal microbiota plays an important role in flax lignan metabolism. The second study was conducted to evaluate the site of administration (rumen and/or abomasum) of flax hulls and/or oil on ingestion, total digestibility, milk yield and composition, and milk fatty acid profile of dairy cows. Treatments were: 1) flax oil and flax hulls administered in the rumen (RUM/RUM); 2) flax oil and flax hulls infused in the abomasum (ABO/ABO); 3) flax oil administered in the rumen and flax hulls infused in the abomasum (RUM/ABO); and 4) flax oil infused in the abomasum and flax hulls administered in the rumen (ABO/RUM). Intake of DM was different among treatments.

Total digestibility of DM was lower for RUM/RUM and RUM/ABO treatments, and NDF and ADF digestibilities were lower on the RUM/RUM treatment. Yield of 4% FCM decreased when flax products were administered in the rumen but milk composition was similar among treatments. Concentration of SCFA in milk fat on RUM/RUM treatment was reduced by 26 % when compared to other treatments. The RUM/RUM treatment resulted in the highest concentration of octadecenoic acid in milk fat. Proportion of *cis*9,*trans*11-CLA in milk fat was higher with ruminal than abomasal administration of flax oil. Although RUM/RUM treatment increased the n-6:n-3 FA ratio in milk fat compared to other treatments, ratios were lower than 4:1, which is ideal for human health. The third study was performed to evaluate the effects of feeding flax hulls and increased amount of flax oil infused in the abomasum on EL and PUFA concentration in milk of cows and to evaluate the effect of the presence of flax oil in the small intestine on lignan metabolism. Six multiparous Holstein cows averaging 650 kg of BW and 95 DIM were used in a 6 × 6 Latin square design with a 2 × 3 factorial arrangement of treatments. The six treatments were two different diets (CON *vs* FHU) and three levels of flax oil infused in the abomasum (0, 250 and 500 g/d), with six 21-d experimental periods. Feeding the FHU ration and with the highest amount of flax oil infused in the abomasum decreased DMI of cows. Feeding the FHU ration increased apparent digestibility of DM, CP and EE, probably as a result of partial replacement of cracked corn grain and soybean meal by flax hulls. Feeding the FHU ration decreased milk yield, and, subsequently yields of protein, fat, total solids and lactose (kg/d). Cows fed FHU ration had lower concentrations of SCFA, MCFA, SFA and n-6, and higher concentrations of LCFA, MUFA, PUFA and n-3 in milk fat, which resulted in a lower, n-6:n-3 FA ratio in milk fat of cows. Concentrations of EL in plasma, milk and urine were higher for cows fed the FHU ration. Amount of flax oil infused in the abomasum had no effect on EL concentration in milk of cows.

Keywords: lignans, milk quality, milk yield, omega-3

INTRODUÇÃO

Nos últimos 10.000 anos, desde as Revoluções da Agricultura e da Indústria, alterações gigantescas ocorreram na dieta humana e dos animais. Alimentos (carne magra, peixe, folhas verdes de vegetais, frutas, nozes, grãos e mel) utilizados pelos nossos ancestrais (pré-agrícolas) foram os que modelaram as exigências genético-nutricionais do homem. A grande utilização de grãos de cereais (ricos em carboidratos e ácidos graxos da série n-6, e, pobres em gorduras da série n-3) na nutrição humana e de animais domésticos (origem de carne, leite e ovos), devido ao crescimento da produção agrícola e do agronegócio, causou desequilíbrio na razão ômega-6/ômega-3 (n-6/n-3) que em associação com o estresse e o sedentarismo, provavelmente é um dos fatores pelo aparecimento de doenças cardiovasculares, câncer, obesidade e hipertensão nos dias atuais (Simopoulos, 2002).

A ingestão de gordura animal saturada, particularmente os ácidos graxos mirístico (14:0) e palmítico (16:0), e a associação com problemas de saúde humana, vem despertando o interesse em estudos dos hábitos alimentares das populações, e também aos efeitos benéficos ou maléficos de determinados alimentos. Sendo assim, a indústria alimentícia apresenta grande interesse em promover o desenvolvimento de alimentos enriquecidos com nutrientes essenciais, os quais, em condições alimentares modernas, estão em déficit na mesa do consumidor. Evidentemente, este interesse da indústria visa favorecer lucros, no entanto, o potencial efeito benéfico desses alimentos enriquecidos em questões de saúde pública, não deve ser negligenciado.

De acordo com legislações de vários países, o alimento funcional pode ser definido como, o alimento elaborado que além de nutrir e fornecer energia possui substâncias capazes de promover uma melhora nas condições de saúde e bem-estar, prevenindo o aparecimento precoce de doenças degenerativas. Segundo Borges (2001),

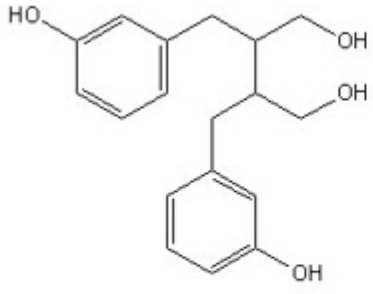
o alimento funcional deve fazer parte da alimentação usual e proporcionar efeitos positivos, obtido com quantidades não tóxicas e que não se destine a tratar ou curar doenças, estando seu papel ligado à redução do risco em contraí-las.

A semente de linhaça (*Linum usitatisimum* L.) tem sido o foco de estudos de vários pesquisadores devido à presença de grande quantidade de ácido alfa-linolênico (LNA), um ácido graxo poli-insaturado (PUFA) que representa aproximadamente 59% do total de ácidos graxos desta semente. A utilização da linhaça na nutrição humana está associada à redução da incidência de diversas doenças, como as cardiovasculares, osteoporose, hipercolesterolemia, sintomas da pós-menopausa e cânceres (Prasad, 1997; Murkies et al., 1998; Thompson e Ward, 2002; Adlercreutz, 2007). Alguns destes efeitos benéficos podem ser parcialmente mediados pelas lignanas presente na linhaça, principalmente na casca da semente (Adlercreutz e Mazur, 1997).

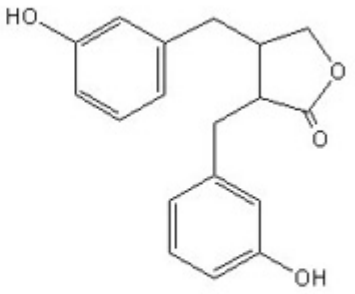
As lignanas pertencem ao grupo dos fitoestrógenos, assim como as isoflavonas e coumestanas, possui uma gama de atividade biológica, incluindo a antioxidante, propriedade fracamente estrogênica e antiestrogênica, e de inibir enzimas relacionadas ao metabolismo de hormônios sexuais (Martin et al., 1996; Kitts et al., 1999).

As lignanas vegetais secoisolariciresinol diglicosídeo (SDG) e matairesinol (MAT) são as mais abundantes em alimentos como grãos de cereais, oleagionosas, legumes e vegetais (Mazur, 1998). A SDG representa mais de 95% do total de lignanas encontradas na semente de linhaça. A SDG, MAT e seus glicosídeos são convertidos em lignanas de mamíferos através da fermentação bacteriana no trato intestinal (Setchell et al., 1980; Stitch et al., 1980; Setchell et al., 1981).

As duas lignanas de mamíferos mais importantes foram identificadas como *trans*-2,3-bis (3-hidroxi-benzil)-γ-butirolactona (enterolactona, EL) e 2,3-bis (3-hidroxibenzil) butano-1,4-diol (enterodiol, ED), sendo as mais abundantes no sangue, urina, bile e fluído seminal de humanos e animais (Setchell et al., 1980; Setchell et al., 1981). Pelo fato desses dois compostos serem produzidos unicamente em animais, utiliza-se o termo enterolignanas para diferenciá-las de seus precursores vegetais, as lignanas SDG e MAT. As enterolignanas (Figura 1) diferem das lignanas por possuir o grupo hidróxi-fenólico apenas na posição *meta* do anel aromático.



Enterodiol



Enterolactona

Figura 1. Estrutura química das lignanas de mamíferos, enterodiol e enterolactona.

Desde sua identificação, a EL recebeu crescente atenção devido as suas propriedades biológicas (estrogênicas) e interação farmacológica com várias enzimas e proteínas. Devido ao seu efeito inibitório de enzimas envolvidas no metabolismo de hormônios sexuais, a EL provavelmente reduz os níveis plasmáticos de estradiol e testosterona, afetando desta forma, o desenvolvimento de doenças hormônio-dependentes (Adlercreutz et al., 1993), como câncer de mama (Mousavi e Adlercreutz, 1992) e proliferação de células tumorígenas do cólon (Serraino e Thompson, 1992; Jenab e Thompson, 1996). As maiores concentrações de fitoestrógenos no plasma e urina foram encontradas em pessoas que habitavam regiões com baixa incidência de câncer e doenças coronarianas e, os menores valores foram encontrados em pacientes com câncer de mama ou em mulheres com alto risco de adquirir este tipo de câncer (Adlercreutz e Mazur, 1997).

A produção de EL através de sua precursora SDG (Figura 2) ocorre no trato intestinal e depende da presença da microbiota intestinal (Setchell et al., 1981). Em animais não-ruminantes, a SDG é convertida a secoisolariciresinol (SECO) através da ação de glicosidases do intestino, e posteriormente, a microbiota do cólon converte SECO para enterolignanas, ED e EL (Setchell et al., 1980; Saarinen et al., 2002). As enterolignanas são absorvidas pelo intestino e, através da ação de enzimas específicas, são conjugadas em sulfatos e glicuronídeos na parede intestinal ou no fígado (Barnes et al., 1996; Jansen et al., 2005). Esses compostos conjugados são excretados nos fluídos fisiológicos (plasma e urina) ou retornam para o lúmen intestinal via circulação

enterohepática (Setchell e Adlercreutz, 1988; Hoikkala et al., 2003). As formas conjugadas de enterolignanas são fracamente absorvidas pelo intestino devido a sua característica hidrofóbica. As enterolignanas são resistentes à enzimas celulares mas não às enzimas microbianas como a beta-glicuronidase, considerada importante para ótima absorção das enterolignanas (Jenab e Thompson, 1996; Raffaelli et al., 2002). Variações na microflora intestinal são responsáveis pela variação interindividual no metabolismo de SDG (Raffaelli et al., 2002). Além disso, ratos alimentados com EL pura excretaram cinco vezes mais EL na urina que ratos alimentados com lignana vegetal (Saarinen et al., 2002), assim, sugere-se que a absorção intestinal de enterolignanas é maior que de lignana vegetal. Segundo Saarinen et al. (2002), a EL desconjugada é absorvida passivamente ao longo do intestino enquanto que a lignana vegetal deve, primeiramente, ser convertida a EL pelos microrganismos do cólon intestinal antes da absorção. Sendo assim, o enriquecimento do leite com EL, alimento consumido diariamente e por um grande número de pessoas, seria uma interessante estratégia para popularizar os efeitos do aporte de enterolignanas na saúde humana.

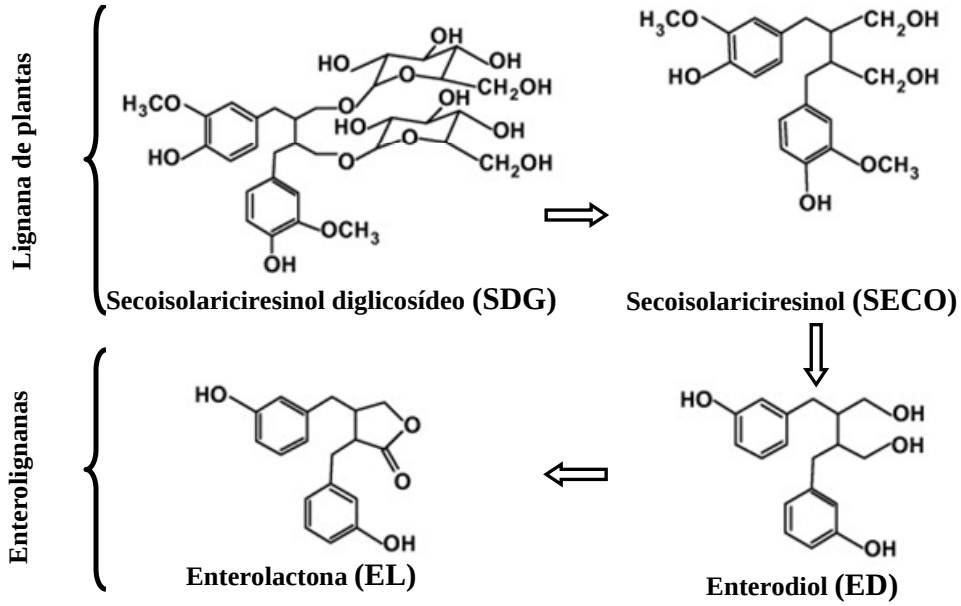


Figura 2. Estrutura química da lignana vegetal SDG e seus metabólitos.
Fonte: Côrtes et al. (2008)

Alguns estudos têm confirmado a presença de compostos polifenólicos como equol, daidzeína, genisteína (Bannwart et al., 1988; King et al., 1988) e EL (Antignac et

al., 2004) no leite de vacas leiteiras. Vacas alimentadas com torta de linhaça foram capazes de aumentar linearmente a concentração de EL no leite, porém, a enterolignana ED não foi detectada (Petit et al., 2009). Em não ruminantes, a fermentação intestinal desempenha importante papel na conversão de lignana vegetal à enterolignanas. Recentes estudos "in vitro" mostraram que a principal enterolignana produzida a partir da casca de linhaça (alimento rico em SDG) pela microbiota ruminal em vacas leiteiras foi a EL, enquanto que a microbiota fecal permitiu principalmente a produção de ED (Côrtes et al., 2008). Como apenas a EL é encontrada no leite, os micro-organismos ruminais parecem ter efeito decisivo na concentração de EL no leite, uma vez que a microbiota intestinal de vacas leiteiras produziu essencialmente ED no estudo "in vitro" de Côrtes et al. (2008). Em estudo "in vivo" com microbiota ruminal de cabras, observou-se a conversão de SDG para ED e EL, porém a EL foi a principal enterolignana presente no fluido ruminal e no plasma (Zhou et al., 2009).

A semente de linhaça contém 40% de óleo (Mustafa et al., 2003; Petit, 2003) e a casca de linhaça contém em torno de 28% de óleo. Sabe-se que o excesso de lipídios na dieta de ruminantes pode alterar a fermentação ruminal, causando diminuição de até 50% na fermentação ruminal de carboidratos estruturais quando 10% de gordura é adicionada à dieta (Ikwuegbu e Sutton, 1982; Jenkins e Palmquist, 1984). A interferência da gordura na fermentação ruminal é principalmente atribuída à formação de uma camada lipídica sobre as partículas de alimentos no rúmen, a qual prejudicará o contato físico das bactérias ruminais e consequentemente a digestão da celulose (Jenkins, 1993). A via metabólica ruminal dos lipídios desempenha um papel protetor aos microrganismos ruminais contra os efeitos tóxicos dos ácidos graxos insaturados, no qual por meio do processo de bio-hidrogenação, os ácidos graxos insaturados são transformados em saturados (Jenkins, 1993). Desta forma, a fermentação ruminal pode ser prejudicada pela ação antimicrobiana dos ácidos graxos insaturados.

Portanto, conhecimentos gerados em estudos "in vivo" sobre a importância do rúmen e dos ácidos graxos poli-insaturados no metabolismo de lignanas provenientes da semente linhaça, poderão ser uma ferramenta eficaz na otimização da transferência de EL e ácidos graxos poli-insaturados para o leite. A aplicação de tais conhecimentos proporcionaria um avanço no desenvolvimento de um leite com propriedades nutricionais capazes de melhorar a saúde do consumidor.

LITERATURA CITADA

Adlercreutz H., C. Bannwart, K. Wähälä, T. Mäkelä, G. Brunow, T. Hase, P.J. Arosemena, J.T. Kellis Jr., L.E. Vickery, 1993. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J. Steroid Biochem. Mol. Biol. 44:147-153.

Aldercreutz, H. and W. Mazur. 1997. Phyto-oestrogens and western diseases. Ann. Med. 29:95-120.

Adlercreutz, H. 2007. Lignans and human health. Crit. Rev. Clin. Lab. Sci. 44:483-525.

Antignac, J. P., R. Cariou, B. Le Bizec, and F. André. 2004. New data regarding phytoestrogens content in bovine milk. Food. Chem. 87:275-281.

Bannwart, C., H. Adlercreutz, K. Wähälä, T. Kotiaho, A. Hesso, G. Brunow, and T. Hase. 1988. Identification of the phyto-oestrogen 3',7-dihydroxyisoflavan, an isomer of equol, in human urine and cow's milk. Biomed. Environ. Mass Spectrom. 17:1-6.

Barnes, S., J. Sfakianos, L. Coward, and M. Kirk. 1996. Soy isoflavonoids and cancer prevention. Adv. Exp. Med. Biol. 401:87–100.

Borges, V. C. 2001. Alimentos funcionais: prebióticos, probióticos, fitoquímicos e simbióticos. In: Waitzberg DL. Nutrição Enteral e Parenteral na Prática Clínica. São Paulo: Atheneu.

Côrtes, C., N. Gagnon, C. Benchaar, D. da Silva, G. T. D. Santos, and H. V. Petit. 2008. In vitro metabolism of flax lignans by ruminal and faecal microbiota of dairy cows. J. Appl. Microbiol. 105:1585-1594.

Hoikkala, A. A., E. Schiavoni, and K. Wahala. 2003. Analysis of phyto-oestrogens in biological matrices. Br. J. Nutr. 89 Suppl 1:S5-18.

Ikwuegbu, O. A. and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr. 48:365–375.

Jansen, G. H. E., I. C. W. Arts, M. W. F. Nielen, M. Muller, P. C. H. Hollman, and J. Keijer. 2005. Uptake and metabolism of enterolactone and enterodiol by human colon epithelial cells. Arch. Biochem. Biophysics. 435:74-82.

Jenab, M. and L. U. Thompson. 1996. The influence of flaxseed and lignans on colon carcinogenesis and β-glucuronidase activity. Carcinogenesis. 17:1343-1348.

Jenkins, T. C. and D. L. Palmquist. 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. J. Dairy Sci., 67:978-986.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851-3863.

King, R. A., M. M. Mano, and R. J. Head. 1998. Assessment of isoflavonoid concentrations in Australian bovine milk samples. J. Dairy Res. 65:479-489.

Kitts, D. D., Y. V. Yuan, A. N. Wijewickreme, and L. U. Thompson. 1999. Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. Mol. Cell Biochem. 202:91-100.

Martin, M. E., M. Haourigui, C. Pelissero, C. Benassayag, and E. A. Nunez. 1996. Interactions between phytoestrogens and human sex steroid binding protein. Life Sci. 58:429-436.

Mazur, W. 1998. Phytoestrogen content in foods. Baillieres Clin. Endocrinol. Metab. 12:729–742.

Mousavi Y., and H. Adlercreutz, 1992. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. J. Steroid. Biochem. Mol. Biol. 41:615-619.

Murkies, A. L., G. Wilcox and S. R. Davis. 1998. Phytoestrogens. J. Clin. Endocrinol. Metab. 83:297-303.

Mustafa, A. F., P. Y. Chouinard, and D. A. Christensen. 2003. Effects of feeding micronised flaxseed on yield and composition of milk from Holstein cows. J. Sci. Food Agric. 83:920–926.

Petit, H. V. 2003. Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. J. Dairy Sci. 86:2637-2646.

Petit, H. V., N. Gagnon, P. Mir, R. Cao, and S. Cui. 2009. Milk concentration of the mammalian lignan enterolactone, milk production, milk fatty acid profile, and digestibility of dairy cows fed diets containing whole flaxseed or flaxseed meal. J. Dairy Res. doi:10.1017/S0022029909003999.

Prasad, K. 1997. Dietary flax seed in prevention of hypercholesterolemic atherosclerosis. Atherosclerosis. 132:69-76.

Raffaelli, B., A. Hoikkala, E. Leppälä, and K. Wähälä. 2002. Enterolignans. J. Chromatogr. B. 777:29-43.

Saarinen, N. M., A. Smeds, S. I. Mäkelä, J. Ämmälä, K. Hakala, J. M. Pihlava, E. L. Ryhänen, R. Sjöholm, and R. Santti. 2002. Structural determinants of plant lignans for the formation of enterolactone in vivo. J. Chromatogr. B. 777:311–319.

Serraino M., and L.U. Thompson, 1992. Flaxseed supplementation and early markers of colon carcinogenesis. Cancer Lett. 63:159-165.

Setchell, K. D. R. and H. Adlercreutz. 1988. Mammalian lignans and phytoestrogens. In The role of the gut flora in toxicity and cancer, pp.15-346. [I Rowland, editor]. London: Academic Press.

Setchell, K. D., A. M. Lawson, F. L. Mitchell, H. Adlercreutz, D. N. Kirk, and M. Axelson. 1980. Lignans in man and in animal species. Nature. 287:740–742.

Setchell, K. D. R., A. M. Lawson, S. P. Harkness, H. Gordon, D. M. L. Morgan, D. N. Kirk, H. Adlercreutz, L. C. Anderson, M. Axelson. 1981. Lignan formation in man – microbial involvement and possible roles in relation to cancer. The Lancet. July 4:4-7.

Simopoulos, A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 56:365-379.

Stitch, S. R., J. K. Toumba, M. B. Groen, C. W. Funke, J. Leemhuis, J. Vink, G. F. Woods. 1980. Excretion, isolation and structure of a new phenolic constituent of female urine. Nature. 287:738-740.

Thompson, L. U. and W. E. Ward. 2002. Flaxseed lignans: health benefits, bioavailability, and safety. In Phytoestrogens and Health. pp. 405-426 [S Gilani and J Anderson, editors], Champaign, IL: AOCS Press.

Zhou, W., G. Wang, Z. Han, W. Yao, and W. Zhu. 2009. Metabolism of flaxseed lignans in the rumen and its impact on ruminal metabolism and flora. Anim. Feed Sci. Technol. 150:18-26.

OBJETIVOS GERAIS

Os objetivos do trabalho foram avaliar as concentrações de enterolactona e ácidos graxos poli-insaturados no leite de vacas recebendo casca e óleo de linhaça em dois locais do trato gastrintestinal (rúmen e/ou abomaso); avaliar o impacto de três níveis de óleo de linhaça infundido no abomaso sobre a concentração de enterolactona no leite de vacas alimentadas com casca de linhaça, bem como, avaliar a ingestão de matéria seca, a digestibilidade das dietas e a produção e composição do leite de vacas recebendo casca e óleo de linhaça.

CAPÍTULO I

(Normas: British Journal of Nutrition; doi:10.1017/S0007114509344104)

RUMINAL METABOLISM OF FLAX LIGNANS BY COWS

Ruminal metabolism of flaxseed (*Linum usitatissimum*) lignans to the mammalian lignan enterolactone and its concentration in ruminal fluid, plasma, urine, and milk of dairy cows

Abbreviations: ABO/ABO, oil and hulls administered in the abomasum; ABO/RUM, oil infused in the abomasum and hulls administered in the rumen; ED, enterodiol; EL, enterolactone; RUM/ABO, oil administered in the rumen and hulls administered in the abomasum; RUM/RUM, oil and hulls administered in the rumen; SDG, secoisolariciresinol diglucoside; SECO, secoisolariciresinol; VFA, volatile fatty acids.

Abstract

Secoisolariciresinol diglucoside is the main flax lignan that is converted to mammalian lignans enterodiol (ED) and enterolactone (EL) by gastrointestinal microbiota. The objectives of the present study were to investigate the role of ruminal microbiota and the effects of flax oil on *in vivo* metabolism of flax lignans and concentration of EL in biological fluids. Four rumen-cannulated dairy cows were used in a 4 × 4 Latin square design. There were four periods of 21 d each and four treatments utilizing flax hulls (1800 g/d) and oil (400 g/d) supplements. The treatments were: 1) oil and hulls administered in the rumen and abomasal infusion of water; 2) oil and hulls administered in the abomasum; 3) oil infused in the abomasum and hulls administered in the rumen; and 4) oil administered in the rumen and hulls administered in the abomasum. Samples were collected during the last week of each period and subjected to chemical analysis. The site of supplementation of oil and hulls had no effect on ruminal EL concentration. Supplementing flax oil in the rumen and the abomasum led to similar EL concentrations in urine, plasma, and milk. Concentrations of EL were higher in urine, plasma, and milk of cows supplemented with hulls in the rumen than in those administered with hulls in the

abomasum. The present study demonstrated that ruminal microbiota plays an important role in metabolism of flax lignans.

Key words: lignan, antioxidant, flaxseed

Introduction

Flaxseed (*Linum usitatissimum*) is an excellent source of polyunsaturated oil in the form of α-linolenic acid and is the richest source of plant lignans[1]. Lignans are polyphenolic compounds with a range of biological activities, including antioxidant, antitumor, weakly estrogenic and antiestrogenic properties, and they also inhibit enzymes involved in the metabolism of sex hormones [2,3]. Greater intakes of flaxseed by human have been linked to potential health benefits, especially in the prevention of cardiovascular diseases, hypercholesterolemia, menopausal symptoms, breast and prostate cancers [4-6]. The study of Saarinen *et al*.[7] on the distribution of absorbed lignans in rat models fed ^{3}H-SDG showed the presence of lignans in liver, kidney, breast tumors, spleen, skin, lung, brain, uterus and adipose tissues. This indicates that lignans may have local effects in tissues such as tumor tissue that may contribute to human breast cancer prevention.

Secoisolariciresinol diglucoside (SDG) represents over 95 % of the total lignans in flaxseed[8]. Flax lignans are concentrated in the outer fibre-containing layers[9], thus resulting in higher concentration of SDG in hulls than seeds[10]. In non-ruminant animals, SDG is converted into secoisolariciresinol (SECO) under the action of intestinal glycosidases and the colonic microbiota convert SECO to mammalian lignans, enterodiol (ED) and enterolactone (EL)[11,12]. Flaxseed also contains small quantities of the plant lignan matairesinol, which is also converted by the colonic microbiota to EL[13]. Mammalian lignans are absorbed by the intestine and under the action of specific enzymes, they are conjugated as sulphate and glucuronide in the intestinal wall and liver[14,15]. They are excreted in physiological fluids (e.g. plasma and urine) or returned to the intestinal lumen via enterohepatic circulation[16,17]. The conjugated forms of mammalian lignans are poorly absorbed by the intestine; deconjugation increases the hydrophobicity of lignans and allows their reabsorption. The mammalian lignans are more resistant to cellular enzymes but are readily cleaved by microbial enzymes such as β-glucuronidase. This enzyme is important for optimal absorption of mammalian

lignans[18,19]. Variation in intestinal microflora plays an important role in the inter-individual variation metabolism of SDG[19]. Moreover, there is a fivefold increase in urine excretion of EL in rats fed pure EL compared with those fed plant lignans[11], thus suggesting that absorption of mammalian lignans is higher than that of plant lignans. According to Saarinen *et al.*[11], deconjugated EL may be passively absorbed along the intestine while plant lignans must first be converted by microbes to EL in the colon before being absorbed. Therefore, milk with elevated EL content may be interesting as a dietary source of EL and an excellent strategy to optimize the effects of lignans on human health.

Different studies have confirmed the presence of polyphenolic compounds such as equol, daidzein, and genistein[20,21] and mammalian lignan EL[22] in the milk of dairy cows. Feeding flax meal to dairy cows resulted in a linear increase of EL concentration in milk but ED was not detected[23]. In non-ruminants animal, intestinal fermentation plays an important role in the metabolism of plant lignans to mammalian lignans, ED and EL. Recent *in vitro* results have shown that the main mammalian lignan metabolite produced from flax hulls by ruminal microbiota of dairy cows was EL, while fecal microbiota led mainly to the net production of ED[10]. Other *in vivo* results have reported that ruminal microbiota of goats convert SDG to ED and EL, and that EL is the main lignan metabolite present in ruminal fluid and plasma[24]. However, feeding PUFA is known to modify ruminal microbiota[25,26], which could interfere with the ruminal metabolism of flax lignans, affecting the concentration of its metabolites in biological fluids. Therefore, the objective of the present study was to investigate the importance of the site of administration (rumen and abomasum) of flax oil and flax hulls on the conversion of plant lignans into the mammalian lignan EL and the consequent concentration of EL in blood, ruminal fluid, urine, and milk of dairy cows. This was achieved by supplementing flax products (hulls and oil) in the rumen or the abomasum in four experimental treatments.

Materials and methods

Animals and experimental treatments

Four primiparous lactating Holstein cows fitted with ruminal cannulas (10 cm, Bar Diamond Inc., Parma, ID, USA) were used in a 4 × 4 Latin square design with four 21-d periods balanced for residual effect and four treatments. The cows averaged 92 (SE 12) days

in milk at the start of the experiment with an average body weight of 575 (SE 25) kg and an average body condition score of 3·00 (SE 0·15; 5-point scale)[27]. No antibiotics were given for at least 16 weeks before initiation of the experiment. The cows were kept in individual stalls and had free access to water. Cows were milked twice a day at 08.30 and 20.00 hours. All cows were fed for ad libitum intake (10 % refusals on as fed basis) twice a day (08.30 and 14.30 hours). The diet (Table 1) was formulated to meet requirements for cows that were 575 kg of body weight and producing 35 kg/d of milk with 3·8 % fat[28]. The experimental protocol complied with the *Guide to Care and Use of Experimental Animals*[29] and was approved by the local Animal Care Committee.

Table 1. Ingredient and chemical composition of the total mixed diet

Composition	Amount
Ingredient (g/kg DM)	
Grass silage	320·0
Corn silage	322·0
Cracked corn grain	119·0
Ground barley	77·0
Soya meal	102·0
Protein supplement*	28·0
Calcium carbonate	7·0
Mineral†	25·0
Chemical	
Dry matter (g per 100 g diet)	41·8
Crude protein (g per 100 g DM)	16·0
Neutral detergent fibre (g per 100 g DM)	29·7
Acid detergent fibre (g per 100 g DM)	20·4
Total lipids (g per 100 g DM)	2·33
NE_L (Mj/kg DM)‡	6·61

*Contained 20% of canola meal, 30% of corn gluten meal, 20% of soybean meal, and 30% of brewer's corn.

†The premix contained (per kg diet): 2.3 g Ca, 1.2 g P, 1.2 g Mg, 0.4 g S, 3.5 g Na, 0.4 g K, 51.7 mg Fe, 68 mg Zn, 11.2 mg Cu, 45.4 mg Mn, 1.7 mg I, 0.2 mg Co, 0.5 mg Se, 11,300 IU of vitamin A, 1,450 IU of vitamin D_3 and 67,300 IU of vitamin E.

‡Calculated using published values of feed ingredients (NRC, 2001).

The four experimental treatments were: 1) oil and hulls administered in the abomasum (ABO/ABO); 2) oil administered in the rumen and hulls administered in the abomasum (RUM/ABO); 3) oil and hulls administered in the rumen and abomasal infusion of water

(RUM/RUM); and 4) oil infused in the abomasum and hulls administered in the rumen (ABO/RUM). Abomasal infusions consisted of 20 kg of tap water (RUM/RUM treatment), 400 g of flax oil + 20 kg of tap water (ABO/RUM treatment) or 1800 g of flax hulls suspended in 18·2 kg of tap water (ABO/ABO and RUM/ABO treatments). Flax oil (Brenntag Canada Inc., Montreal, QC, Canada) contained, expressed as a percentage of total fatty acids, 5·3% of 16:0, 3·8% of C18:0, 18·4% of C18:1*cis*-9, 0·8% of C18:1*cis*-11, 15·8% of C18:2*cis*-9,*cis*-12, 52·7% of C18:3*cis*-9,*cis*-12,*cis*-15, and 3·2% others. Flax hulls (Natunola Health Inc; Nepean, ON, Canada) contained, expressed as a percentage of dry matter, 23·5% of crude protein, 19·4% of neutral detergent fibre, 14·3% of acid detergent fibre, 29·8% of total lipids, and 0·99% of SDG, and 6·86 kJ of NE_L per gram of dry matter. During the first seven days of each 21-d period, only 30% of the experimental dose of oil and hulls were administered in the abomasum over a 7-h period. From d 8 to d 21, infusion in the abomasum was conducted with 100% of the experimental dose of oil and hulls over a 23-h period. Administration in the rumen was done by adding one third each of oil and hulls three times daily (09.30, 14.30, and 21.30 hours) during all the experiment. Samples of the diet and of flax hulls were taken daily from d 14 to d 21 and pooled within period. All samples were frozen at -20^{o}C for subsequent drying at 55^{o}C and analyzed according to the procedures used by Petit & Benchaar[30]. Infusates of flax products were prepared daily and the appropriate amount of infusate for each cow was weighed into tared buckets that were stirred continuously while being infused. To perform abomasal infusions, an infusion line was inserted through the rumen cannula and the sulcus omasi into the abomasum as described by Gressley *et al.*[31]. Placement of the infusion lines was monitored daily to ensure postruminal delivery. Suspensions were pumped into the abomasum by using peristaltic pumps (Masterflex L/S; Cole-Parmer Canada Inc., Montreal, QC, Canada). The daily amount of oil supplied by flax hulls was similar to that supplied by flax oil. Half of the lipids were provided by flax oil and the other half by flax hulls for a total amount of 800 g daily, thus resulting in a fat intake below 6 to 7% of the DM, which is known to have little effect on DM intake[28].

Sampling

Feed intake and milk yield were measured daily. Milk samples were taken twice daily from d 16 to d 21, pooled on a 6-d basis proportionally to the corresponding milk yield, and frozen at -20°C for lignan analysis. On d 20, blood was withdrawn into K_3EDTA-vacutainer tubes (Becton Dickinson and Cie, Rutherford, NJ, USA) from the jugular vein 6 h after the morning meal. Plasma samples were kept frozen at -20°C until lignan analysis. On d 21, a sample of urine was taken 2 h after the morning meal by hand stimulation of the perineal region and kept frozen at -20°C for lignan analysis. Also, ruminal contents were collected 0, 2, 4, and 6 h after the morning meal from different locations within the rumen (the anterior dorsal, anterior ventral, medium ventral, posterior dorsal, and posterior ventral locations) to obtain a representative sample. Ruminal pH was monitored immediately after sample collection with a portable pH meter (OAKTON; Eutech Instruments, Singapor). The ruminal contents were then strained through four layers of cheesecloth. A 350-ml sample of strained ruminal fluid was mixed with ruminal retentate (26 g) and frozen at -20°C until assay for ß-glucuronidase activity. One portion of filtered ruminal fluid was acidified to pH 2 with 50% H_2SO_4 and frozen at - 20°C for later determination of volatile fatty acids (VFA) and ammonia N concentrations. Concentrations of NH_3 and VFA in ruminal fluid were analyzed, respectively, with the indophenol-blue method[32] and a GLC Waters Alliance 2695 system (Milford, MA) fitted with an IR detector autosampler according to the procedure used by Petit & Benchaar[30]. Another portion of ruminal fluid was kept at -20°C and freeze-dried for further analysis of lignans. As previous results (Gagnon *et al.* unpublished results) have shown that there is no variation in ruminal lignan concentration after feeding, ruminal samples for the three postfeeding times (2, 4, and 6 h) were pooled within cow and period to obtain only one composite sample for lignan analysis. Moreover, fecal grab samples (250 g) were collected directly from the rectum 2, 4, 6, and 8 h postfeeding on the same day (d 21). Fecal pH was monitored immediately after sample collection. Fecal samples were pooled and kept frozen at -20°C for further ß-glucuronidase analysis.

Lignan extraction

Lignans in ruminal fluid, plasma, urine, and milk samples were hydrolysed and extracted according to the method of Frank & Custer[33] with some modifications. Freeze-dried samples of filtered ruminal fluid were resuspended in Milli-Q purified water (20 mg/ 0·5 ml) as decribed by Heinonen *et al.*[34]. Five hundred microliters of warmed (40°C) milk and resuspended ruminal samples (0 h and pooled postfeeding samples) were mixed with 5 µl of ß-glucuronidase/arylsulfatase from *Helix pomatia* (Roche-Diagnostics, Laval, QC, Canada) while 500 µl of plasma and urine were mixed with 500 µl of 0·1 mol/l sodium acetate buffer (pH 5) and 5 µl of ß-glucuronidase/arylsulfatase. Milk samples were incubated for 1·5 h while plasma, urine, and ruminal samples were incubated overnight at 37°C in a shaking waterbath. After hydrolysis, all samples were acidified with 10 µl of 6 N HCl. Acidified milk samples were washed with 3 ml of hexane before extraction to remove lipids[19]. All samples were extracted with 2 ml of diethyl ether. The samples were vortex-mixed twice for 2 min. Organic layer was separated by freezing. The remaining liquid phase was submitted to a second extraction under the same conditions. The organic layers were pooled and evaporated by vacuum (Speed-Vac; Thermo Savant, Holbrook, NY, USA) at room temperature for 40 min. The dry extract was redissolved in 500 µl of enzyme immunoassay (EIA) buffer and warmed at 37°C. Four serial dilutions were done in EIA buffer for EL analysis using an EIA kit (Cayman Chemical, Ann Arbor, MI, USA). The starting dilutions were 1:50, 1:200, 1:1600, and 1:60 000 for milk, plasma, ruminal fluid, and urine samples, respectively. The kit is a competitive assay that recognizes both enantiometric forms of EL and utilizes a standard curve ranging from 15.6 to 2 000 pg/mL. The assay exhibits a limit of quantification (defined as 80% B/B_0) of 70 pg/mL and an IC50 (50% B/B_0) of 240 pg/mL. The recoveries of EL ranged from 85 to 104%.

ß-glucuronidase activity

The determination of ß-glucuronidase activity was based on a modified method of Jenab & Thompson[18]. Briefly, fecal samples (5 g) were homogenized with a Polytron (Kinematica AG, Lucerne, Switzerland) in a final volume of 20 ml of cold KH_2PO_4 pH 6·8 for 15 sec while ruminal samples were homogenized using a stomacher (A. J. Seward & Co. Ltd, London, UK). Samples were then filtered through two layers of cheesecloth, sonicated on

ice (two bursts, 1 min; Sonics and Materials Inc., Danbury, CT, USA) and centrifuged at 10 000***g*** for 15 min at 4°C. The supernatant fraction was stored at -80°C until enzyme assay. Activity of the ß-glucuronidase was quantified by mixing 25 µl of extracted sample with 125 µl of 0·04 mol/l KH_2PO_4 (pH 6·8), 50 µl of 0·5 mmol/l EDTA, and 50 µl of 5 mmol/l phenolphthalein diglucuronide (Sigma-Aldrich, Oakville, ON, Canada). The assay was performed in quadruplicate with one duplicate used as a baseline and the other one was incubated at 37°C for 60 min. The reaction was stopped by adding 1·25 ml of 0·2 mol/l glycine buffer (pH 10·4) containing 0·2 mol/l NaCl. After 10 min of incubation at room temperature, 200 µl of each replicate was transferred in 96-well flat-button plate and the plate was read on a Spectra Max 250 ELISA reader (Molecular Devices, Sunnyvale, CA, USA) at 540 nm. The absorbance values directly correlate to the amount of the phenolphthalein released based on a phenolphthalein standard curve. The specific activity of ß-glucuronidase was calculated by the formula: (nmol phenolphthalein 60 min - nmol phenolphthalein 0 min)/(60 min * mg of protein). Protein was determined by bicinchoninic acid protein assay kit (Sigma-Aldrich) using bovine serum albumin as the standard.

Statistical analysis

All data were analyzed using the MIXED procedure of SAS (SAS 2000; SAS Institute, Cary, NC, USA) according to the model:

$Y_{ijk} = \mu + a_i + \beta_j + \tau_k + e_{ijk}$

where Y_{ijk} = response variable, μ = overall mean, a_i = random effect of cow *i*, β_j = effect of period *j*, τ_k = effect of treatment *k*, and e_{ijk} = residual error. The residual effect was initially included in the model but was removed because it was not significant. Data on EL concentrations were transformed (log) as previously performed by Hausner *et al.*[35] and Nesbitt *et al.*[36] but results in Table 2 are reported as the adjusted mean value (with confidence interval) on the original scale of measurements. The model for specific ß-glucuronidase activity and pH was augmented with time and time by treatment interaction for repeated measurements and values are reported with their adjusted mean values with standard errors. The two-sided level of significance was set at $P<0·05$, although probability values up to $P<0·1$ are shown in the text if the data suggest a trend. When a significant *F*-test was detected, multiple comparisons were done using a Tukey's adjustment for the

probability. A specific contrast was also used a posteriori to compare the site of administration of either oil or hulls.

Results

Oil supplementation and dry matter intake

The actual amounts of oil administered in the rumen and the abomasum averaged, respectively, 450 and 440 g per day. The total amount of oil provided by both flax products averaged 912 g per day and there was no difference (P=0·12) among treatments. Intake of dry matter differed (P=0·002) among diets and averaged, respectively, 13.3, 16.3, 15.4, and 13.8 kg per day for treatments RUM/RUM, ABO/ABO, RUM/ABO, and ABO/RUM. Total input of dry matter (intake of dry matter + flax products added in the rumen or the abomasum) was also different (P=0·02) among treatments with an average of 15.4, 18.5, 17.5, and 15.9 kg per day for treatments RUM/RUM, ABO/ABO, RUM/ABO, and ABO/RUM, respectively.

Concentration of EL in ruminal fluid

Treatment had no effect (P=0·26) on EL concentration of ruminal fluid collected before feeding (Table 2). Similarly, the site of administration of oil and hulls had no effect (P=0·18) on EL concentration of postfeeding pooled ruminal samples.

Concentration of EL in plasma

Concentration of EL in plasma was different (P=0·04) among treatments (Table 2). Supplementation of flax hulls in the rumen (ABO/RUM and RUM/RUM) resulted in higher (P=0·008) concentration of EL in the plasma than when hulls were supplemented in the abomasum (ABO/ABO and RUM/ABO). The site of oil infusion had no significant effect on plasma EL concentration.

Concentration of EL in urine

Concentration of EL in urine was different (P<0·0001) among treatments (Table 2). Cows supplemented with flax hulls in the rumen (RUM/RUM and ABO/RUM) had higher (P<0·0001) EL concentration in urine than those receiving hulls in the abomasum

(RUM/ABO and ABO/ABO). There was no difference (P=0·71) between RUM/RUM and ABO/RUM treatments. Moreover, there was no difference (P=0·18) in urine concentration of EL between cows on ABO/ABO and those on RUM/ABO treatments.

Concentration of EL in milk

Concentration of EL in milk differed (P=0·001) among treatments (Table 2). Milk concentration of EL was similar (P=0·44) for cows on ABO/RUM and RUM/RUM treatments and it was significantly higher (P=0·0002) than that of cows on RUM/ABO and ABO/ABO treatments. There was no difference (P=0·90) in milk EL concentration between cows on ABO/ABO and those on RUM/ABO.

Ruminal fermentation characteristics

There were an interaction (P=0·01) between sampling time and treatment for the acetate to propionate ratio and the acetate + butyrate to propionate ratio in the rumen (data not shown). Ammonia N concentration in the rumen was similar among treatments (Table 3). Concentration of total VFA in the rumen was higher (P=0·02) for cows on ABO/RUM and RUM/ABO treatments than for those on RUM/RUM treatment. Cows supplemented with oil in the rumen (RUM/RUM and RUM/ABO) had lower (P=0·05) concentration of total VFA in the rumen than those receiving oil in the abomasum (ABO/RUM and ABO/ABO). Cows on the ABO/ABO treatment had higher (P=0·02) and lower (P=0·004) proportions of acetate and propionate in the rumen, respectively, than those on RUM/RUM treatment. Treatment had no effect on proportions of butyrate (P=0·27), isobutyrate (P=0·18), and lactate (P=0·51) in the rumen.

Table 2. Concentration of enterolactone in biological fluids of Holstein cows receiving oil and hulls administered in the abomasum (ABO/ABO); oil administered in the rumen and hulls administered in the abomasum (RUM/ABO); oil and hulls administered in the rumen (RUM/RUM); and oil infused in the abomasum and hulls administered in the rumen (ABO/RUM)

	Treatment								
	ABO/ABO		RUM/ABO		RUM/RUM		ABO/RUM		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	*P*
Ruminal fluid (μmol/l)									
0h	15·1	(8·92, 25·5)	21·7	(12·9, 36·7)	28·3	(16·7, 47·8)	31·3	(18·5, 52·9)	0·26
Pool (2h, 4h, 6h)	13·3	(6·30, 28·2)	19·2	(9·07, 40·6)	21·5	(10·2, 45·5)	35·4	(16·7, 74·8)	0·17
Urine (μmol/l)	34·4[b]	(21·0, 56·3)	67·5[b]	(41·3, 111)	540[a]	(330, 884)	735[a]	(449, 1203)	<0·0001
Plasma (μmol/l)	0·50	(0·23, 1·07)	0·48	(0·03, 0·22)	1·59	(0·74, 3·41)	1·78	(0·89, 3·80)	0·04
Milk (μmol/l)	0·04[b]	(0·02, 0·81)	0·06[b]	(0·03, 0·10)	0·52[a]	(0·28, 0·96)	0·29[a]	(0·16, 0·54)	0·001

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).

Table 3. Ruminal fermentation characteristics of Holstein cows receiving oil and hulls administered in the abomasum (ABO/ABO); oil administered in the rumen and hulls administered in the abomasum (RUM/ABO); oil and hulls administered in the rumen (RUM/RUM); and oil infused in the abomasum and hulls administered in the rumen (ABO/RUM)

	Treatment					
	ABO/ABO	RUM/ABO	RUM/RUM	ABO/RUM	SEM	*P*
Ammonia-N (mmol/l)	8·51	7·92	8·34	10·1	1·16	0·60
Volatile fatty acids (mmol/l)	107[a,b]	110[a]	100[b]	113[a]	1·91	0·02
Molar proportions (mmol/mol)						
Acetate	644[a]	626[a,b]	590[b]	625[a,b]	8·97	0·02
Propionate	202[b]	214[b]	266[a]	231[a,b]	7·23	0·004
Butyrate	94·5	97·8	90·8	89·8	2·74	0·27
Isobutyrate	7·34	5·78	6·96	6·17	0·47	0·18
Valerate	11·0	9·68	12·0	9·89	0·46	0·07
Isovalerate	15·5	15·0	20·8	15·1	1·47	0·08
Lactate	25·8	31·6	13·3	22·6	8·22	0·51
Molar ratio						
Acetate:propionate[*]	32·2[a]	29·6[a]	22·9[b]	27·8[a]	0·93	0·002
(Acetate + butyrate):propionate[*]	37·0[a]	34·1[a,b]	26·4[c]	31·8[b]	1·04	0·002

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

[*] Interaction between treatment and hour ($P<0.05$).

Activity of ß-glucuronidase in ruminal fluid and feces

There was no interaction (P=0·22) between hour and treatment for specific ß-glucuronidase activity in ruminal fluid and feces. Therefore, only mean values for the 6-h ruminal sampling period are presented in Figure 1A. Cows on RUM/ABO treatment tended to have lower (P=0·06) ruminal ß-glucuronidase activity than those on ABO/RUM treatment. Infusing oil directly in the rumen (RUM/ABO and RUM/RUM) reduced (P=0·01) ruminal ß-glucuronidase activity compared with infusing oil in the abomasum (ABO/ABO and ABO/RUM). There was no difference (P=0·97) between ABO/ABO and ABO/RUM treatments indicating that ruminal ß-glucuronidase activity was not modulated by flax hulls supplementation in the rumen. Treatment had no effect (P=0·46) on fecal ß-glucuronidase activity (Figure 1B) although activity was on average 5 times higher (P=0·0001) in feces than in ruminal fluid (9·77 and 1·96 nmol phenolphthalein/min/mg protein, respectively).

Ruminal fluid pH

There was no significant interaction (P=0·43) between hour and treatment for ruminal fluid pH. There was a decrease (P<0·0001) in ruminal fluid pH after feeding for all treatments and the pH remained stable afterwards (Figure 2A). Cows on ABO/RUM treatment had lower (P=0·04) pH values than cows on ABO/ABO treatment. The ruminal fluid pH tended (P=0·07) to be lower when hulls were supplemented in the rumen (ABO/RUM and RUM/RUM) than when they were administered in the abomasum (ABO/ABO and RUM/ABO).

Fecal pH

There was no interaction (P=0·20) between hour and treatment and no effect (P=0·20) of sampling time for fecal pH (Figure 2B). However, fecal pH tended (P=0·07) to differ among treatments. Supplementation of flax hulls in the abomasum (ABO/ABO and RUM/ABO) resulted in lower (P=0·015) fecal pH than when hulls were supplemented in the rumen (ABO/RUM and RUM/RUM).

Fig. 1A

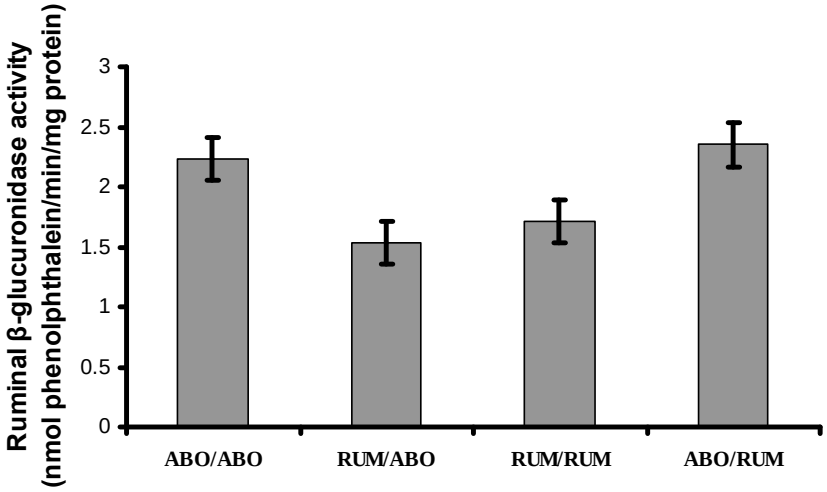


Fig. 1B

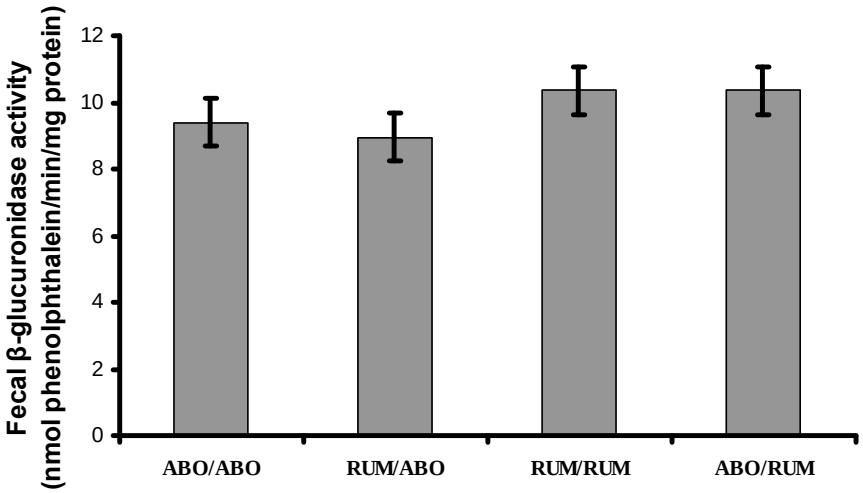


Fig. 1. Postfeeding activity of ß-glucuronidase in ruminal fluid (A) and feces (B) of dairy cows supplemented with flax hulls and flax oil. Treatments were: 1) oil and hulls administered in the abomasum (ABO/ABO); 2) oil administered in the rumen and hulls administered in the abomasum (RUM/ABO); 3) oil and hulls administered in the rumen (RUM/RUM); and 4) oil infused in the abomasum and hulls administered in the rumen (ABO/RUM). Data are the mean values with standard errors represented by vertical bars. There was an effect (P=0·01) of infusion site of oil on ruminal ß-glucuronidase activity.

Fig. 2A

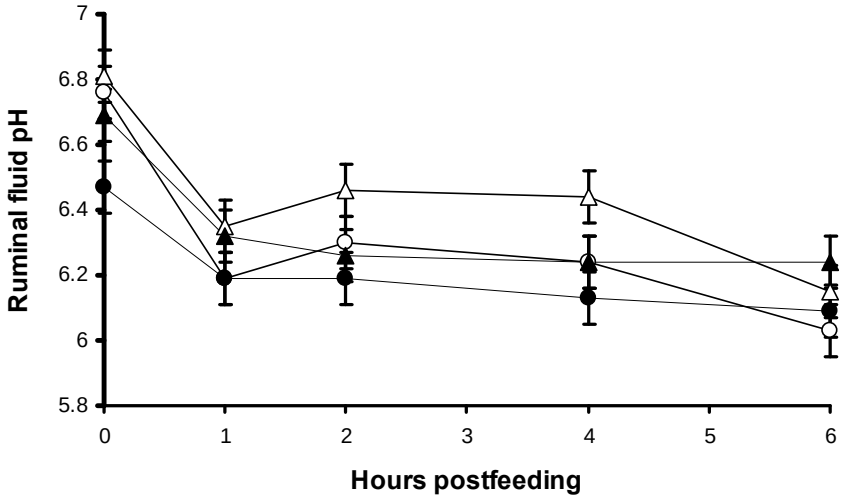


Fig. 2B

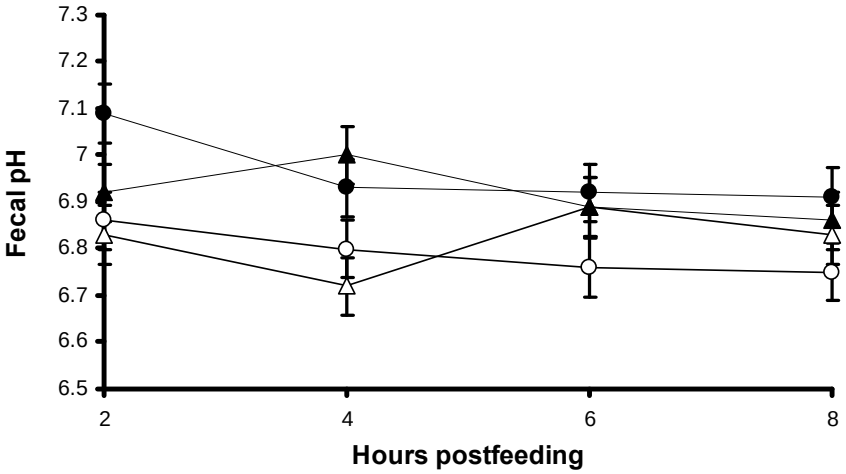


Fig. 2. Postfeeding pH in ruminal fluid (A) and feces (B) of dairy cows supplemented with flax hulls and flax oil. Treatments were: 1) oil and hulls administered in the abomasum (-Δ-, ABO/ABO); 2) oil administered in the rumen and hulls administered in the abomasum (-○-, RUM/ABO); 3) oil and hulls administered in the rumen (-▲-, RUM/RUM); and 4) oil infused in the abomasum and hulls administered in the rumen (-●-, ABO/RUM). Data are the mean values with standard errors represented by vertical bars. The effect of infusion site of flax hulls on ruminal pH tended (P=0·07) to be significant and this effect was significant for fecal pH.

Discussion

Many studies have reported that intestinal microorganisms play an important role in phytoestrogen metabolism[37,38]. In ruminants, fermentation processes occur first in the rumen as opposed to non-ruminant animals, where they take place in the colon. Previous studies with ruminant animals have shown that lignans are metabolized by both ruminal and fecal microbiota[10,24] and lignan metabolites are present in biological fluids such as plasma, semen, and milk[22,39,40]. To our knowledge, this is the first *in vivo* study investigating the importance of rumen microbiota in the conversion of flax lignans into the mammalian lignan EL and its concentration in physiological fluids of dairy cows.

According to our *in vitro* results[10], ruminal microbiota of dairy cows has the ability to metabolize lignans from flax products to mammalian lignan EL. Other dietary feed ingredients such as forages and cereals also contain lignans[41,42], which may lead to the production of EL and explain the presence of EL in ruminal fluid of cows on all present treatments. The levels of EL in ruminal fluid were not significantly different among treatments, but numerically higher values of EL were observed in ruminal fluid of cows supplemented with flax hulls in the rumen (ABO/RUM and RUM/RUM). Ruminal fluid pH tended to decrease when flax hulls were added in the rumen (ABO/RUM and RUM/RUM) compared to when they were administered in the abomasum (ABO/ABO and RUM/ABO). Similarly, infusing the plant lignan SDG into the rumen of goats led to increased ruminal EL concentration and decreased pH[24]. Ruminal fluid pH in the present experiment decreased within one hour of feeding likely as a result of the production of volatile fatty acids, which are the end products of ruminal fermentation following the supply of substrate[10]. Flax hulls are a rich source of fermentable carbohydrates, which may contribute to lower pH in ruminal fluid of cows supplemented with flax hulls in the rumen (ABO/RUM and RUM/RUM) as compared to the abomasum (ABO/ABO and RUM/ABO) due to greater availability of carbohydrates in the rumen[43]. However, the concentration of VFA was not affected by the site of oil infusion when flax hulls were administered in the abomasum. On the other hand, in the presence of flax hulls in the rumen, VFA concentration decreased with oil administered in the rumen as compared to the abomasum likely as a result of oil modulating microbial population. Moreover, flax hulls administered in the rumen may contribute to increase the proportion of propionate due

to the presence of highly fermentable products. Results from a recent *in vitro* study[26] reported that growth of ruminal bacteria can be affected by PUFA such as omega-3, although sensitivity differs among species.

In our study, SDG metabolism in the rumen was not affected by flax oil, which is rich in omega-3 fatty acids, as indicated by similar ruminal fluid EL concentrations among treatments. On the other hand, ruminal fluid activity of microbial β-glucuronidase was influenced by the presence of flax oil in the rumen as shown by lower activity for cows supplemented with flax oil in the rumen (RUM/ABO and RUM/RUM) than for those with flax oil bypassing the rumen (ABO/ABO and ABO/RUM). The activity of β-glucuronidase has been attributed to bacteria belonging to the dominant human intestinal microbiota, such as *Ruminococcus*, *Bacteroides*, *Bifidobacterium*, and *Eubacterium*[44]. Henderson[25] showed that the growth of some strains of important rumen bacteria such as *Butyrivibrio, Ruminococcus* and *Methanobrevibacter* is strongly inhibited by the presence of long-chain fatty acids. The data of the present study may indicate that species of ruminal microbiota with β-glucuronidase activity are more sensitive to the presence of PUFA than ruminal species involved in the conversion of plant SDG into the mammalian lignan EL. This may indicate that a diet rich in PUFA will not affect the conversion of SDG in EL in the rumen but will decrease β-glucuronidase activity. However, the importance of deconjugation in the rumen for further absorption of mammalian lignan is not well understood in ruminant animals.

In a study carried out with colonic microbiota from rats, Jenab & Thompson[18] observed a positive correlation between SDG dietary concentration and microbial β-glucuronidase activity. In goats, Zhou *et al.*[24] suggested that SDG supplementation stimulates the growth of *Ruminococcus gnavus*, which plays a role in glucuronidase activity of the rumen. In the present study, supplementation with plant lignans SDG had no effect on ruminal fluid and fecal β-glucuronidase activity of dairy cows. One explanation for the lack of an effect of flax hulls supplementation on β-glucuronidase activity in ruminal fluid and feces may be the small number of cows used in the present study. Microbiota plays an important role in β-glucuronidase activity and there is a large variation in microbiota composition among cows[45,46], suggesting that a greater number of cows is required to detect any difference in β-glucuronidase activity. Moreover, differences in

animal species (e.g. non-ruminant vs. ruminant animal) and plant lignan concentration used in experiments could explain discrepancies between results on the effect of flax hulls supplementation on β-glucuronidase activity.

Specific ß-glucuronidase activity was on average 5 times higher in feces than in ruminal fluid, indicating that deconjugation activity may be more important in the large intestine than in the rumen of dairy cows. This is the first time that both fecal and ruminal microbial β-glucuronidase activity were compared in ruminants. Further investigations on microbial β-glucuronidase activity along the gastrointestinal tract of cattle are required to better understand the reabsorption of deconjugated metabolites such as mammalian lignans in dairy cows to improve the understanding of their metabolic pathway in dairy cows, in order to enable targeted manipulation of their quantities in milk. Microbial ß-glucuronidase activity is important for the absorption of mammalian lignan in humans and this enzyme is inducible and positively correlated with the level of plant lignans and urinary excretion of mammalian lignans[18]. In the present experiment, milk EL concentration followed a profile similar to that of urinary EL excretion, which may suggest that microbial ß-glucuronidase activity can contribute to increase the amount of lignans in milk as a result of a greater absorption of mammalian lignans in the body.

Lower fecal pH for cows supplemented with flax hulls in the abomasum compared to that of cows supplemented with flax hulls in the rumen may result of greater amount of undigested plant lignans (dietary source of carbohydrates) reaching the colon and being fermented by intestinal microflora due to rumen bypass of flax hulls. Fermentation of carbohydrates in the colon results in the production of short chain volatile fatty acids that lower colonic pH and serve as an energy source for the colonocytes[43].

After absorption by the intestine, conjugated mammalian lignans return to the intestinal lumen via enterohepatic circulation or are excreted in physiological fluids. Many studies have shown the presence of lignans in plasma and milk of dairy cows[40,42] but none has investigated the role of the rumen in the transfer of mammalian lignans in biological fluids such as plasma, urine, and milk. The present study shows that concentration of EL obtained in plasma, urine, and milk depends on ruminal metabolism of plant lignans. A significant increase in EL concentration was observed in plasma, milk, and urine of cows when a source of lignans (flax hulls) was administered directly in the rumen compared to when

lignans bypassed the rumen through administration in the abomasum. Therefore, it appears that the main site for metabolism of flax lignans in dairy cows is the rumen and that the small and large intestine are not as efficient to metabolize plant SDG into mammalian EL. The response to plant lignan SDG supplementation was more important in urine and milk than blood. Increases in EL concentration of milk and urine were, respectively, 12 and 16 times higher for cows receiving both flax products (hulls + oil) in the rumen compared to those administered with flax products directly in the abomasum while plasma EL concentration was only 3 times higher. It appears that the best biomarkers for lignan metabolism of flax products in cattle may be obtained by measuring the concentration of mammalian lignan EL in urine and milk rather than in ruminal fluid and plasma. Ruminal fluid and blood are dynamic media with body exchanges such as absorption while milk and urine may accumulate overtime (e.g. in the mammary gland). As a result, mammalian lignan EL is more likely concentrated in urine and milk than in ruminal fluid and blood.

It has been shown that people with higher blood concentrations of EL have lower incidence of cardiovascular diseases[47]. However, it is unknown if health effects of mammalian lignans are similar in dairy cows. Further investigations are required to better understand the impact of increasing mammalian lignans in biological fluids on reproduction, lactation, and health of dairy cows. The protective effects of lignans in humans are well documented and production of value-added milk naturally enriched with mammalian lignans could then contribute to better human health.

In conclusion, flax oil supplementation has no influence on ruminal metabolism of lignans supplied as flax hulls. Moreover, the results demonstrate that the main site for metabolism of flax lignans in dairy cows is the rumen. Therefore, ruminal microbiota may be the most important flora to target for plant lignan metabolism in order to increase the concentration of mammalian lignan EL in milk of dairy cows.

References

1. Thompson LU, Robb P, Serraino M & Cheung F (1991) Mammalian lignan production from various foods. *Nutr. Cancer* **16**, 43-52.
2. Kitts DD, Yuan YV, Wijewickreme AN & Thompson LU (1999) Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Mol Cell Biochem* **202**, 91-100.
3. Martin ME, Haourigui M, Pelissero C, *et al.* (1996) Interactions between phytoestrogens and human sex steroid binding protein. *Life Sci* **58**, 429-436.
4. Prasad K (1997) Dietary flax seed in prevention of hypercholesterolemic atherosclerosis. *Atherosclerosis* **132**, 69-76.
5. Thompson LU & Ward WE (2002) Flaxseed lignans: health benefits, bioavailability, and safety. In *Phytoestrogens and Health*. pp. 405-426 [S Gilani and J Anderson, editors], Champaign, IL: AOCS Press.
6. Adlercreutz H (2007) Lignans and human health. *Crit Rev Clin Lab Sci* **44**, 483-525.
7. Saarinen NM, Power KA, Chen J & Thompson LU (2008) Lignans are accessible to human breast cancer xenografts in athymic mice. *Nutr Cancer* **60**, 245-250.
8. Liu Z, Saarinen NM & Thompson LU (2006) Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed *in vitro* and in rats. *J Nutr* **136**, 906–912.
9. Adlercreutz H & Mazur W (1997) Phyto-oestrogens and western diseases. *Ann Med* **29**, 95-120.
10. Côrtes C, Gagnon N, Benchaar C, *et al.* (2008) *In vitro* metabolism of flax lignans by ruminal and fecal microbiota of dairy cows. *J Appl Microbiol* **105**, 1585-1594.
11. Saarinen NM, Smeds A, Mäkelä SI, *et al.* (2002) Structural determinants of plant lignans for the formation of enterolactone *in vivo. J Chromatogr B* **777**, 311-319.
12. Setchell KD, Lawson AM, Mitchell FL, *et al.* (1980) Lignans in man and in animal species. *Nature* **287**, 740-742.
13. Axelson M, Sjovall J, Gustafsson BE & Setchell KDR (1982) Origin of lignans in mammals and identification of a precursor from plants. *Nature* **298**, 659-60.
14. Barnes S, Sfakianos J, Coward L & Kirk M (1996) Soy isoflavonoids and cancer

prevention. *Adv Exp Med Biol* **401**, 87–100.

15. Jansen GHE, Arts ICW, Nielen MWF, *et al.* (2005) Uptake and metabolism of enterolactone and enterodiol by human colon epithelial cells. *Arch Biochem Biophysics* **435**, 74-82.
16. Setchell KDR & Adlercreutz H (1988) Mammalian lignans and phytoestrogens. In *The role of the gut flora in toxicity and cancer*, pp.15-346. [I Rowland, editor]. London: Academic Press.
17. Hoikkala AA, Schiavoni E & Wahala K (2003) Analysis of phyto-oestrogens in biological matrices. *Br J Nutr* **89 Suppl 1**, S5-18.
18. Jenab M & Thompson LU (1996) The influence of flaxseed and lignans on colon carcinogenesis and β-glucuronidase activity. *Carcinogenesis* **17**, 1343-1348.
19. Raffaelli B, Hoikkala, A, Leppälä E & Wähälä K (2002) Enterolignans. *J Chromatogr B* **777**, 29-43.
20. Bannwart C, Adlercreutz H, Wähälä K, *et al.* (1988) Identification of the phyto-oestrogen 3',7-dihydroxyisoflavan, an isomer of equol, in human urine and cow's milk. *Biomed Environ Mass Spectrom* **17**, 1-6.
21. King RA, Mano MM & Head RJ (1998) Assessment of isoflavonoid concentrations in Australian bovine milk samples. *J Dairy Res* **65**, 479-489.
22. Antignac JP, Cariou R, Le Bizec B & André F (2004) New data regarding phytoestrogens content in bovine milk. *Food Chem* **87**, 275-281.
23. Petit HV, Gagnon N, Giguère A & Mir P (2005) Transfer of enterolactone in the milk of dairy cows fed flaxseed or flaxseed meal P69 in 4th NIZO Dairy Conference, Prospects for health, well-being and safety Papendal, The Netherlands.
24. Zhou W, Wang G, Han Z, *et al.* (2008) Metabolism of flaxseed lignans in the rumen and its impact on ruminal metabolism and flora. *Anim Feed Sci Technol* **150**, 18-26.
25. Henderson C (1973) The effects of fatty acids on pure cultures of rumen bacteria. *J Agric Sci Cambridge* **81**, 107–112.
26. Maia MRG, Chaudhary LC, Figueres L & Wallace RJ (2007) Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie Van Leeuwenhoek* **91**, 303-314.

27. Edmonson A J, Lean I J, Weaver LD, *et al.* (1989) A body condition scoring chart for Holstein for dairy cows. *J Dairy Sci* **72**, 68-78.
28. National Research Council. (2001) *Nutrient requirements of dairy cattle*, 7th ed., pp. 258-280. Washington: National Academies Press.
29. Canadian Council on Animal Care (1993) *Guide to Care and Use of Experimental Animals*. Vol.1 [ED Offert, BM Cross and AA McWilliam, editors]. CCAC, Ottawa, ON, Canada.
30. Petit HV & Benchaar C (2007) Milk production, milk composition, blood composition, and conception rate of transition dairy cows fed different profiles of fatty acids. *Can J Anim Sci* **87**, 591-600.
31. Gressley TF, Reynal SM, Colmenero JJO, *et al.* (2006) Technical note: Development of a tool to insert abomasal infusion lines into dairy cows. *J Dairy Sci* **89**, 3965-3967.
32. Novozamsky I, Van Eck R, Van Schouwenburg JC, *et al.* (1974) Total nitrogen determination in plant material by means of the indophenol-blue method. *Neth J Agric Sci* **22**, 3–5.
33. Frank AA & Custer LJ (1996) Diadzein and geneistein concentrations in human milk after soy consumption. *Clin Chem* **42**, 955-964.
34. Heinonen S, Nurmi T, Liukkonen K, *et al.* (2001) *In vitro* metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterediol .*J Agric Food Chem* **49**, 3178-3186.
35. Hausner H, Johnsen NF, Hallund J & Tetens I (2004) A single measurement is inadequate to estimate enterolactone levels in Danish postmenopausal women due to large intraindividual variation. *J Nutr* **10**, 1197-1200.
36. Nesbitt PD, Lam Y & Thompson LU (1999) Human metabolism of mammalian lignan precursors in raw and processed flaxseed. *Am J Clin Nutr* **69**, 549-555.
37. Borriello SP, Setchell KDR, Axelson M & Lawson AM (1985) Production and metabolism of lignans by the human faecal flora. *J Appl Bacteriol* **58**, 37-43.
38. Bowey E, Adlercreutz H & Rowland I (2003) Metabolism of isoflavones and lignans by the gut microflora, a study in germ-free and human flora associated rats. *Food Chem Toxicol* **41**, 631–636.

39. Dehennin L, Reiffsteck A, Jondet M &Thibier M (1982) Identification and quantitative estimation of a lignan in human and bovine semen. *J Reprod Fert* **66**, 305–309.
40. Hansen-Møller J & Kristensen NB (2004) Determination of phytoestrogens in bovine plasma using liquid chromatography and tandem mass spectrometry. *J Anim Feed Sci* **13 (Suppl 1)**, 315-318.
41. Penalvo JL, Haajanen KM, Botting N & Adlercreutz H (2005) Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry. *J Agric Food Chem* **53**, 9342–9347.
42. Steinshamn H, Purup S, Thuen E & Hansen-Møller J (2008) Effects of clover-grass silages and concentrate supplementation on the content of phytoestrogens in dairy cow milk. *J Dairy Sci* **91**, 2715-2725.
43. Slavin JL (2000) Mechanisms for the impact of whole grain foods on cancer risk. *J Am Coll Nutr* **19**, 300-307.
44. Beaud D, Tailliez P & Anba-Mondoloni J (2005) Genetic characterization of the beta-glucuronidase enzyme from a human intestinal bacterium, *Ruminococcus gnavus*. *Microbiol* **151**, 2323-2330.
45. Weimer PJ, Waghorn GC, Odt CL & Mertens DR (1999) Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. *J Dairy Sci* **82**, 122-134.
46. Wiedemann S, Gurtler P & Albrecht C (2007) Effect of feeding cows genetically modified maize on the bacterial community in the bovine rumen. *Appl Environ Microbiol* **73**, 8012-8017.
47. Vanharanta M, Voutilainen S, Lakka TA, *et al.* (1999) Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. *Lancet* **354**, 2112-2115.

CAPÍTULO II

(Normas: Journal of Dairy Science)

FLAXSEED ON MILK QUALITY OF COWS

Effects of Abomasal or Ruminal Infusion of Flax Oil and Hulls on Milk Production, Digestibility, and Milk Fatty Acid Profile in Dairy Cows

ABSTRACT

Four rumen-cannulated primiparous lactating Holstein cows were assigned to a 4 × 4 Latin square design to determine the site effects of infusion (rumen and/or abomasum) of flax oil and flax hulls on milk fatty acid (FA) profile and digestibility parameters. The treatments were: 1) oil and hulls administered in the rumen and abomasal infusion of water (RUM/RUM); 2) oil and hulls infused in the abomasum (ABO/ABO); 3) oil administered in the rumen and hulls infused in the abomasum (RUM/ABO); and 4) oil infused in the abomasum and hulls administered in the rumen (ABO/RUM). Infusion site of flax products affected DMI of dairy cows. Apparent DM digestibilities were lower on RUM/RUM and RUM/ABO than ABO/ABO and ABO/RUM treatments. Dairy cows on the RUM/RUM treatment showed the lowest mean value of NDF and ADF digestibilities. Greater concentration of fat in the rumen probably caused a detrimental effect on fiber digestibility. Apparent digestibilities of CP and EE were higher when flax hulls were administered in the rumen than when they were infused in the abomasum. Production of 4% FCM was significantly reduced when flax products were administered in the rumen. Milk composition was similar among treatments. Concentration of SCFA in milk fat on RUM/RUM treatment was reduced by 26% when compared to the other treatments. Cows on the RUM/RUM treatment showed the highest concentration of octadecenoic acid in milk fat. Proportion of *cis*9,*trans*11-CLA in milk fat was higher with ruminal administration of flax oil. Abomasal infusion of flax oil resulted in greater proportions of 18:3 n-3 FA in milk fat and RUM/RUM treatment resulted in the highest n-6:n-3 ratio in milk fat. The site of infusion of flax oil and flax hulls had important effects on milk FA concentrations, DMI and digestibility parameters in dairy cows.

Key Words : dairy production, milk fat, omega 3, PUFA

INTRODUCTION

Flaxseed has been the focus of many researchers due to its high content in alpha-linolenic acid (LNA), a polyunsaturated fatty acid (PUFA) which is linked to many potential health benefits, such as decreased incidence of cancer, prevention of cardiovascular diseases, hypertension, arthritis, and improvement in visual acuity (Simopoulos, 1996; Wright et al., 1998). Milk fat contains high levels of saturated fatty acids (SFA), which have hypercholesterolemic properties (Kennelly, 1996). Health-conscious consumers are demanding dairy products that are richer in polyunsaturated long-chain fatty acids (LCFA), particularly in LNA.

Milk fat of dairy cows fed flaxseed shows reduced concentrations of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA), and increased concentrations of LCFA (Mustafa et al., 2003; Petit, 2003). However, the increase in milk fat concentration of LNA is small due hydrolysis of ester linkages of lipids and biohydrogenation of unsaturated FA in the rumen. The major substrates for lipid biohydrogenation are linoleic (LA) and LNA, and the rate of ruminal biohydrogenation is faster as the degree of unsaturation increases (Bauman et al., 2003). Biohydrogenation of unsaturated fatty acids in the rumen results in the formation of *trans*11-18:1 (vaccenic acid, VA) from LA and LNA, and *cis*9,*trans*-11-CLA (conjugated linoleic acid) from LA. Most of *cis*9,*trans*-11-CLA found in ruminants are synthesized in the mammary gland and adipose tissue that involves the enzyme delta-9 desaturase with rumen-derived vaccenic acid as substrate (Bauman et al., 2003; Tanaka, 2005).

Many researchers evaluated ruminal lipid biohydrogenation and transfer efficiency of LCFA in milk of dairy cows fed seeds (processed or whole seeds) rich in oil (Petit, 2002, Petit, 2003; Gonthier et al., 2005; Bell et al., 2006; Da Silva et al., 2007) or by intestinal infusion of vegetable oil (Benson et al., 2001). However, there is no information on ruminal metabolism of PUFA from flax hulls in dairy cows and their effects on digestibility, milk composition, and milk fatty acids profile. Flax hulls are produced in Canada and are rich in fat (28% of the DM) and also LNA, suggesting that it can be a good source of dietary PUFA to transfer in milk of dairy cows.

Thus, the objective of the present study was to evaluate the effects of site (rumen and/or abomasum) of administration of flax oil and flax hulls on digestibility, milk composition, and milk fatty acid profile of dairy cows.

MATERIALS AND METHODS

Animals and Treatments

The experiment was conducted at Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada. Four primiparous lactating Holstein cows fitted with ruminal cannulas (10 cm, Bar Diamond Inc., Parma, ID) were used in a 4 × 4 Latin square design with four treatments and four 21-d periods balanced for residual effect. The cows averaged 92 ± 12 DIM at the start of the experiment with an average BW of 575 ± 25 kg, and an average BCS of 3.00 ± 0.15 (5-points scale; Edmonson et al., 1989). The cows were kept in individual stalls and had free access to water. Cows were milked twice a day at 0830 and 2000 h. All cows were fed the same total mixed diet for *ad libitum* intake (10 % refusals on as fed basis) twice a day (0830 and 1430 hours). The diet (Table 1) was formulated to meet requirements for cows that were 575 kg of BW and producing 35 kg/d of milk with 3.8% fat (NRC, 2001). The experimental protocol complied with the *Guide to Care and Use of Experimental Animals* (CCAC, 1993) and was approved by the local animal care committee.

The four experimental treatments were: 1) oil and hulls administered in the rumen and abomasal infusion of water (RUM/RUM); 2) oil and hulls infused in the abomasum (ABO/ABO); 3) oil administered in the rumen and hulls infused in the abomasum (RUM/ABO); and 4) oil infused in the abomasum and hulls administered in the rumen (ABO/RUM). Abomasal infusions consisted of 20 kg of tap water (RUM/RUM), 1.8 kg of flax hulls suspended in 18.2 kg of tap water (ABO/ABO and RUM/ABO) and 0.4 kg of flax oil + 20 kg of tap water (ABO/RUM).

Chemical composition of flax hulls and fatty acids profile of flax oil and hulls are shown in Table 1 and 2, respectively. During the first seven days of each 21-d period, only 30% of experimental dose of oil and hulls were infused in the abomasum over a 7-h period. From d 8 to d 21, infusion in the abomasum was conducted with 100% of the experimental dose of oil and hulls over a 23-h period. Administration in the rumen was done by adding one third each of oil and hulls three times daily (0930, 1430, and 2130 h) during experimental periods. Infusates of flax products were prepared daily and the appropriate amount of infusate for each cow was weighed into tarred buckets that were stirred continuously while being infused. To perform abomasal infusions, an infusion line was inserted through the rumen cannula and the *sulcus omasi* into the abomasum as described by Gressley et al. (2006). Placement of the infusion lines was monitored daily to ensure postruminal delivery. Solutions were pumped into the

abomasum by using peristaltic pumps (Masterflex L/S; Cole-Parmer Canada Inc., Montreal, QC, Canada). The daily amount of oil supplied by flax hulls was similar to that supplied by flax oil. Half of the lipids were provided by flax oil and the other half by flax hulls for a total amount of 800 g daily, thus resulting in a fat intake below 6 to 7% of the DM, which is known to have little effect on DM intake (NRC, 2001). Fatty acid profiles of flax oil and hulls are shown in Table 2.

Table 1. Ingredient composition of basal diet and nutrient contents as analyzed in the basal diet and flax hulls

	Feeds	
	Basal diet	Flax hulls
Ingredient	—— % of DM ——	
Grass silage	32.0	
Corn silage	32.2	
Cracked corn grain	11.9	
Ground barley	7.7	
Soybean meal	10.2	
Top supplement[1]	2.8	
Mineral[2]	2.5	
Ca carbonate	0.7	
Nutrient	—— % of DM ——	
DM, %	41.8	93.2
CP	16.0	23.5
EE	2.3	29.4
NDF	29.7	19.4
ADF	20.4	14.3
NE_L, Mcal/kg of DM	1.58	1.65

[1]Contained 20% of canola meal, 30% of corn gluten meal, 20% of soybean meal, 30% of brewer's corn.
[2] Contained 9.02% Ca, 4.90% P, 4.89% Mg, 1.76% S, 14% Na, 1.43% K, 2068 mg/kg Fe, 2718 mg/kg Zn, 447 mg/kg Cu, 1814 mg Mn, 69 mg/kg I, 7 mg/kg Co, 20 mg/kg Se, 452,000 IU/kg of vitamin A, 58,000 UI/kg of vitamin D_3, and 2692 IU/kg of vitamin E.

Sampling

Cows were weighed on three consecutive days at the beginning and the end of each period after the am milking.

Samples of diets, flax hulls and flax oil were taken daily from d 14 to d 21, pooled by cow within period, and stored at -20°C. To predict fecal output and digestibility, a capsule of chromic oxide (Cr_2O_3) was inserted in the rumen once daily at 0900 h from d-11 to d-20 of each period, supplying a total of 10 g of Cr_2O_3/d. Fecal

grab samples were taken twice daily from day 15 to day 19 at 0830 and 1630 h, subsequently they were dried at 55ºC for 48 h and composited on a DM basis by cow within period.

Table 2. Fatty acid profile of flax oil and flax hulls

Fatty acids*	Flax oil	Flax hulls
14:0	0.41	0.66
16:0	5.38	6.95
*cis*9-16:1	0.39	0.43
18:0	3.90	2.36
*cis*9-18:1	18.81	18.06
*cis*11-18:1	0.84	1.07
*cis*9,12-18:2	16.05	17.18
*cis*9,12,15-18:3	53.69	52.91
22:0	0.34	0.09
24:0	0.19	0.29

* Percentage of total fatty acids

From day 22 to 28, milk samples were taken from each cow for 14 consecutive milkings and pooled on a yield basis to obtain one composite milk sample per cow within period. One sample was kept frozen without preservative for further analyses of milk fat and fatty acids profile. Another sample was stored at + 4°C with a preservative (bronopol-B2) until analyzed for protein, urea N and lactose. All samples were frozen at -20°C until subsequent chemical analyses.

Chemical analysis

Oven-dried (fecal) and freeze-dried (flax hulls and diets) samples were ground through a 1-mm screen in a Wiley mill. Determination of DM of diets was conducted in a forced-air oven according to the procedure 934.01 (AOAC, 1990). Total N content of total mixed ration (TMR) and feces was determined by thermal conductivity (LECO model FP-428 Nitrogen Determinator, LECO, St. Joseph, MI) and CP was calculated as N x 6.25. Concentration of NDF in TMR and feces was determined as described by Van Soest et al. (1991) without the use of sodium sulfite and with the inclusion of heat stable α-amylase. Determination of ADF content in TMR and feces was conducted according to AOAC (1990; Method 973.18). Determinations of NDF and ADF were adapted for use in an ANKOM^{200} Fibre Analyzer (ANKOM Technology Corp., Fairport, NY).

Concentration of ether extract in diets and feces were conducted with Tecnal TE–044/1 (Piracicaba, São Paulo, Brazil) according to the method No. 7.060 (AOAC, 1990). Protein, lactose, total solids, and urea N concentrations in milk samples were analyzed by infrared spectrophotometer (System 4000 Milkoscan; Foss Electric of Hillerod, Denmark). Milk fat concentration was determined by the method of Roese-Goettlib (AOAC, 1990). Milk fatty acids was extracted and methylated according to the method described by Chouinard et al. (1997) while in situ transesterification was performed on diets according to Park and Goins (1994). Fatty acids methyl ester profiles were measured by GLC on a Hewlett-Packard 6890 chromatograph (Hewlett-Packard Lteé, Montreal, Qc, Canada) with a G1315A auto sampler equipped with a flame ionization detector and a split-splitless injector as described by Delbecchi et al. (2001).

Statistical analysis

All data were analyzed using the MIXED procedure of SAS (SAS 2000; SAS Institute, Cary, NC, USA) according to the model:

$$Y_{ijk} = \mu + a_i + \beta_j + \tau_k + e_{ijk},$$

where Y_{ijk} = response variable, μ = overall mean, a_i = random effect of cow i, β_j = effect of period j, τ_k = effect of treatment k, and e_{ijk} = residual error. The residual effect was initially included in the model but was removed because it was not significant. Significance was declared at $P \leq 0.05$ and a trend at $P \leq 0.10$, unless otherwise stated. When a significant F-test was detected, multiple comparisons were done using a Tukey's adjustment for the probability.

RESULTS AND DISCUSSION

Feed Intake and Diet Total Digestibility

Site of flax hulls and oil supplementation affected ($P < 0.01$) DMI and total DM input (DMI + flax products administered in the rumen or infused in the abomasum) by dairy cows (Table 3). Depression in DMI was correlated to the amount of fat delivered in the rumen, which averaged 2.3%, 4.3%, 7.8%, and 5.2% of DM, for ABO/ABO, RUM/ABO, RUM/RUM, and ABO/RUM treatments, respectively. Increased intake of fat contributed to DM and fiber digestibilities depression.

In general, fat supplementation reduces DM digestibility and DMI of lactating dairy cows as reported by the review of Allen (2000). High amounts of fat in the rumen (> 5% of DM) may cause a detrimental effect on fiber degradation due to a layer of lipid

that coats the feed particles, thus resulting in less contact between microbes and cellulose, and also due to cytotoxic effects of unsaturated fatty acids on membrane function of eucaryotic cells (Jenkins, 1993). Our data agree with those theories because although cows on the ABO/ABO treatment presented the lowest concentration of fat in the rumen, they also showed higher total DM digestibility than those on RUM/RUM and RUM/ABO treatments. Moreover, total digestibilities of NDF and ADF were lower when both flax products were administered in the rumen (RUM/RUM treatment).

Table 3. Dry matter intake, total dry matter input, and apparent digestibility of diets in dairy cows supplemented with flax hulls and flax oil administered in the rumen and/or infused in the abomasum

	Treatments				SE	*P*-value
	ABO/ ABO	RUM/ ABO	RUM/ RUM	ABO/ RUM		
	kg/d					
DMI	16.4[a]	15.3[ab]	13.4[c]	13.6[bc]	0.73	0.003
Total DM input[1]	18.6[a]	17.5[a]	15.5[b]	15.7[b]	0.74	0.003
	% of BW					
DMI	2.8[a]	2.6[ab]	2.3[b]	2.4[b]	0.10	0.006
Total DM input	3.2[a]	3.0[ab]	2.7[b]	2.8[b]	0.10	0.005
Total digestibility	% of DM					
DM (%)	73.3[a]	70.5[b]	70.4[b]	72.7[ab]	1.79	0.02
CP	73.6[ab]	70.9[b]	74.9[a]	74.4[a]	1.40	0.01
EE	90.1[b]	88.7[b]	93.2[a]	93.6[a]	0.85	0.003
NDF	63.0[a]	60.1[a]	54.6[b]	60.7[a]	1.99	0.004
ADF	63.5[a]	62.4[a]	55.8[b]	63.1[a]	2.58	0.02

ABO/ABO: oil and hulls infused in the abomasum; RUM/ABO: oil administered in the rumen and hulls infused in the abomasum; RUM/RUM: oil and hulls administered in the rumen and abomasal infusion of water; ABO/RUM: oil infused in the abomasum and hulls administered in the rumen.
[1]total DM input = DMI + flax products administered in the rumen or infused in the abomasum

Cows on treatments with flax hulls administered in the rumen (RUM/RUM and ABO/RUM) showed higher CP apparent digestibility ($P < 0.01$) than those on RUM/ABO treatment. Ruminal fermentation of CP from flax hulls may contribute for higher CP digestibility on RUM/RUM and ABO/RUM treatments, suggesting that flax hulls had more time to be digested in the rumen and gastro-intestinal tract. Decreased DMI when both flax products in the rumen (RUM/RUM treatment) compared to when flax hulls were administered in the abomasum (ABO/ABO and RUM/ABO treatments)

may contribute to decrease the solid rumen outflow rate and increase the total amount of fat present in the rumen, thus decreasing fiber digestion. Moreover, when fat supplements interfere with ruminal fermentation, ruminal protein metabolism is affected (Jenkins, 1993). According to Gonthier et al. (2004), inclusion of flaxseed at 12.7% of the DM in the diet of dairy cows reduced microbial CP flow to the duodenum and microbial efficiencies (true and apparent) and increased undegradable protein flow to the duodenum, which is usually accompanied by a reduction in ruminal fiber digestion (Sauvant and Bas, 2001). Ruminal flaxseed oil infusion in sheep promoted a depression in protein digestion in the rumen accompanied by decreased ammonia concentration and increased nitrogen flow to the duodenum (Ikwuegbu and Sutton, 1982).

Total digestibility of ether extract was higher ($P < 0.001$) when flax hulls were administered in the rumen (RUM/RUM and ABO/RUM) than when they were infused in the abomasum (ABO/ABO and RUM/ABO). Thus, as previous reported for CP digestibility, ruminal bacteria may have more time to digest flax hulls, which may contribute to improve ether extract digestibility. On the other hand, when flax hulls were infused in the abomasum, increased intestinal digesta rate of passage may not allow efficient hydrolyzation and absorption of fatty acids by intestinal mucosa cells, resulting in a reduction in EE digestibility on ABO/ABO and RUM/ABO treatments.

Milk Production and Composition

Yields of 4% FCM, milk protein and lactose (Table 4) were reduced ($P < 0.05$) when all flax products were administered in the rumen (RUM/RUM treatment), which may be due to depressed DMI, and DM, NDF and ADF digestibilities. According to Petit et al. (2005), dairy cows fed a ration with whole flaxseed, containing 7.6% of EE and 16.0% of CP, showed a decrease in DMI and milk yield when compared to cows fed a control ration with 2.8% of EE; however, when rations had 18.0 % of CP, no milk yield depression was observed. Cows fed supplemental fat require greater amount of protein to maintain milk protein yield in response to fat supplementation (Gamsworthy, 1997).

Yields of milk fat and total solids and SCS were similar among treatments. Protein, fat, total solids, and lactose concentrations in milk were also similar among treatments, suggesting that infusion site of flax oil and hulls has no effect on milk composition in mid-lactating dairy cows. Urea N concentration (mg/dl) was lower when flax oil was administered in the rumen (RUM/RUM and RUM/ABO) than when it was

infused in the abomasum (ABO/ABO and ABO/RUM), suggesting that the presence of flax oil in the rumen allows better N utilization. These data agree with the fact that presence of fat in the rumen may increase efficiency of microbial protein synthesis by reduction of protozoal numbers and lower recycling of bacterial N (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984).

Table 4. Milk production and composition of dairy cows supplemented with flax hulls and flax oil administered in the rumen and/or infused in the abomasum

	Treatments				SE	*P*-value
	ABO/ ABO	RUM/ ABO	RUM/ RUM	ABO/ RUM		
	kg/d					
Milk	27.4[ab]	29.1[a]	26.2[b]	27.1[b]	1.79	0.01
4% FCM	24.6[a]	25.0[a]	20.4[b]	24.8[a]	1.57	0.02
Protein	0.97[a]	0.95[ab]	0.86[b]	0.88[ab]	0.05	0.03
Fat	0.95	0.76	0.64	0.93	0.09	0.22
Total solids	3.56	3.42	3.03	3.31	0.22	0.12
Lactose	1.31[ab]	1.38[a]	1.24[b]	1.30[ab]	0.10	0.05
Milk composition	%					
Protein	3.79	3.42	3.40	3.48	0.10	0.07
Fat	3.56	2.56	2.45	3.02	0.36	0.22
Total solids	13.1	11.7	11.6	12.3	0.44	0.16
Lactose	4.77	4.73	4.75	4.79	0.05	0.72
Urea N, mg/dL	14.8[a]	10.2[b]	9.38[b]	12.8[a]	1.76	0.001
SCS[1]	1.21	1.38	1.38	1.17	0.48	0.88

ABO/ABO: oil and hulls infused in the abomasum; RUM/ABO: oil administered in the rumen and hulls infused in the abomasum; RUM/RUM: oil and hulls administered in the rumen and abomasal infusion of water; ABO/RUM: oil infused in the abomasum and hulls administered in the rumen.
[1] Somatic Cell Score = $\log_{10}$SCC

Milk Fatty Acid Profile

Concentration of short-chain fatty acids (SCFA) in milk fat on RUM/RUM treatment was reduced ($P < 0.01$) by 26 % when compared to other treatments (11.35 % of total fatty acids on RUM/RUM treatment *vs* 15.42 % of total fatty acids for ABO/ABO, RUM/ABO, and ABO/RUM treatments, respectively) as shown at Table 5. Moreover, RUM/RUM treatment showed the highest concentration of octadecenoic acid in milk fat, followed by RUM/ABO and ABO/RUM treatment, and finally by ABO/ABO treatment. Similarly, Dhiman et al. (1995) reported that a greater dietary supply of 18:2 and 18:3 fatty acids increases milk fat concentration of 18:1 through ruminal biohydrogenation.

Concentration of total *trans* fatty acids, *cis*9,*trans*11-CLA, and *trans*11-18:1 were affected by the presence of flax oil in the rumen, and the highest values were reported on RUM/ABO and RUM/RUM treatments. On the other hand, the opposite was observed on ABO/ABO treatment due to PUFA from flax products that were delivered directly in the abomasum, thus escaping ruminal biohydrogenation. Diet plays important role on milk fat concentration of CLA whereas the major source of CLA in milk fat is endogenously synthesized by enzyme delta-9 desaturase from rumen derived *trans*11-18:1, an intermediate formed by ruminal biohydrogenation of PUFA (Griinari et al., 2000; Corl et al., 2001).

Table 5. Fatty acid concentrations (% of total fatty acids) in milk of dairy cows supplemented with flax hulls and flax oil administered in the rumen and/or infused in the abomasum

	Treatments				SE[1]	*P*-value
	ABO/ ABO	RUM/ ABO	RUM/ RUM	ABO/ RUM		
4:0	4.64	5.51	4.13	5.21	0.40	0.17
5:0	0.04	0.05	0.05	0.06	0.01	0.90
6:0	2.42^{a}	2.31^{a}	1.58^{b}	2.33^{a}	0.17	0.002
7:0	0.08	0.10	0.08	0.10	0.01	0.56
8:0	1.46^{a}	1.26^{b}	0.91^{c}	1.27^{b}	0.09	0.001
9:0	0.11	0.11	0.09	0.09	0.02	0.49
10:0	3.43^{a}	2.57^{b}	1.73^{c}	2.56^{b}	0.21	0.0003
11:0	0.13	0.11	0.14	0.10	0.01	0.27
12:0	3.81^{a}	2.90^{b}	2.29^{c}	2.63^{bc}	0.21	0.0007
*cis*11-12:1	0.18	0.18	0.20	0.12	0.03	0.22
13:0	0.14^{ab}	0.14^{ab}	0.16^{a}	0.11^{b}	0.01	0.03
14:0	9.32^{a}	9.36^{a}	8.46^{b}	7.67^{c}	0.22	< 0.0001
*cis*9-14:1	0.58^{bc}	0.72^{b}	1.04^{a}	0.44^{c}	0.07	0.0006
15:0	0.84^{ab}	0.90^{a}	0.96^{a}	0.70^{b}	0.05	0.01
16:0	20.11^{a}	19.16^{ab}	18.67^{b}	16.05^{c}	0.47	0.0001
*trans*9-16:1	0.07^{b}	0.16^{a}	0.19^{a}	0.08^{b}	0.01	0.0006
*cis*9-16:1	1.11^{bc}	1.36^{ab}	1.79^{a}	0.89^{c}	0.12	0.003
17:0	0.52^{bc}	0.59^{a}	0.56^{ab}	0.45^{c}	0.02	0.005
18:0	8.54^{b}	12.79^{a}	11.63^{a}	14.13^{a}	0.77	0.005
*trans*9-18:1	0.19^{c}	0.42^{b}	0.71^{a}	0.29^{bc}	0.04	0.0002
*trans*11-18:1	0.89^{b}	2.71^{a}	2.88^{a}	1.29^{b}	0.17	< 0.0001
*cis*6-18:1	0.36^{c}	2.06^{b}	4.38^{a}	1.61^{b}	0.35	0.0006
*cis*9-18:1	17.31^{c}	21.82^{b}	26.48^{a}	21.71^{b}	1.19	< 0.0001
*cis*11-18:1	0.63^{b}	0.76^{ab}	0.87^{a}	0.70^{ab}	0.06	0.04
19:0	0.17^{c}	0.74^{b}	1.78^{a}	0.59^{bc}	0.09	< 0.0001
*trans*9,12-18:2	0.10^{bc}	0.15^{b}	0.25^{a}	0.07^{c}	0.02	0.001
*cis*9,12-18:2	6.14^{a}	3.31^{c}	2.52^{d}	5.03^{b}	0.22	< 0.0001
20:0	0.29	0.36	0.35	0.28	0.03	0.14

*cis*8-20:1	0.19[b]	0.23[ab]	0.28[a]	0.18[b]	0.02	0.02
*cis*11-20:1	0.16	0.17	0.20	0.15	0.02	0.09
*cis*9,12,15-18:3	14.37[a]	4.78[b]	2.33[c]	11.61[a]	0.59	< 0.0001
*cis*9,*trans*11-18:2	0.38[c]	1.12[ab]	1.66[a]	0.53[bc]	0.18	0.002
*trans*10,*cis*12-18:2	0.01	0.02	0.02	0.01	0.01	0.32
*cis*11,14-20:2	0.14[a]	0.14[a]	0.03[b]	0.12[a]	0.02	0.01
*cis*11,14,17-20:3	0.24[a]	0.17[a]	0.07[b]	0.20[a]	0.02	0.006
22:0	0.18	0.18	0.14	0.11	0.04	0.46
*cis*8,11,14-20:3	0.10	0.10	0.07	0.08	0.02	0.22
*cis*5,8,11,14-20:4	0.15	0.13	0.11	0.13	0.01	0.92
*cis*5,8,11,14,17-20:5	0.22[a]	0.14[b]	0.09[b]	0.16[ab]	0.03	0.006
*cis*7,10,13,16,19-22:5	0.22	0.22	0.12	0.18	0.04	0.10
Total trans	1.64[b]	4.58[a]	5.71[a]	2.27[b]	0.34	0.0001
MUFA[2]	21.68[d]	30.58[b]	39.02[a]	27.46[c]	1.30	< 0.0001
PUFA[2]	22.08[a]	10.28[b]	7.28[b]	18.12[a]	0.78	< 0.0001
SFA[2]	56.24[ab]	59.13[a]	53.71[b]	54.42[b]	1.32	0.01
PUFA/SFA	0.39[a]	0.17[b]	0.14[b]	0.33[a]	0.02	0.0002
SCFA[2]	16.45[a]	15.24[a]	11.35[b]	14.58[a]	0.93	0.002
MCFA[2]	32.56[a]	32.25[a]	31.67[a]	26.27[b]	0.76	< 0.0001
LCFA[2]	50.99[d]	52.51[c]	56.98[b]	59.14[a]	0.88	< 0.0001
n-3[3]	15.05[a]	5.32[b]	2.62[c]	12.15[a]	0.61	< 0.0001
n-6[4]	6.53[a]	3.67[c]	2.73[d]	5.36[b]	0.22	< 0.0001
n-6:n-3	0.43[a]	0.69[a]	1.04[b]	0.44[a]	0.06	0.0004

ABO/ABO: oil and hulls infused in the abomasum; RUM/ABO: oil administered in the rumen and hulls infused in the abomasum; RUM/RUM: oil and hulls administered in the rumen and abomasal infusion of water; ABO/RUM: oil infused in the abomasum and hulls administered in the rumen.

[1]Least squares means with pooled standard error (SE).

[2]MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SCFA = short-chain fatty acids (< C14); MCFA = medium-chain fatty acids (< C18); LCFA = long-chain fatty acids (> C17).

[3] cis9,12,15-18:3 + cis11,14,17-20:3 + cis5,8,11,14,17-20:5 + cis7,10,13,16,19-22-5.

[4] cis9,12-18:2 + cis11,14-20:2 + cis8,11,14-20:3 + cis5,8,11,14-20:4.

Although daily amounts of oil supplied by flax hulls and flax oil were similar, RUM/ABO treatment resulted in higher concentrations of *trans*11-18:1 and total *trans* fatty acids than ABO/RUM treatment in milk fat, suggesting that oil was released more slowly from flax hulls than free oil in the rumen. As a result, biohydrogenation of unsaturated fatty acids from flax oil was higher than that of flax hulls, thus increasing concentration of total *trans* fatty acids in milk fat.

Abomasal infusion of flax oil was mainly responsible for increased concentrations of 18:3 n-3 and 18:2 n-6 fatty acids in milk fat as shown by results obtained with ABO/ABO and ABO/RUM treatments. However, rumen bypass of flax hulls when flax oil was infused in the rumen (RUM/ABO) showed a significant contribution on n-3 FA and PUFA concentrations in milk fat as shown by enhanced

concentrations for cows on RUM/ABO compared to those on RUM/RUM treatment (Table 5). Similar availability of PUFA occurs when processed or treated oil seeds are fed to dairy cows, which results in PUFA enriched milk (Dhiman et al., 1995; Gonthier et al., 2005; Da Silva et al., 2007).

Flax products administered in the rumen promoted the highest n-6:n-3 ratio ($P < 0.01$) in milk fat, but all n-6:n-3 ratios were lower than the 4:1 ratio considered as ideal for reduction of coronary heart diseases and improved human health (Sim, 1998).

CONCLUSIONS

Greater concentration of lipids in the rumen affected negatively dry matter intake which was accompanied by a depression in dry matter and fiber digestibilities and a reduction in milk yield. Lipids from flax hulls were likely released more slowly than that of free oil in the rumen, thus resulting in less biohydrogenation of unsaturated fatty acids and less total *trans* fatty acids in milk fat. Flax products administered in the rumen increased the n-6:n-3 fatty acid ratio in milk fat, but the n-6:n-3 FA ratio was still considered a good ratio to improve human health. Overall, these results suggest that flax oil and flax hulls may be added to the diet of dairy cows to produce a n-3 enriched milk for better human health.

REFERENCES

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

AOAC. 1990. Official Methods of Analysis. 15th ed. AOAC, Washington, DC.

Bauman, D. E., J. W. Perfield II, M. J. Veth, and A. L. Lock. 2003. News perspectives on lipid digestion and metabolism in ruminants. Pages 175-189 in Proc. Cornell Nutr. Conf., Cornell Universtiy, Ithaca, NY.

Bell, J. A., J. M. Griinari, and J. J. Kennelly. 2006. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. J. Dairy Sci. 89:733–748.

Benson, J. A., C. K. Reynolds, D. J. Humphries, S. M. Rutter, and D. E. Beever. 2001. Effects of abomasal infusion of long-chain fatty acids on intake, feeding behavior and milk production in dairy cows. J. Dairy Sci. 84:1182–1191.

CCAC - Canadian Council on Animal Care. 1993. Guide to Care and Use of Experimental Animals. Vol.1, E. D. Offert, B. M. Cross and A. A. McWilliam, ed. CCAC, Ottawa, ON, Canada.

Chouinard, P. Y., V. Girard, and G. J. Brisson. 1997. Performance and profiles of milk fatty acids of cows fed full-fat, heat-treated soybeans using various processing methods. J. Dairy Sci. 80:334-342.

Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips, and D. E. Bauman. 2001. The role ofΔ9-desaturase in the production of cis-9, trans-11 CLA. J. Nutr. Biochem. 12:622–630.

Da Silva, D. C., G. T. D. Santos, A. F. Branco, J. C. Damasceno, R. Kazama, M. Matsushita, J. A. Horst, W. B. R. dos Santos, and H. V. Petit. 2007. Production performance and milk composition of dairy cows fed whole or ground flaxseed with or without monensin. J. Dairy Sci. 90:2928-2936.

Delbecchi, L., C. E. Ahnadi, J.J. Kennelly, and P. Lacasse. 2001. Milk fatty acid composition and mammary lipid metabolism in Holstein cows fed protected or unprotected canola seeds. J. Dairy Sci. 84:1375-1381.

Dhiman, T. R., K. V. Zanten, and L. D. Satter. 1995. Effect of dietary fat source on fatty acid composition of cows' milk. J. Sci. Food Agric. 69:101–107.

Edmonson, A. J., Lean, I. J., Weaver, L. D., Farver, T., and Webster, G. 1989. A body condition scoring chart for Holstein for dairy cows. J. Dairy Sci. 72:68-78.

Garnsworthy, P. C. 1997. Fats in dairy cow diets. Pages 87–13 in Recent Advances in Animal Nutrition. P. C. Garnsworthy and D. J. A. Cole, ed. University of Nottingham Press, Nottingham, UK.

Gonthier, C., A. F. Mustafa, R. Berthiaume, H. V. Petit, R. Martineau, and D. R. Ouellet. 2004. Effects of Feeding Micronized and Extruded Flaxseed on Ruminal Fermentation and Nutrient Utilization by Dairy Cows. J. Dairy Sci. 87:1854-1863.

Gonthier, C., A. F. Mustafa, D. R. Ouellet, P. Y. Chouinard, R. Berthiaume, and H. V. Petit. 2005. Feeding Micronized and Extruded Flaxseed to Dairy Cows: Effects on Blood Parameters and Milk Fatty Acid Composition. J. Dairy Sci. 88:748-756.

Gressley, T. F., Reynal, S. M., Colmenero, J. J. O., Broderick, G. A., and Armentano, L. E. 2006. Technical note: Development of a tool to insert abomasal infusion lines into dairy cows. J. Dairy Sci. 89:3965-3967.

Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating cows by Δ9-desaturase. J. Nutr. 130:2285–2291.

Ikwuegbu, O. A. and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr. 48:365-375.

Jenkins, T. C. 1993. Lipid Metabolism in the Rumen. J. Dairy Sci. 76:3851-3863.

Jenkins, T. C. and D. L. Palmquist. 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. J Dairy Sci. 67:978-986.

Kennelly, J. J. 1996. The fatty acid composition of milk as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60:137–152.

Mustafa, A. F., P. Y. Chouinard, and D. A. Christensen. 2003. Effects of feeding micronised flaxseed on yield and composition of milk from Holstein cows. J. Sci. Food Agric. 83:920–926.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Nat. Acad. Press, Washington, DC.

Park, P. W., and R. E. Goins. 1994. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food Sci. 59:1262-1266.

Petit, H. V. 2002. Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. J. Dairy Sci. 85:1482-1490.

Petit, H. V. 2003. Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. J. Dairy Sci. 86:2637-2646.

Petit, H. V., M. Ivan, and P. S. Mir. 2005. Effects of flaxseed on protein requirements and N excretion of dairy cows fed diets with two protein concentrations. J. Dairy Sci. 88:1755–1764.

SAS Institute, Inc. 2000. SAS/STAT® user's guide. Release 8.02. Cary NC, USA.

Sauvant, D., and P. Bas. 2001. La digestion des lipids chez le ruminant. INRA Prod. Anim. 14:303-310.

Sim, J. S. 1998. Designer eggs and their nutritional and functional significance. World Rev. Nutr. Diet. 23:89–101.

Simopoulos, A. P. 1996. Omega-3-fatty acids and public health. Pages 5–28 in Proc. Flax Council of Canada Conf. Flax, the Next Decade, Winnipeg, MB, Canada.

Tanaka, K. 2005. Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. Animal Science J. 76:291-303.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wright, T., B. McBride, and B. Holub. 1998. Docosahexaenoic acid enriched milk. World Rev. Nutr. Diet. 83:160-165.

CAPÍTULO III

(Normas: Journal of Dairy Science)

FLAX LIGNANS CONCENTRATIONS IN BIOLOGICAL FLUIDS

Effects of Abomasal or Ruminal Infusion of Flax Oil and Hulls on Concentration of the Mammalian Lignan Enterolactone in Ruminal Fluid, Urine, Plasma, and Milk of Dairy Cows

ABSTRACT

Six rumen-cannulated multiparous Holstein cows were used in a 2 × 3 factorial arrangement of treatments and six 21-d periods to determine the effects of flax hulls supplementation in the diet and three levels of abomasal flax oil infusion on feed intake, digestibility, milk production and composition, fatty acids profile of milk fat and enterolactone (EL) concentrations in ruminal fluid, urine, plasma and milk of dairy cows. Treatments were flax hulls supplementation (without: CON vs. with ?% in the DM: FHU) and 3 different amounts of flax oil infused in the abomasum (0, 250 and 500 g/d). The FHU treatment and increased amounts of abomasal flax oil infusion decreased DMI. The FHU treatment increased apparent digestibilities of DM, CP and EE probably due to partial replacement of cracked corn grain and soybean meal by flax hulls. Feeding FHU treatment decreased milk yield and yields of protein, fat, total solids and lactose. Cows fed flax hulls showed lower SCFA, MCFA and SFA, and higher LCFA, MUFA and PUFA concentrations in milk fat. Flax hulls supplementation and abomasal flax oil infusion decreased 14:0 and 16:0 concentrations in milk fat. Supplementation of flax hulls likely increased the amount of *cis*11-18:1, *cis*9,12-18:2 and *cis*9,12,15-18:3 reaching the rumen, thus increasing concentrations of 18:0, *cis*9-18:1, *trans* dienes, *trans* monoenes and total *trans* in milk fat. Flax hulls supplementation increased concentrations of n-3 FA in milk fat and decreased those of n-6 FA, thus resulting in a reduced n-6:n-3 ratio in milk fat of dairy cows. Concentrations of EL in plasma, milk, and urine of dairy cows fed FHU diet were higher than those of cows on the CON diet. Different amounts of abomasal flax oil infusion did not affect EL transfer in milk of dairy cows.

Key words: enterolignans, milk quality, linolenic acid, secoisolariciresinol diglycosidae

INTRODUCTION

Flaxseed (*Linum usitatissimum*) consumption by human has been linked to enhance health benefits, such as prevention of cardiovascular diseases, osteosporosis, hypercholesterolemia, postmenopausal symptoms, and cancer (Murkies et al., 1998; Prasad, 1997; Thompson and Ward, 2002; Adlercreutz, 2007). These health properties of flaxseed may be due to its high content in alpha-linolenic (LNA) acid and plant lignans (Thompson et al., 1991). Plant lignans are classified as phytoestrogens like isoflavones and coumestans (Stopper et al., 2005). Flax lignans are present in the form of secoisolariciresinol diglucoside (SDG) and SDG represents 95% of the total plant lignans (Liu et al., 2006). Flax lignans are concentrated in the outer fibre-containing layers (Adlercreutz and Mazur, 1997).

Beneficial effects of lignans on human health depends on the metabolism of plant SDG. In non-ruminants, SDG is converted to secoisolariciresinol (SECO) under action of intestinal glycosidases and the colonic microbiota convert SECO to mammalian lignans, enterodiol (ED) and enterolactone (EL; Setchell et al., 1980; Saarinen et al., 2002). Mammalian lignans are then absorbed by the intestine (Borrielo et al., 1985; Saarinen et al., 2002; Setchell et al., 1980). Mammalian lignans are conjugated as sulphate and glucuronide in the intestinal wall and liver under the action of specific enzymes (Barnes et al., 1996; Jansen et al., 2005). They are excreted in physiological fluids (e.g. urine) or returned to the intestinal lumen via enterohepatic curculation (Hoikkala et al., 2003). Conjugated forms of mammalian lignans are poorly absorbed by the intestine and they must be cleaved by microbial enzymes such as β-glucuronidase for optimal absorption (Jenab and Thompson, 1996; Raffaelli et al., 2002). Fermentation process and activity of intestinal microbiota play important roles in the metabolism and absorption of lignans.

According to Saarinen et al. (2002), there is a fivefold increase in urine excretion of EL in rats fed pure EL compared with those fed plant lignans, suggesting that absorption of mammalian lignans is higher than that of plant lignans. Thus, the transfer of EL into milk could be interesting for consumers keen to improve their health by consumption of EL enriched dairy products.

A previous study showed a linear increase in milk EL concentration when dairy cows are fed a ration with increased levels of flaxseed meal (Petit et al., 2009). Côrtes et al. (2008) showed that SDG from flax (seed and hulls) was converted to mammalian

lignan by *in vitro* ruminal and faecal microbiota of dairy cows, and that the main metabolite from flax hulls produced by ruminal microbiota was EL.

Greater absorption of mammalian lignans may contribute to improve human health as it has been shown that people with higher blood concentrations of EL have lower incidence of cardiovascular diseases (Vanharanta et al. 1999). On the other hand, feeding polyunsaturated fatty acids is known to modify ruminal microbiota (Henderson, 1973; Maia et al., 2007), which could interfere with the metabolism of flax lignans and affect the concentration of its metabolites in biological fluids.

Therefore, the objective of the present study was to evaluate total digestibility of diets, milk production and composition, and EL concentration in ruminal fluid, plasma, urine and milk of dairy cows fed a ration with or without flax hulls, and with three levels of abomasal flax oil infusion.

MATERIALS AND METHODS

Animals and Treatments

The experiment was conducted at Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada. Six multiparous lactating Holstein cows fitted with ruminal cannulas (10 cm, Bar Diamond Inc., Parma, ID) were used in a 2 × 3 factorial arrangement of treatments and six 21-d periods balanced for residual effect. Cows averaged 95 ± 20 DIM at the start of the experiment with an average BW of 650 ± 36, and an average BCS of 3.00 ± 0.15 (5-points scale; Edmonson et al., 1989). Cows were kept in individual stalls and had free access to water. Cows were milked twice a day at 0830 and 2000 h. All cows were fed for ad libitum intake (10 % refusals on as fed basis) twice a day (0830 and 1430 hours). The diets (Table 1) were formulated to meet requirements for cows that were 575 kg of BW and producing 35 kg/d of milk with 3.8% fat (NRC, 2001). The experimental protocol complied with the Guide to Care and Use of Experimental Animals (CCAC, 1993) and was approved by the local animal care committee.

Treatments were flax hulls supplementation (without: CON vs. with 15.9% in the DM: FHU) and 3 different amounts of flax oil infused in the abomasum (0, 250 and 500 g/d). During the first seven days of each 21-d period, only 30 % of the experimental dose of oil was infused in the abomasum over a 7-h period. From d 8 to d 21, infusion in the abomasum was conducted with 100 % of the experimental dose of oil over a 23-h period. The appropriate amount of oil used for infusion was prepared daily for each cow

and was weighed into tarred bottles. To perform abomasal infusions, an infusion line was inserted through the rumen cannula and the sulcus omasi into the abomasum as described by Gressley *et al.* (2006). Placement of the infusion lines was monitored daily to ensure postruminal delivery. Solutions were pumped into the abomasum by using peristaltic pumps (Masterflex L/S; Cole-Parmer Canada Inc., Montreal, QC, Canada).

Table 1. Ingredient composition and chemical analysis of the total mixed ration

	Rations	
	CON	FHU
Ingredient, % of DM		
Grass silage	31.8	30.3
Corn silage	32.1	30.5
Cracked corn grain	11.7	5.86
Ground barley	8.09	7.57
Soybean meal	10.8	4.49
Flaxseed hull[1]	0.00	15.9
Top supplement[2]	2.77	2.78
Mineral[3]	2.41	2.27
Ca carbonate	0.39	0.37
Chemical analysis		
DM, %	42.0	45.1
CP, % of DM	16.5	16.2
Ether extract, % of DM	2.47	7.32
NDF, % of DM	32.4	31.5
ADF, % of DM	21.7	21.4
SDG, %	0.01	0.23
NE_L, Mcal/kg of DM[4]	1.58	1.61
Fatty acids, % of total fatty acids		
4:0	0.40	0.17
6:0	0.29	0.00
12:0	0.71	0.33
*cis*11-12:1	0.54	0.25
14:0	0.63	0.12
15:0	0.22	0.05
16:0	16.5	9.32
*cis*9-16:1	0.33	0.02
17:0	0.18	0.08
18:0	1.88	2.23
*cis*9-18:1	17.0	18.1
*cis*11-18:1	1.07	0.99
*cis*9,12-18:2	45.7	24.4
*cis*6,9,12-18:3	0.08	0.00
*cis*9,12,15-18:3	13.1	43.2
20:0	0.22	0.30
*cis*11-20:1	0.59	0.00
*cis*11,14-20:2	0.08	0.03
22:0	0.20	0.18

24:0	0.33	0.26
MUFA[5]	19.6	19.5
PUFA[5]	59.0	67.9
SFA[5]	21.5	13.1
PUFA/SFA	2.75	5.19
SCFA[5]	1.94	0.75
MCFA[5]	17.7	9.55
LCFA[5]	80.3	90.1
n3[6]	13.1	43.4
n6[7]	45.9	24.5
n6:n3	3.55	0.57

[1]Mean of six samples that were prepared by compositing seven daily samples collected and accumulated during the digestibility trial. Contained 29.8% ether extract, 23.7% CP, 14.25% ADF and 19.2% NDF, 0.99% SDG of DM.
[2]Contained 20% of canola meal, 30% of corn gluten meal, 20% of soybean meal, and 30% of brewer's corn.
[3]Contained 9.02% Ca, 4.90% P, 4.89% Mg, 1.76% S, 14% Na, 1.43% K, 2068 mg/kg Fe, 2718 mg/kg Zn, 447 mg/kg Cu, 1814 mg Mn, 69 mg/kg I, 7 mg/kg Co, 20 mg/kg Se, 452,000 IU/kg of vitamin A, 58,000 UI/kg of vitamin D_3, and 2692 IU/kg of vitamin E.
[4]Calculated using published values of feed ingredients (NRC, 2001).
[5]MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids.
[6]*cis*9,12,15-18:3 + *cis*11,14,17-20:3.
[7]*cis*9,12-18:2 + *cis*11,14-20:2.

Sampling

Cows were weighted on three consecutive days at the beginning and the end of each period after the am milking.

Samples of diets, orts and flax hulls were taken daily from d 14 to d 21 and pooled by cow within period, and stored at -20°C. To predict fecal output and digestibility, a capsule of chromic oxide (Cr_2O_3) was inserted in the rumen once daily at 0900 h from d-11 to d-20 of each period, supplying a total of 10 g of Cr_2O_3/d.

Fecal grab samples were taken twice daily from day 15 to day 19 at 0830 and 1630 h. Fecal samples were dried at 55ºC for 48 h and composited on a DM basis by cow within period for later chemical analysis. Fecal and feed samples were ground through a 1-mm screen in a Wiley mill for further analysis.

During the last week of each period, milk samples were taken from each cow for 14 consecutive milkings and pooled on a yield basis to obtain one composite milk sample per cow within period. One sample was kept frozen without preservative for further analyses of milk fat, FA profile and EL. Another sample was stored at + 4°C

with a preservative (bronopol-B2) until analyzed for protein, urea N and lactose. All samples were frozen at -20°C until subsequent chemical analyses.

On d 20, blood was withdrawn into K_3EDTA-vacutainer tubes (Becton Dickinson and Cie, Rutherford, NJ, USA) from the jugular vein 6 h after the morning meal. Plasma samples were kept frozen at -20^{o}C until lignan analysis. On d 21, samples of urine were taken 2 h after the morning meal and kept frozen at -20^{o}C for lignan analysis. Also, ruminal contents were collected 0, 2, 4, and 6 h after the morning meal from different locations within the rumen (the anterior dorsal, anterior ventral, medium ventral, posterior dorsal, and posterior ventral locations) to obtain a representative sample. Ruminal pH was monitored immediately after sample collection with a portable pH meter (OAKTON; Eutech Instruments, Singapore). The ruminal contents were then strained through four layers of cheesecloth. Ruminal fluid was kept at -20°C and freeze-dried for further analysis of lignans. As previous results (Gagnon *et al.* unpublished results) have shown no variation in ruminal lignan concentration after feeding, ruminal samples for the three postfeeding times (2, 4, and 6 h) were pooled within cow and period to obtain only one composite sample for lignan analysis.

Chemical analysis

Oven-dried (fecal) and freeze-dried (flax hulls and diets) samples were ground through a 1-mm screen in a Wiley mill for later analysis. Dry matter of diets was determined in a forced-air oven according to the procedure 934.01 (AOAC, 1990). Total N content of TMR and feces was determined by thermal conductivity (LECO model FP-428 Nitrogen Determinator, LECO, St. Joseph, MI) and CP was calculated as N x 6.25. The concentration of NDF in TMR and feces was determined as described by Van Soest et al. (1991) without the use of sodium sulfite and with the inclusion of heat stable α-amylase. The ADF content in TMR and feces was determined according to AOAC (1990; Method 973.18). The NDF and ADF procedures were adapted for use in an ANKOM^{200} Fibre Analyzer (ANKOM Technology Corp., Fairport, NY). Ether extract concentration in diets and feces were conducted with Tecnal TE–044/1 (Piracicaba, São Paulo, Brazil) according to the method No. 7.060 (AOAC, 1990). Protein, lactose, total solids, and urea N concentrations in milk samples were analyzed by infrared spectrophotometer (System 4000 Milkoscan; Foss Electric of Hillerod, Denmark). Milk fat concentration was determined by the method of Roese-Goettlib (AOAC, 1990). Milk fat was extracted and FA were methylated according to the method described by

Chouinard et al. (1997) while in situ transesterification was performed on diets according to Park and Goins (1994). Fatty acid methyl ester profiles were measured by GLC on a Hewlett-Packard 6890 chromatograph (Hewlett-Packard Lteé, Montreal, Qc, Canada) with a G1315A auto sampler equipped with a flame ionization detector and a split-splitless injector as described by Delbecchi et al. (2001).

Lignan extraction

Lignans in ruminal fluid, plasma, urine, and milk samples were hydrolysed and extracted according to the method of Frank and Custer (1996) with some modifications. Freeze-dried samples of filtered ruminal fluid were resuspended in Milli-Q purified water (20 mg/ 0·5 ml) as decribed by Heinonen et al. (2001). Five hundred microliters of warmed (40°C) milk and resuspended ruminal samples (0 h and pooled postfeeding samples) were mixed with 5 µl of ß-glucuronidase/arylsulfatase from *Helix pomatia* (Roche-Diagnostics, Laval, QC, Canada) while 500 µl of plasma and urine were mixed with 500 µl of 0·1 mol/l sodium acetate buffer (pH 5) and 5 µl of ß-glucuronidase/arylsulfatase. Milk samples were incubated for 1·5 h while plasma, urine, and ruminal samples were incubated overnight at 37°C in a shaking waterbath. After hydrolysis, all samples were acidified with 10 µl of 6 N HCl. Acidified milk samples were washed with 3 ml of hexane before extraction to remove lipids (Raffaelli et al., 2002). All samples were extracted with 2 ml of diethyl ether. The samples were vortex-mixed twice for 2 min. Organic layer was separated by freezing. The remaining liquid phase was submitted to a second extraction under the same conditions. The organic layers were pooled and evaporated by vacuum (Speed-Vac; Thermo Savant, Holbrook, NY, USA) at room temperature for 40 min. The dry extract was redissolved in 500 µl of EIA buffer and warmed at 37°C for EL analysis using an enzyme immunoassay kit (EIA; Cayman Chemical, Ann Arbor, MI, USA). The kit is a competitive assay that utilizes a standard curve ranging from 15.6 to 2 000 pg/mL. The assay exhibits a limit of quantification (defined as 80% B/B_0) of 70 pg/mL and an IC50 (50% B/B_0) of 240 pg/mL.

Statistical analysis

All results were analyzed using the MIXED procedure of SAS (2000) within a 2 x 3 factorial arrangement of treatments. Data on DMI, digestion, milk production, milk

composition and fatty acids in milk were analyzed using a 6 X 6 Latin square design with the following general model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

Where: Y_{ijkl} = dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 6), P_j = fixed effect of period (j = 1 to 6), T_k = fixed effect of treatment (l = CON-0, CON-250, CON-500, FHU-0, FHU-250, FHU-500), and e_{ijk} = random residual error. The residual effect was initially included in the model but was removed when it was not significant. Treatments were compared to provide factorial contrasts: 1) with vs. without flaxseed hulls, 2) with vs. without abomasal flax oil infusion, 3) linear and quadratic effects of 0, 250 and 500 g/d of abomasal flax oil, and 4) the interaction between flaxseed hulls and abomasal flax oil infusion. Significance was declared at $P < 0.05$ and a trend at $P < 0.10$, unless otherwise stated. When a significant *F*-test was detected, multiple comparisons were done using a Tukey's adjustment for the probability. Data on enterolactone concentrations were transformed (log) as previously performed by Gagnon et al. (2009). All results were analyzed according to a 6 x 6 Latin square design; a value was missing for two treatments (CON-0 and CON-500), therefore these treatments had five repetitions instead six.

RESULTS AND DISCUSSION

Dry Matter Intake and Diet Apparent Digestibility

There was an interaction ($P = 0.04$) between flax hulls and flax oil for DMI (% of BW) as shown in Table 2. Flax hulls supplementation in the diet decreased DMI ($P < 0.0001$), and a quadratic reduction in DMI was observed as the amounts of flax oil infused in the abomasum increased (Table 2). Effects of flax oil and hulls supplementation on DMI depression were sharply observed in dairy cows, whereas cows fed the CON-0 treatment (diet without flax products) showed the highest mean value of DMI. According to previous results, abomasal infusion of unsaturated LCFA also decreased DMI (Christensen et al., 1994; Benson et al., 2001; Litherland et al., 2005). Higher concentrations of triglycerides and free fatty acids reaching the small intestine are correlated with increased plasma concentrations of gut hormones such as cholecystokinin and glucagon-like peptide-1, which may lead to satiety and also reduce gut motility (Choi and Palmquist, 1996; Benson and Reynolds, 2001; Litherland et al., 2005). This may likely explain the depression in DMI of cows supplemented with fat from flax hulls and infused with increased levels of flax oil infused in the abomasum.

Table 2. Dry matter intake and total digestibility in cows supplemented or not with flax hulls and increased levels of flax oil infused in the abomasum (0, 250 or 500 g/d) in dairy cows

	Control (CON)			Flax hulls (FHU)				*P* value			
	0	250	500	0	250	500	SE[1]	Flax hulls	Flax oil	L/Q[2]	Interaction
DMI (kg/d)	22.8	20.8	20.2	20.6	19.4	19.4	0.63	< 0.0001	< 0.0001	LQ	0.06
DMI (% BW)	3.5 a	3.2 b	3.1 b	3.2 b	3.0 b	3.0 b	0.09	< 0.0001	< 0.0001	LQ	0.04
Infused Flax oil, g/d	0	256	521	0	258	497					
Total digestibility	% of DM										
DM (%)	70.3	68.7	68.9	70.4	71.1	72.5	1.52	0.02	0.71		0.24
CP	67.1	65.4	65.5	72.0	72.0	72.9	1.77	< 0.0001	0.75		0.55
EE	82.6 c	89.0 b	89.8 b	92.9 a	94.3 a	95.2 a	0.76	< 0.0001	< 0.0001	LQ	0.0007
NDF	42.2	46.5	46.2	44.7	43.2	41.7	2.33	0.20	0.68		0.09
ADF	38.1	41.6	43.3	43.2	42.0	41.4	2.22	0.40	0.61		0.14

[1]Least squares means with pooled standard error (SE). [2]L = Linear and Q = Quadratic (L/Q). Different letters in the same row are different by Tukey test ($P < 0.05$).

Total digestibilities of DM and CP of both rations were not affected by levels of abomasal flax oil infusion ($P > 0.05$), but feeding FHU ration resulted in higher ($P < 0.05$) digestibilities of DM and CP than when feeding the CON ration (Table 2). Partial replacement (Table 1) of cracked corn grain and soybean meal (CON ration) by flax hulls (FHU ration) may have contributed to improve digestibility due to flax hulls containing more digestible nutrients than corn and soybean meal. Similar responses were observed by Gonthier et al. (2004) when late-lactating dairy cows fed a ration with flaxseed (12.5% of DM) showed higher digestibilities of DM and CP than those fed a ration without flaxseed.

There was an interaction ($P < 0.001$) between flax hulls and oil for ether extract (EE) apparent digestibility. Flax hulls supplementation improved EE total digestibility ($P < 0.0001$), which may also be due to partial replacement of cracked corn grain by flax hulls that resulted in a ration with higher concentration of EE (Table 1). Increasing levels of abomasal flax oil infusion improved EE total digestibility of dairy cows fed the CON ration (Table 2).

Neither flax hulls supplementation nor abomasal flax oil infusion affected total digestibilities of NDF and ADF, which averaged 44.1 and 41.6%, respectively.

Milk Production and Composition

There was no interaction ($P > 0.05$) between flax hulls and oil for milk production and composition. Supplementation of flax hulls reduced yields of milk, 4% FCM, protein, fat, total solids and lactose (Table 3). These results are in agreement with the depression in DMI for dairy cows fed the FHU ration (Table 2).

Although CP digestibility of the FHU ration was higher than that of the CON ration ($P < 0.001$), there was a reduction in milk urea N ($P < 0.04$), suggesting that N utilization by cows fed the FHU ration was better than that of cows fed the CON ration.

Higher levels of abomasal flax oil infusion tended ($P < 0.06$) to increase milk fat concentration, and there was also a quadratic increase in concentration of total solids in milk (Table 3). This is in agreement with the results of Drackley et al. (2007) who observed an increase in milk fat and total solid concentration of dairy cows as the amount of high oleic sunflower FA infused in the abomasum increased.

Table 3. Milk production and composition of dairy cows supplemented or not with flax hulls and increased levels of flax oil infused in the abomasum (0, 250 or 500 g/d) in dairy cows

	Control (CON)			Flax hulls (FHU)				*P* value			
	0	250	500	0	250	500	SE[1]	Flax hulls	Flax oil	L/Q[2]	Interaction
	kg/d										
Milk	35.1	34.9	34.0	33.9	32.6	32.4	1.71	0.009	0.24		0.75
4% FCM	27.5	27.0	28.0	25.5	24.7	25.7	1.61	0.002	0.38		0.97
Protein	1.15	1.13	1.12	1.05	1.02	1.06	0.05	0.0007	0.67		0.69
Fat	0.90	0.90	0.96	0.80	0.78	0.85	0.07	0.01	0.17		0.98
Total solids	3.98	3.93	3.98	3.73	3.60	3.71	0.17	0.0004	0.45		0.86
Lactose	1.59	1.59	1.57	1.55	1.48	1.49	0.05	0.01	0.40		0.57
	%										
Protein	3.29	3.24	3.29	3.16	3.14	3.29	0.09	0.14	0.28		0.58
Fat	2.58	2.49	2.81	2.38	2.38	2.65	0.14	0.15	0.06		0.93
Total solids	11.41	11.27	11.71	11.11	11.04	11.54	0.19	0.08	0.02	LQ	0.92
Lactose	4.56	4.57	4.63	4.60	4.55	4.63	0.09	0.72	0.11		0.62
Urea N, mg/dL	11.13	12.48	12.96	10.95	10.50	11.32	0.86	0.04	0.32		0.41
SCS[3]	2.47	1.98	2.04	2.62	2.49	3.56	0.74	0.06	0.46		0.31

[1]Least squares means with pooled standard error (SE). [2]L = Linear and Q = Quadratic (L/Q). [3] Somatic Cell Score = $\log_{10}$SCC

Table 4. Milk fatty acid profile (% of total fatty acids) of dairy cows supplemented or not with flax hulls and increased levels of flax oil infused in the abomasum (0, 250 or 500 g/d) in dairy cows

	Treatments							*P-value*			
	Control (CON)			Flax hulls (FHU)							
	0*	250*	500*	0*	250*	500*	SE[1]	Flax hulls	Flax oil	L/Q[2]	Interaction
4:0	4.89	4.84	4.50	4.70	5.00	4.65	0.25	0.72	0.11		0.47
5:0	0.04	0.05	0.05	0.04	0.05	0.04	0.01	0.75	0.44		0.56
6:0	2.36	2.56	2.42	1.93	2.14	2.04	0.12	< 0.0001	0.10	Q	0.96
7:0	0.10	0.10	0.10	0.05	0.07	0.08	0.02	0.01	0.59		0.68
8:0	1.39	1.54	1.50	1.05	1.16	1.13	0.06	< 0.0001	0.06		0.92
9:0	0.10	0.11	0.10	0.05	0.09	0.09	0.01	0.02	0.08		0.35
10:0	3.28	3.66	3.60	2.08	2.36	2.32	0.18	< 0.0001	0.04	L	0.90
11:0	0.12	0.13	0.12	0.10	0.10	0.10	0.01	0.002	0.70		0.37
12:0	4.05	4.24	4.03	2.49	2.54	2.51	0.18	< 0.0001	0.58		0.78
*cis*11-12:1	0.19	0.21	0.17	0.15	0.13	0.13	0.02	0.001	0.30		0.29
13:0	0.16	0.16	0.14	0.14	0.12	0.12	0.01	0.0002	0.04	L	0.22
14:0	13.25	11.69	10.79	9.24	8.29	7.81	0.32	< 0.0001	< 0.0001	L	0.13
*cis*9-14:1	1.42	0.85	0.69	1.15	0.62	0.59	0.17	0.02	< 0.0001	LQ	0.66
15:0	1.30	1.02	0.91	0.99	0.80	0.75	0.04	< 0.0001	< 0.0001	LQ	0.14
16:0	32.40 a	25.80 b	22.66 c	19.94 d	16.96 e	16.19 e	0.68	< 0.0001	< 0.0001	LQ	< 0.0001
*trans*9-16:1	0.07 bc	0.07 bc	0.06 c	0.14 a	0.09 b	0.09 b	0.01	< 0.0001	0.0003	L	0.002
*cis*9-16:1	2.08	1.35	1.02	1.61	0.94	0.89	0.16	0.001	< 0.0001	LQ	0.25
17:0	0.63	0.58	0.49	0.51	0.45	0.41	0.02	< 0.0001	< 0.0001	L	0.39
18:0	8.49	9.47	8.91	13.48	14.53	12.75	0.68	< 0.0001	0.08	Q	0.45
*trans*9-18:1	0.25	0.22	0.19	0.49	0.39	0.37	0.02	< 0.0001	0.0008	L	0.17
*trans*11-18:1	0.98	0.98	0.84	2.39	1.88	1.87	0.18	< 0.0001	0.15		0.31
*cis*6-18:1	0.57	0.52	0.58	3.37	2.91	2.81	0.22	< 0.0001	0.31		0.34
*cis*9-18:1	16.34 c	15.39 c	15.44 c	24.93 a	21.34 b	20.76 b	0.81	< 0.0001	0.0006	LQ	0.03
*cis*11-18:1	0.55	0.51	0.53	0.76	0.69	0.68	0.04	< 0.0001	0.09		0.47
19:0	0.20	0.19	0.25	1.55	1.25	1.17	0.11	< 0.0001	0.23		0.12
*trans*9,12-18:2	0.06	0.07	0.01	0.13	0.10	0.11	0.02	< 0.0001	0.12	L	0.13
*cis*9,12-18:2	2.09	4.09	5.33	2.13	3.96	5.00	0.15	0.24	< 0.0001	LQ	0.45

20:0	0.24	0.24	0.29	0.23	0.27	0.21	0.06	0.61	0.92		0.63
*cis*6,9,12-18:3	0.05	0.04	0.04	0.002	0.02	0.01	0.01	0.0008	0.87		0.37
*cis*8-20:1	0.19	0.19	0.16	0.21	0.17	0.17	0.01	0.73	0.04	L	0.15
*cis*11-20:1	0.09	0.14	0.11	0.12	0.13	0.15	0.01	0.07	0.09		0.08
*cis*9,12,15-18:3	0.64	7.38	12.41	1.94	8.80	12.30	0.46	0.01	< 0.0001	LQ	0.12
*cis*9,*trans*11-18:2	0.56	0.42	0.38	1.24	0.76	0.77	0.08	< 0.0001	0.0003	LQ	0.06
*trans*10,*cis*12-18:2	0.01	0.01	0.01	0.01	0.02	0.01	0.005	0.14	0.76		0.17
*cis*11,14-20:2	0.08	0.13	0.11	0.05	0.08	0.11	0.02	0.03	0.03	L	0.19
22:0	0.11	0.13	0.15	0.10	0.13	0.11	0.03	0.46	0.59		0.78
*cis*8,11,14-20:3	0.11	0.10	0.08	0.08	0.07	0.07	0.01	0.002	0.02	L	0.30
*cis*11,14,17-20:3	0.05	0.20	0.22	0.08	0.18	0.19	0.02	0.68	< 0.0001	LQ	0.32
*cis*5,8,11,14-20:4	0.13	0.13	0.12	0.10	0.10	0.11	0.01	0.001	0.91		0.54
*cis*5,8,11,14,17-20:5	0.11 bc	0.15 b	0.20 a	0.09 c	0.11 bc	0.12 bc	0.01	< 0.0001	0.0003	L	0.02
*cis*13,16-22:2	0.05	0.05	0.02	0.01	0.02	0.02	0.01	0.03	0.37		0.31
*cis*7,10,13,16,19-22:5	0.17	0.23	0.20	0.14	0.17	0.18	0.02	0.05	0.15		0.56
24:0	0.05	0.06	0.07	0.003	0.01	0.01	0.01	< 0.0001	0.27		0.76
Total *trans*	1.93	1.77	1.49	4.40	3.24	3.22	0.26	< 0.0001	0.009	L	0.13
MUFA[3]	22.73	20.43	19.79	35.32	29.29	28.51	1.19	< 0.0001	< 0.0001	LQ	0.07
PUFA[3]	4.11	13.00	19.13	6.00	14.39	19.00	0.61	0.02	< 0.0001	LQ	0.16
SFA[3]	73.16 a	66.57 b	61.08 c	58.67 c	56.32 cd	52.49 d	1.45	< 0.0001	< 0.0001	L	0.04
PUFA/SFA	0.06	0.20	0.31	0.10	0.26	0.36	0.02	0.0002	< 0.0001	L	0.92
SCFA[3]	16.68	17.60	16.73	12.78	13.76	13.21	0.69	< 0.0001	0.21		0.93
MCFA[3]	51.15 a	41.36 b	36.62 c	33.58 d	28.15 e	26.73 e	0.81	< 0.0001	< 0.0001	LQ	< 0.0001
LCFA[2]	32.17 e	41.04 d	46.65 c	53.64 b	58.09 a	60.06 a	1.13	< 0.0001	< 0.0001	L	0.0006
n-3[4]	0.97	7.96	13.03	2.25	9.26	12.79	0.46	0.02	< 0.0001	LQ	0.10
n-6[5]	2.51	4.55	5.70	2.37	4.25	5.32	0.15	0.03	< 0.0001	LQ	0.63
n-6:n-3	2.59 a	0.57 c	0.44 c	1.05 b	0.46 c	0.42 c	0.08	< 0.0001	< 0.0001	LQ	< 0.0001

[1]Least squares means with pooled standard error (SE). [2]L = Linear and Q = Quadratic (L/Q). [3]MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids. [4]*cis*9,12,15-18:3 + *cis*11,14,17-20:3 + *cis*5,8,11,14,17-20:5 + *cis*7,10,13,16,19-22-5. [5]*cis*9,12-18:2 + *cis*6,9,12-18:3 + *cis*11,14-20:2 + *cis*8,11,14-20:3 + *cis*5,8,11,14-20:4 + *cis*13,16-22:2. Different letters in the same row are different by Tukey test ($P < 0.05$).

Milk Fatty Acids (FA) Profile

Milk FA profile of dairy cows fed the CON-0 treatment is in general agreement with the typical milk fat containing approximately 5% of PUFA, 70% of saturated fatty acids (SFA), and 25% of monounsaturated fatty acids (MUFA) (Grummer, 1991). There was an interaction ($P < 0.05$) between flax hulls and flax oil for medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA) and SFA concentrations of milk fat (Table 4). Cows fed the FHU ration presented lower concentrations of short-chain fatty acids (SCFA), MCFA and SFA, and higher concentrations of LCFA, MUFA and PUFA in milk fat (Table 4). A reduction in SCFA and MCFA concentrations of milk fat is typically observed following supplementation of LCFA in the diet (Grummer, 1991; Dhiman et al., 1995; Gonthier et al., 2005).

There was an interaction ($P < 0.0001$) between flax hulls and oil for 16:0 concentration in milk fat (Table 4). Flax hulls supplementation and abomasal flax oil infusion decreased milk fat concentrations of 14:0 and 16:0 probably by enhancing those of LCFA, MUFA and PUFA (Table 4).

Flax hulls likely supplied a greater amount of oleic (*cis*11-18:1), linoleic (*cis*9,12-18:2, LA) and linolenic (*cis*9,12,15-18:3, LNA) acids in the rumen than the control diet. Moreover, cows fed flax hulls had higher concentrations of 18:0, *cis*9-18:1, *trans* dienes (*cis* 9,*trans* 11-18:2-CLA and *trans* 9,12-18:2), *trans* monoenes (*trans* 9-18:1 and *trans* 11-18:1) and total *trans* in milk fat, thus suggesting that flax hulls supplementation increased 18-carbon FA in milk fat by ruminal biohydrogenation of PUFA, mainly for *cis*9,*trans*11-CLA and *trans*11-18:1 that are intermediates of complete biohydrogenation of LA to stearic acid (Tanaka, 2005). According to Chilliard et al. (2000), flax oil greatly increases milk fat CLA content, which is accompanied by a large increase in production of ruminal *trans*11-18:1, that can be desaturated by delta-9 desaturase enzyme in the mammary gland for CLA synthesis.

In contrast, increased amounts of abomasal flax oil infusion provided a post-rumen delivery of fat and lower concentrations of milk fat CLA and total *trans* fatty acids, resulting in higher values of LA and LNA, linear increase in concentrations of LCFA and quadratic increase in PUFA concentration (Table 4).

There was an interaction ($P < 0.0001$) between flax hulls and flax oil for the n-6:n-3 FA ratio in milk fat (Table 4). Flax hull supplementation increased concentrations of n-3 FA ($P = 0.02$) in milk fat and decreased those of n-6 FA ($P = 0.03$), thus decreasing the n-6:n-3 ratio (Table 4). Increasing levels of abomasal flax oil infusion

resulted in a quadratic increase of both n-3 and n-6 FA ($P < 0.0001$) concentrations in milk fat, and a quadratic reduction in the n-6:n-3 FA ratio in milk fat (Table 4). However, all treatments resulted in a n-6:n-3 FA ratio less than 4:1, which is the ideal ratio to reduce potential risk of coronary heart diseases and improve human health (Sim, 1998).

Enterolactone (EL)

Dairy cows fed the FHU ration showed higher values of EL concentrations ($P < 0.0001$) in ruminal fluid before feeding (T0) and postfeeding than those fed the control diets (Table 5). According to the literature, other feeds such as forages and cereals also contain lignans (Penalvo et al., 2005; Steinshamm et al., 2008), which may lead to EL synthesis and could explain the presence of EL in ruminal fluid of dairy cows fed the CON ration. Ruminal pH (Figure1) of cows fed the FHU ration was lower ($P < 0.01$) than that of cows on the CON ration; however all pH values were within a normal range of 6.1 to 6.3. Similarly, Zhou et al. (2009) infused the plant lignan SDG in the rumen of goats and observed an increase in ruminal EL concentration and a depression in ruminal pH.

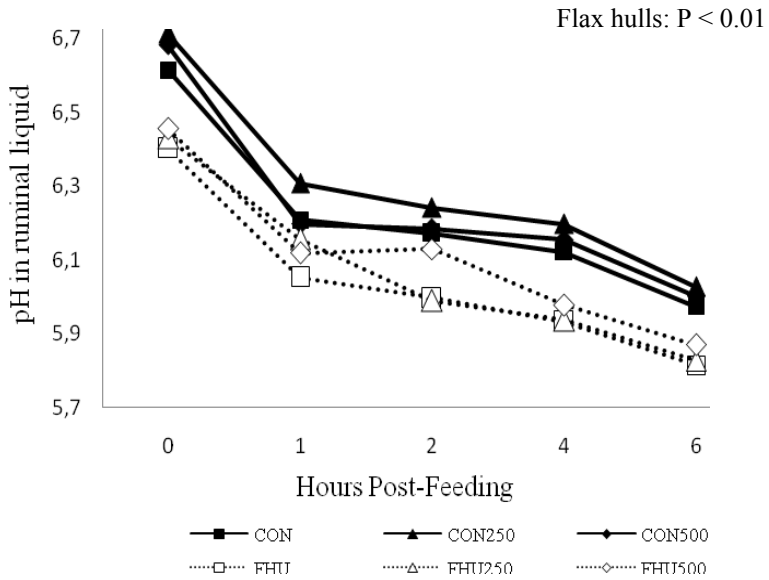


Figure 1. Ruminal pH of dairy cows fed a ration without (CON) or with (FHU) flax hulls and with increased amounts of flax oil infused in the abomasum (0, 250 and 500 g/d).

Table 5. Adjusted mean values of enterolactone concentration (EL, nmole/L) with their confidence intervals (*italic numbers*) in plasma, urine, milk and ruminal liquor in Holstein cows in factorial arrangement 2X3: two diets (Control or Flaxseed hulls) and three doses of flaxseed oil infused in the abomasum (0, 250 or 500 g/d)

	Treatments						*P-value*		
	Control (CON)			Flaxseed hulls (FHU)			Flaxseed hulls	Flaxseed oil	Interaction
	0	250	500	0	250	500			
EL LR T0	3935 *2431 – 6370*	4779 *2952 - 7736*	4342 *2682 - 7029*	17883 *11047 - 28948*	11026 *6811- 17848*	13649 *8432 - 22094*	<.0001	0.63	0.11
EL LR Pool	6573 *5027 - 8593*	7309 *5590 - 9557*	7195 *5503 - 9407*	19937 *15249 - 26068*	18157 *13887 - 23740*	15234 *11652 - 19918*	<.0001	0.49	0.16
EL plasma	493 *339 – 718*	468 *321 - 681*	481 *330 - 700*	1721 *1182 - 2505*	1343 *923 - 1955*	1333 *915 - 1940*	<.0001	0.64	0.78
EL urine	40135 *25596 - 62930*	27077 *17269 - 42456*	46280 *29515 - 72565*	291569 *185953 -457174*	313498 *199938 -491557*	200523 *127886 -314416*	<.0001	0.70	0.07
EL milk	39.3 *20.2 – 76.3*	47.2 *24.3 – 91.8*	49.2 *25.3 – 95.6*	309 *159 - 601*	299 *154 - 582*	321 *165 - 625*	<.0001	0.86	0.89

Concentration of EL increased 3-fold in plasma, 6.8-fold in milk, and 7.1-fold in urine of dairy cows fed FHU ration compared to those fed the CON ration. Petit et al. (2009) reported that EL concentration in milk increased linearly with greater amount of flaxseed meal fed to dairy cows.

Although higher levels of flax oil infused in the abomasum decreased DMI (Table 2) and consequently flax hulls intake, concentrations of EL in milk of cows fed the FHU ration were not affected, thus suggesting that the metabolism of flax lignans in the intestine is not affected by the amount of flax oil present in the small intestine of dairy cows fed flax hulls.

CONCLUSIONS

Dry matter intake was decreased by flax hulls supplementation and increased amount of flax oil infused in the abomasum. Feeding flax hulls improved digestibility of the diet but reduced milk yield of cows. Flax hulls and flax oil increased concentrations of polyunsaturated and long-chain fatty acids and decreased concentrations of saturated fatty acids and the n-6:n-3 FA ratio in milk fat, which would improve milk quality and its nutritive value for better human health. Dairy cows fed flax hulls produced a milk enriched in enterolactone. Greater amounts of flax oil infused in the abomasum had no effect on milk enterolactone concentration and presence of polyunsaturated fatty acids in the intestine had no effect on metabolism of lignans.

REFERENCES

Aldercreutz, H. and W. Mazur. 1997. Phyto-oestrogens and western diseases. Ann. Med. 29:95-120.

Adlercreutz, H. 2007. Lignans and human health. Crit. Rev. Clin. Lab. Sci. 44:483-525.

AOAC. 1990. Official Methods of Analysis. 15th ed. AOAC, Washington, DC.

Barnes, S., J. Sfakianos, L. Coward, and M. Kirk. 1996. Soy isoflavonoids and cancer prevention. Adv. Exp. Med. Biol. 401:87-100.

Benson, J. A., and C. K. Reynolds. 2001. Effects of abomasal infusion of long-chain fatty acids on splanchnic metabolism of pancreatic and gut hormones in lactating dairy cows. J. Dairy Sci. 84:1488–1500.

Benson, J. A., C. K. Reynolds, D. J. Humphries, S. M. Rutter, and D. E. Beever. 2001. Effects of abomasal infusion of long-chain fatty acids on intake, feeding behavior and milk production in dairy cows. J. Dairy Sci. 84:1182–1191.

Borriello, S. P., K. D. R. Setchell, M. Axelson, and A. M. Lawson. 1985. Production and metabolism of lignans by the human faecal flora. J. Appl. Bacteriol. 58:37-43.

CCAC - Canadian Council on Animal Care. 1993. Guide to Care and Use of Experimental Animals. Vol.1, E. D. Offert, B. M. Cross and A. A. McWilliam, ed. CCAC, Ottawa, ON, Canada.

Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Ann. Zootech. 49:181–205.

Choi, B. R., and D. L. Palmquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating dairy cows. J. Nutr. 126:2913–2919.

Chouinard, P. Y., V. Girard, and G. J. Brisson. 1997. Performance and profiles of milk fatty acids of cows fed full-fat, heat-treated soybeans using various processing methods. J. Dairy Sci. 80:334-342.

Christensen, R. A., J. K. Drackley, D. W. LaCount, and J. H. Clark. 1994. Infusion of four long-chain fatty acid mixtures into the abomasum of lactating dairy cows. J. Dairy Sci. 77:1052–1069.

Côrtes, C., N. Gagnon, C. Benchaar, D. da Silva, G. T. D. Santos, and H. V. Petit. 2008. In vitro metabolism of flax lignans by ruminal and faecal microbiota of dairy cows. J. Appl. Microbiol. 105:1585-1594.

Delbecchi, L., C. E. Ahnadi, J.J. Kennelly, and P. Lacasse. 2001. Milk fatty acid composition and mammary lipid metabolism in Holstein cows fed protected or unprotected canola seeds. J. Dairy Sci. 84:1375-1381.

Dhiman, T. R., K. V. Zanten, and L. D. Satter. 1995. Effect of dietary fat source on fatty acid composition of cows' milk. J. Sci. Food Agric. 69:101–107.

Drackley, J. K., T. R. Overton, G. Ortiz-Gonzalez, A. D. Beaulieu, D. M. Barbano, J. M. Lynch, and E. G. Perkins. 2007. Responses to increasing amounts of high-oleic sunflower fatty acids infused into the abomasum of lactating dairy cows. J. Dairy Sci. 90:5165-5175.

Edmonson, A. J., Lean, I. J., Weaver, L. D., Farver, T., and Webster, G. 1989. A body condition scoring chart for Holstein for dairy cows. J. Dairy Sci. 72:68-78.

Frank, A. A. and L. J. Custer. 1996. Diadzein and geneistein concentrations in human milk after soy consumption. Clin. Chem. 42:955-964.

Gagnon, N., C. Côrtes, D. Silva, R. Kazama, C. Benchaar, G. Santos, L. Zeoula, and H. V. Petit. 2009. Ruminal metabolism of flaxseed (*Linum usitatissimum*) lignans to the mammalian lignan enterolactone and its concentration in ruminal fluid, plasma, urine and milk of dairy cows. Br. J. Nutr. doi: 10.1017/S0007114509344104.

Gonthier, C., A. F. Mustafa, R. Berthiaume, H. V. Petit, R. Martineau, and D. R. Ouellet. 2004. Effects of Feeding Micronized and Extruded Flaxseed on Ruminal Fermentation and Nutrient Utilization by Dairy Cows. J. Dairy Sci. 87:1854-1863.

Gonthier, C., A. F. Mustafa, D. R. Ouellet, P. Y. Chouinard, R. Berthiaume, and H. V. Petit. 2005. Feeding Micronized and Extruded Flaxseed to Dairy Cows: Effects on Blood Parameters and Milk Fatty Acid Composition. J. Dairy Sci. 88:748-756.

Gressley, T. F., Reynal, S. M., Colmenero, J. J. O., Broderick, G. A., and Armentano, L. E. 2006. Technical note: Development of a tool to insert abomasal infusion lines into dairy cows. J. Dairy Sci. 89:3965-3967.

Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244–3257.

Heinonen, S., T. Nurmi, K. Liukkonen, K. Poutanen, K. Wähälä, T. Deyama, S. Nishibe, and H. Adlercreutz. 2001. In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterediol. J. Agric. Food Chem. 49:3178-3186.

Henderson, C. 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. Cambridge. 81:107-112.

Hoikala, A. A., E. Schiavoni, K. Wahala. 2003. Analysis of phyto-oestrogens in biological matrices. Br. J. Nutr. 89:5-18.

Jansen, G. H. E., I. C. W. Arts, M. W. F. Nielen, M. Müller, P. C. H. Hollman, J. Keijer. 2005. Uptake and metabolism of enterolactona and enterodiol by human colon epithelial cells. Arch. Biochem. Biophysics. 435:74-82.

Jenab, M. and L. U. Thompson. 1996. The influence of flaxseed and lignans on colon carcinogenesis and β-glucuronidase activity. Carcinogenesis. 17:1343-1348.

Litherland, N. B., S. Thire, A. D. Beaulieu, C. K. Reynolds, J. A. Benson, and J. K. Drackley. 2005. Dry matter intake is decreased more by abomasal infusion of unsaturated free fatty acids than by unsaturated triglycerides. J. Dairy Sci. 88:632-643.

Liu, Z., N. M. Saarinen, and L. U. Thompson. 2006. Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed *in vitro* and in rats. J. Nutr. 136:906-912.

Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie Van Leeuwenhoek. 91:303-314.

Murkies, A. L., G. Wilcox and S. R. Davis. 1998. Phytoestrogens. J. Clin. Endocrinol. Metab. 83:297-303.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Nat. Acad. Press, Washington, DC.

Nesbitt, P. D., Y. Lam, and L. U. Thompson. 1999. Human metabolism of mammalian lignan precursors in raw and processed flaxseed. Am. J. Clin. Nutr. 69:549-555.

Park, P. W., and R. E. Goins. 1994. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food Sci. 59:1262-1266.

Penalvo, J. L., K. M. Haajanen, N. Botting, and H. Adlercreutz. 2005. Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry. J. Agric. Food Chem. 53:9342–9347.

Petit, H. V., N. Gagnon, P. Mir, R. Cao, and S. Cui. 2009. Milk concentration of the mammalian lignan enterolactone, milk production, milk fatty acid profile, and digestibility of dairy cows fed diets containing whole flaxseed or flaxseed meal. J. Dairy Res. doi:10.1017/S0022029909003999.

Prasad, K. 1997. Dietary flax seed in prevention of hypercholesterolemic atherosclerosis. Atherosclerosis. 132:69-76.

Raffaelli, B., A. Hoikkala, E. Leppälä, and K. Wähälä. 2002. Enterolignans. J Chromatogr B. 777:29-43.

Saarinen, N. M., A. Smeds, S. I. Mäkelä, J. Ämmälä, K. Hakala, J. M. Pihlava, E. L. Ryhänen, R. Sjöholm, and R. Santti. 2002. Structural determinants of plant lignans for the formation of enterolactone in vivo. J. Chromatogr. B. 777:311–319.

SAS Institute, Inc. 2000. SAS/STAT® user's guide. Release 8.02. Cary NC, USA.

Setchell, K. D., A. M. Lawson, F. L. Mitchell, H. Adlercreutz, D. N. Kirk, and M. Axelson. 1980. Lignans in man and in animal species. Nature. 287:740–742.

Sim, J. S. 1998. Designer eggs and their nutritional and functional significance. World Rev. Nutr. Diet. 23:89–101.

Steinshamn, H., S. Purup, E. Thuen, and J. Hansen-Møller. 2008. Effects of clover-grass silages and concentrate supplementation on the content of phytoestrogens in dairy cow milk. J. Dairy Sci. 91:2715-2725.

Stopper, H., E. Schmitt, and K. Kobras. 2005. Genotoxicity of phytoestrogens. Mutation Res. 574:139-155.

Tanaka, K. 2005. Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. Anim. Sci. J. 76:291-303.

Thompson, L. U. and W. E. Ward. 2002. Flaxseed lignans: health benefits, bioavailability, and safety. In Phytoestrogens and Health. pp. 405-426 [S Gilani and J Anderson, editors], Champaign, IL: AOCS Press.

Thompson, L. U., P. Robb, M. Serraino, and F. Cheung. 1991. Mammalian lignan production from various foods. Nutr. Cancer. 16:43-52.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vanharanta M., S. Voutilainen, T. A. Lakka, M. Van der Lee, H. Adlercreutz, J. T. Salonen. 1999. Risk of acute coronary events according to serum concentrations of enterolactona: a prospective population-based case-control study. Lancet. 354:2112-2115.

Zhou, W., G. Wang, Z. Han, W. Yao, and W. Zhu. 2009. Metabolism of flaxseed lignans in the rumen and its impact on ruminal metabolism and flora. Anim. Feed Sci. Technol. 150:18-26.

CONSIDERAÇÕES FINAIS

Os locais de administração do óleo de linhaça e da casca de linhaça são fatores importantes para os parâmetros de digestibilidade e para a transferência de determinados ácidos graxos para o leite de vacas.

A presença de gordura no rúmen com quantidades acima de 5% da matéria seca da dieta, provenientes da casca e do óleo de linhaça, pode diminuir a ingestão de alimento e consequentemente a produção de leite das vacas. A casca de linhaça mostra-se como fonte proteica e energética de alta digestibilidade.

A suplementação de casca de linhaça na dieta de vacas aumenta a concentração de ácidos graxos mono e poli-insaturados e, reduz a concentração de ácidos graxos saturados na gordura do leite.

O fornecimento de óleo de linhaça diretamente no abomaso reduz a concentração de ácidos graxos saturados na gordura do leite. A presença ou associação entre casca e óleo de linhaça proporciona um aumento na concentração de ácido alfa-linolênico, reduzindo assim, a razão ômega-6/ômega-3 da gordura do leite.

Vacas alimentadas com casca de linhaça na dieta promove um aumento de quase sete vezes a concentração de enterolactona no leite. A presença de óleo poli-insaturado no rúmen e no abomaso não interfere o metabolismo das lignanas, não prejudicando desta forma, a transferência de enterolactona para o leite.

O rúmen apresenta-se como principal local para o metabolismo das lignanas, sendo assim, para experimentos futuros, o principal alvo de manipulação com intuito de melhorar a eficiência de transferência de enterolactona para o leite de vacas leiteiras seria a microbiota ruminal. Além disso, tendo em vista que a enterolactona é um potente antioxidante, pesquisas futuras poderão determinar o poder antioxidante de um leite enriquecido, ao mesmo tempo, em ácidos graxos poli-insaturados e enterolactona.

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