UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

FONTES DE LIPÍDIOS EM VACAS NO PERÍODO DE TRANSIÇÃO

Autor: Rodolpho Martin do Prado
Orientador: Prof^{o} Dr. Ivanor Nunes do Prado
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MARINGÁ
Estado do Paraná
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Dissertação apresentada como parte das exigências para obtenção do título de MESTRE EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá – Área de Concentração Produção Animal.

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TITULAÇÃO: Mestre em Zootecnia - Área de Concentração Produção Animal

APROVADA em 04 de abril de 2012.

Prof. Dr. Júlio Cesar Damasceno

Prof. Dr. Laudí Cunha Leite

Prof. Dr. Geraldo Tadeu dos Santos

"I am enough of an artist to draw freely upon my imagination. Imagination is more important than knowledge. Knowledge is limited. Imagination encircles the world. "

Albert Eistein

A

Deus,

pelo dom da vida.

Ao

meu pai,

por ser minha inspiração profissional.

À

minha mãe,

por cada batalha vencida.

Aos

meus irmãos,

pelas risos e brigas de criança, pelas felicidades e satisfações de adultos.

À

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RODOLPHO MARTIN DO PRADO, filho de Ivanor Nunes do Prado e Marlene Martin do Prado, nasceu em Rennes, França, no dia 15 de janeiro de 1986.

Em dezembro de 2008 concluiu o Curso de Zootecnia pela Universidade Estadual de Maringá.

Em março de 2010 iniciou no mestrado em Produção Animal no Programa de Pós-Graduação em Zootecnia na Universidade Estadual de Maringá.

Em março de 2010 foi contemplado com bolsa no Programme d'Adjoint de Recherche pelo governo do Canadá para estágio no Dairy and Swine Research and Development Centre – Agriculture and Agri-Food Canada, na cidade de Sherbrooke, província do Québec, Canadá, no qual participou de projetos na área de produção em bovinocultura leiteira.

No dia 04 de abril de 2012, submeteu-se à banca para defesa da Dissertação e foi aprovado para receber titulação de Mestre em Produção Animal pelo Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá.

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ADF	acid detergent fiber
BCS	body condition score
BHBA	β-hydroxybutyrate
BW	body weight
CAT	catalase
CLA	conjugated linoleic acid
DM	dry matter
DMI	dry matter intake
EDTA	ethylenediaminotetraacetate
EE	ether extract
FA	fatty acid
GPx	glutathione peroxidase
MEG	diet supplemented with Megalac®
NDF	neutral detergent fiber
NEB	negative energy balance
NEFA	non esterified fatty acids
NE_L	net energy for lactation
PBS	phosphate-buffered saline
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acid
SCC	somatic cell count
SDG	secoisolariciresinol diglucoside
SOD	superoxide dismutase
TG	triglyceride
TMR	total mixed rations
VLDL	very-low-density lipoprotein
WFL	diet supplemented with whole flaxseed
WLO	diet supplemented with whole linola

RESUMO

Foram blocadas 29 vacas Holstein de acordo com a previsão de parto e alocadas em três tratamentos para avaliar o efeito de diferentes fontes de gordura na produção e qualidade do leite, parâmetros sanguíneos e hepáticos em vacas durante o período de transição. Os tratamentos experimentais iniciaram quatro semanas antes da previsão do parto e duraram até 12 semanas depois do parto. Foram formuladas dietas isonitrogênicas e isoenergéticas suplementadas com semente de linhaça (WFL), semente de linola (WLO) ou com uma fonte de sais de cálcio de óleo de palma (MEG). Vacas do tratamento MEG tiveram maior produção leiteira e maior produção diária de lactose, porém estavam em balanço energético negativo mais severo. Vacas no tratamento WLO tiveram maior produção do ácido graxo *cis*-9, *trans*-11 18:2 quando comparado ao tratamento WFL, que foram similar ao tratamento MEG. A produção do ácido graxo *cis*-9, *cis*-12, *cis*-15 foi maior para vacas do tratamento WFL. Não houve efeito de tratamento para os parâmetros plasmáticos. Não houve efeito de tratamento para as concentrações de lipídios totais, triglicerídeos e glicogênio hepático. Ainda, não houve efeito de tratamento para as atividades enzimáticas da superóxido dismutase e glutationa peroxidase, porém vacas do tratamento WFL tiveram maior atividade enzimática da catalase na quarta semana pós-parto. A suplementação da semente de linhaça em vacas de leite no período de transição não foi eficiente em melhorar os parâmetros produtivos, plasmáticos e hepáticos.

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

ABSTRACT

Twenty nine Holstein cows were blocked according to the expecting calving dates and were allocated in three treatments: to evaluate the effect of different fat sources on milk production and quality, and plasmatic and hepatic parameters during the transition period. Cows were fed experimental diets through four weeks before calving to 12 weeks after calving. Diets were isonitrogenous and isoenergetic supplemented with whole flaxseed (WFL), whole linola (WLO) and a source of calcium salts of palm oil. Cows from MEG treatment had higher milk production and higher daily lactose yield, but had the severest negative energetic balance. Cows from WLO treatment had higher production of *cis*-9, *trans*-11 18:2 fatty acids when compared to WFL, but were similar to MEG. The production of *cis*-9, *cis*-12, *cis*-15 was higher for cows from MEG treatment. There was no treatment effect for the plasmatic parameters. There was no treatment effect on the hepatic concentrations of total lipids, triglyceride and glycogen. Still, there was no treatment effect for the enzymatic activities of superoxide dismutase and glutathione peroxidase; however cows from WFL treatment had higher enzymatic activity of catalase on week four postpartum. Supplementing whole flaxseed in dairy cows diets during the transition period was not an effective way to improve production, plasmatic and hepatic parameters.

Key-words: hepatic metabolism, milk quality, omega-3 milk production

INTRODUÇÃO

Os esforços para obtenção de lucro no setor da bovinocultura leiteira focam-se principalmente na maximização da produção de leite e barateamento do custo da ração, tendo pouca consideração com outros custos como, por exemplo, a saúde da vaca. A produção leiteira aumentou substancialmente durante as últimas décadas e médias anuais de 9.000 kg de leite não são mais incomuns. Esses ganhos são resultados de intensa seleção genética, além da nutrição e manejo. Entretanto, além da produção em si, hoje os profissionais da área preocupam-se com baixa fertilidade e problemas de saúde do rebanho. Segundo van Knegsel (2005), entre 1992 e 2002, a média anual da produção de leite aumentou aproximadamente 1.100 kg, enquanto a fertilidade do rebanho diminui cerca de cinco pontos percentuais no mesmo período (Figura 1).

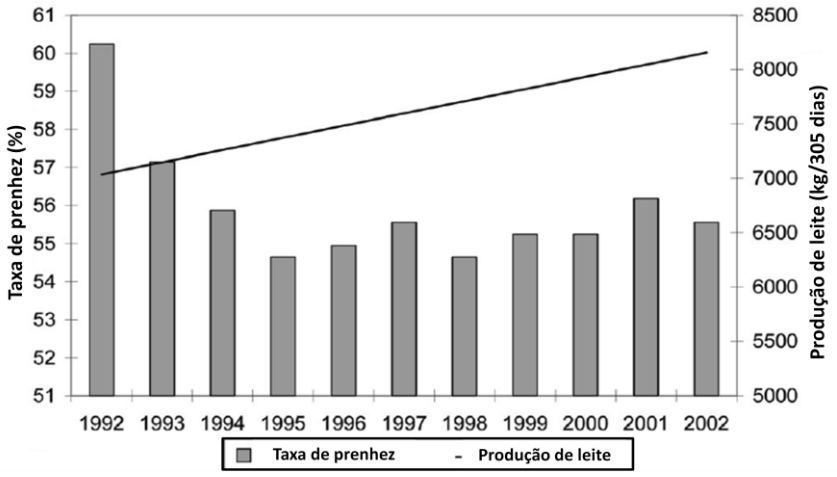

Figura 1. Taxa de fertilidade e produção leiteira anual de vacas leiteiras na Holanda de 1992-2002; n > 1 milhão de partos por ano
Fonte: van Knegsel et al. (2005)

O período de transição em vacas leiteiras, que conceitualmente dura de três semanas antes a três semanas depois do parto (Grummer et al., 1995), é crítico na vida

do animal e tem papel importante na sua saúde, produção e reprodução. Durante este período, o animal está mais suscetível à distocia, febre do leite, cetose, esteatose hepática, retenção de placenta, metrite, mastite e laminite (Drackley, 1999). Limitações nutricionais e de manejo durante esta fase podem impedir a vaca de alcançar seu potencial produtivo. Observa-se na Figura 2 que é nos dias próximos ao parto que se concentram o maior número de doenças. Nesse sentido, Zamet et al. (1979) constataram que vacas que tiveram um ou mais problemas reprodutivos, nutricionais ou de saúde 30 dias antes do parto, ingeriram 19% menos MS durante o período de transição quando comparadas à vacas sadias. O reflexo disso pode ser encontrado no trabalho de Drackley (1999), em que o autor afirma que vacas com qualquer problema de saúde no mesmo período produziram 7,2 kg de leite por dia a menos do que vacas sadias. Assim, com o intuito de melhorar os ganhos da atividade leiteira, deve-se minimizar problemas de saúde, especialmente no período de transição.

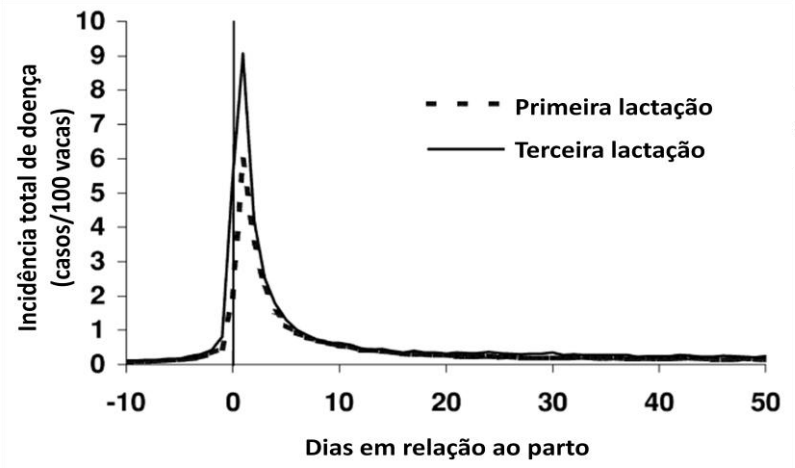

Figura 2. Incidência total de doenças (soma da incidência de mastite, cetose, desordens digestivas e laminite) no período de transição, em vacas de primeira e terceira lactação (n= 93.347 e 58.459, respectivamente).
Fonte: Adaptado de Ingvartsen et al. (2003).

A esteatose hepática é uma doença metabólica que acomete os animais. De acordo com Bobe at al. (2004), ela ocasiona gastos com tratamentos veterinários estimados em U$ 60 milhões ao ano nos Estados Unidos. Sua maior incidência acontece durante as quatro primeiras semanas após o parto, quando aproximadamente 50% das vacas acumulam lipídios no fígado (Jorritsma et al., 2000). A deposição de lipídios no fígado é resultado do excesso de metabolismo de ácidos graxos não-esterificados (AGNE's) provenientes de reservas corporais. Em períodos normais da vida produtiva da vaca, os AGNE's circulantes são absorvidos pelo fígado e em parte são re-

esterificados a triglicerídeos (TG) antes de serem secretados no sangue como lipoproteínas de baixa densidade (Herdt et al., 1988). Entretanto, quando as concentrações de AGNE's intra-hepáticas aumentam rapidamente, decorrente de intensa lipólise, o fígado não consegue aumentar a produção de lipoproteína de baixa densidade comparado à síntese de TG. Consequentemente, parte do TG é acumulado, impedindo o bom funcionamento do fígado, resultando em diminuição de produtividade leiteira, ingestão de matéria seca (IMS) e da performance reprodutiva do rebanho leiteiro (Herdt et al., 1988). Assim, para que o metabolismo dos AGNE's seja eficaz, é necessário bom metabolismo hepático de ácidos graxos em ruminantes no período de transição (Figura 3).

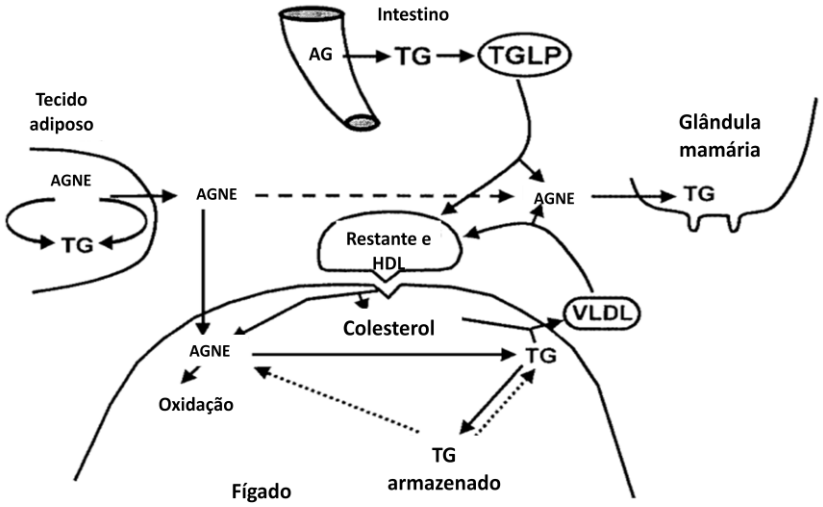

Figura 3. Representação esquemática do metabolismo dos AGNE's. AGNE – ácidos graxos não-esterificados; HDL – lipoproteína de alta densidade; TG – triglicerídeo; TGLP – lipoproteína rica em triglicerídeo; VLDL – lipoproteína de baixa densidade.
Fonte: Drackley (1999).

O período pré-parto inicia-se três semanas antes da vaca parir e termina no momento do parto. Durante esta fase o balanço energético pode ser próximo do neutro ou mesmo negativo (Figura 4), devido à diminuição de IMS, efeitos hormonais, nutrição do feto, produção de colostro, e no caso de novilhas, do crescimento próprio e desenvolvimento da glândula mamária (Bertics et al., 1992). Assim, os lipídios de reserva são hidrolisados à AGNE's para suprir parte desta demanda. Grummer (1993) observou que os AGNE's do plasma sanguíneo praticamente dobraram entre o 17º dia e o segundo dia antes do parto, e dobraram novamente logo após o parto. O mesmo destaca que este aumento está associado com maior incidência de cetose, deslocamento de abomaso e retenção de placenta (Grummer,1993).

O período pós-parto se prolonga do parto até o final da terceira semana que o sucede. É neste momento que o balanço energético negativo é mais severo (Figura 4), podendo chegar a -20 Mcal EL_L/dia. Durante este período, as vacas têm aumento de IMS e de produção de leite. Todavia, o balanço energético permanece negativo até a quarta semana, pela alta demanda energética para produção de leite. Com o avançar das semanas, a produção leiteira diminui (Figura 5) e o balanço energético volta a ficar positivo.

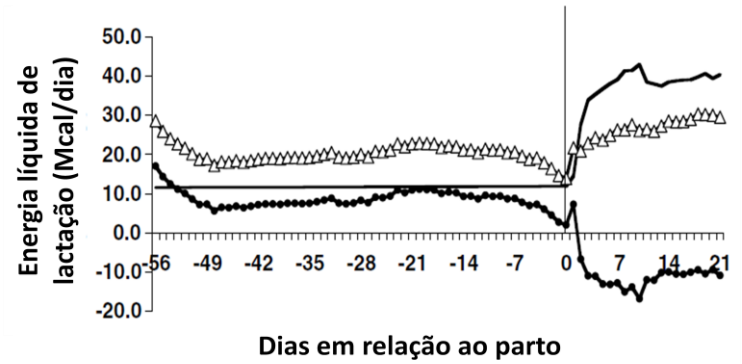

Figura 4. Energia necessária (Mcal EL_L/dia) (-) e consumida (Δ), e balanço energético (●) de vacas.
Fonte: Grummer (2008).

Grum et al. (1996) demonstraram que dietas com alto teor de gordura, fornecidas a vacas no período seco, diminuem a acumulação de TG e aumentam a β-oxidação hepática. Da mesma maneira, Douglas et al. (2006) observaram que dietas com altos níveis de gordura fornecidas a vacas secas tendem a diminuir o acúmulo de TG no fígado no período de parto. Em ratos, a inclusão de gordura na dieta induz mudanças metabólicas no fígado, como aumento de oxidação peroxissômica e β-oxidativa de ácidos graxos (Kumamoto & Ide, 1998), diminuição da esterificação de ácidos graxos (Malewiak et al., 1988), e alteração nos perfis e remoção de lipoproteínas no plasma (Lambert et al., 1998). A maioria das diferenças no metabolismo de gorduras em cobaias foi observada durante jejum de comida ou balanço energético negativo, o que é similar ao balanço energético negativo pós-parto em vacas leiteiras.

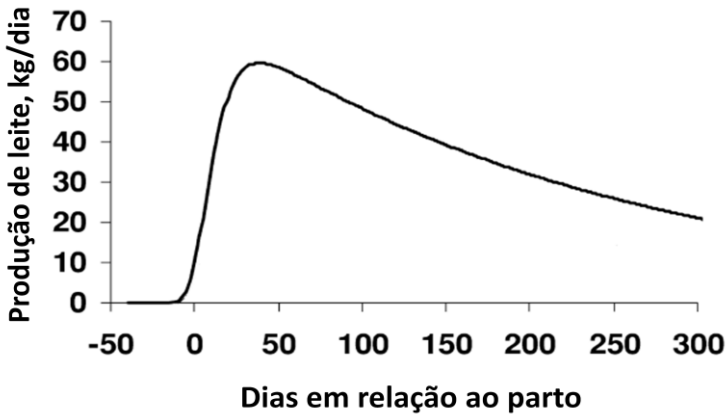

Figura 5. Produção de leite durante a lactação de vacas tendo o pico de lactação de 60 kg de leite por dia
Fonte: Ingvartsen et al. (2003).

A solin (nome comercial linola) é uma planta transgênica da linhaça, desenvolvida na década de 90 pela empresa Commonwealth Scientific and Industrial Research Organisation, AU. Aproximadamente 35% da sua composição em porcentagem de MS é gordura, sendo o ácido graxo C18:2 correspondente a 70% dos ácidos graxos totais. A grande quantidade do ácido graxo C18:2 na linola é interessante quando se deseja aumentar a quantidade de CLA no leite, pois uma parte do CLA do leite é derivado do rúmen, decorrente da bio-hidrogenação incompleta do ácido graxo C18:2. Há poucas informações sobre o fornecimento de linola para vacas leiteiras. Hayirli et al. (2011) suplementaram vacas com semente de linola, linhaça e canola, três semanas antes do parto, para avaliar a performance na lactação e acumulação lipídica no fígado, e encontraram maior produção leiteira para o tratamento linola. Ward et al. (2002) avaliaram a influência das mesmas três oleaginosas na qualidade do leite e concluíram que a adição de linhaça e linola é um meio de aumentar os ácidos graxos C18:1, C18:2 e C18:3 no leite.

A linhaça contém 23% de proteína bruta, 21% de FDN e 35% de extrato etéreo, sendo 48% desta gordura o ácido graxo C18:3; além de ser rica em antioxidantes (Petit & Gagnon, 2009). Isto faz dela um alimento interessante, que pode ser incluído na formulação de ração animal como fonte proteica e energética. De acordo com Petit et al. (2007), o fornecimento de 11% da semente da linhaça em função da matéria seca no períodos pós-parto diminui o risco de desenvolvimento da esteatose hepática, pelo aumento de glicogênio e diminuição do TG hepático. Mashek et al. (2005) infundiram óleo de linhaça em vacas de leite e relataram diminuição das concentrações de AGNE's,

e β-hidroxibutirato no plasma sanguíneo, e houve tendência de diminuição de TG no fígado, quando comparado com infusão de sebo, uma fonte de ácido graxo saturado.

Estudos "in vivo" e "in vitro" demonstraram que, quando cortes de fígado bovino foram incubados em meio suplementado com os ácidos graxos linoleico e linolênico, houve redução da capacidade de esterificação a TG dos ácidos graxos palmítico e esteárico (Mashek et al., 2002; Mashek & Grummer; 2003a, b). Morise et al. (2006) suplementaram ratos com dieta rica em ácido graxo C18:3 e encontraram menor quantidade de TG e melhores taxas de oxidação de ácido graxo no fígado, aumento de expressão gênica e atividade de enzimas envolvidas na β-oxidação, e menor expressão gênica de enzimas lipogênicas. Marsman et al. (2011) induziram esteatose hepática em ratos e depois adicionaram ômega-3 à dieta de um grupo, e encontraram melhor capacidade antioxidativa no fígado daqueles suplementados com ômega-3. Isto sugere que os AGPI's podem limitar o acúmulo de gordura no fígado.

Os mecanismos básicos de mudanças adaptativas, induzidos por uma dieta rica em gordura, ainda não foram completamente elucidados, mas segundo Desvergne et al. (1998) a identificação de um grupo de receptores de hormônios, os receptores ativados por proliferador peroxissomo (PPAR, sigla expressão em inglês *peroxisome proliferator-activated receptor*), leva a crer que estes receptores podem ter importante papel no metabolismo dos ácidos graxos. Os genes controlados pelos PPAR's estão envolvidos no metabolismo de lipase de lipoproteínas, proteína de ligação de ácidos graxos, carnitina palmitoyltransferase, acyl-CoA oxidase, apolipoproteínas, entre outras (Schoonjans et al., 1996). Os PPAR's são ativados pela ligação com ácidos graxos poli-insaturados (AGPI) e também pelos seus derivativos de eicosanoides (Forman et al., 1997, Kliewer et al., 1997), sendo o ômega-3 preferencialmente utilizado nesta ligação (Couet et al., 1997). Estes resultados sugerem que um maior fornecimento de AGPI ômega-3 na dieta de vacas leiteiras pode aumentar a β-oxidação hepática pela maior expressão de PPAR no fígado, o que poderia diminuir a incidência de esteatose hepática em vacas no período de transição.

Além dos efeitos positivos dos ácidos graxos da linhaça no metabolismo de lipídios, a linhaça é fonte rica de antioxidantes que estão concentrados principalmente na casca da semente. De acordo com Kiso (2004), alguns antioxidantes aumentam a expressão de genes envolvidos na β-oxidação de ratos. Além disso, Rajesha et al. (2006) observaram que os antioxidantes da linhaça aumentam a expressão dos genes que

codificam enzimas como superóxido dismutase, catalase e glutationa peroxidase. Essas enzimas estão relacionadas ao combate do estresse oxidativo no organismo animal.

A hipótese do presente trabalho é que os ácidos graxos da dieta poderiam modular o metabolismo lipídico no fígado de vacas de leite no período de transição, evitando a esteatose hepática. Assim, a suplementação de vacas leiteiras com linhaça diminuiria as concentrações os lipídios totais no fígado, e os AGNE's e BHBA do plasma sanguíneo; e aumentaria os níveis de glicogênio, superóxido dismutase, catalase e peroxidase do fígado e as concentrações de glicose no sangue, comparado à dieta controle ou suplementada com linola.

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OBJETIVOS GERAIS

Objetivou-se neste trabalho avaliar:

as concentrações de β-hidroxibutirato, glicose e ácidos graxos não-esterificados plasma sanguíneo de vacas alimentadas com uma dieta controle e dietas contendo sementes de linola ou linhaça;

as concentrações de triglicerídeos, glicogênio e lipídios totais no fígado de vacas alimentadas com uma dieta controle e dietas contendo sementes de linola ou linhaça;

os níveis de atividade enzimática da superóxido dismutase, catalase e peroxidase no fígado de vacas alimentadas com uma dieta controle e dietas contendo sementes de linola ou linhaça;

a qualidade do leite de vacas alimentadas com uma dieta controle e dietas contendo sementes de linola ou linhaça.

CAPÍTULO I

LIPID METABOLISM IN THE LIVER

Sources of Lipids in Dairy Cows during the Transition Period

ABSTRACT

Twenty nine Holsteins cows were allotted, 4 weeks before the expected date of parturition until 12 weeks after parturition, to 10 groups of 3 cows blocked within parity for similar calving date to determine the effects of feeding different fatty acid sources on milk production and quality, plasma metabolites and liver parameters. Cows were fed a isonitrogenous and isoenergetic diet supplemented of whole flaxseed (WFL; 4.82% and 7.59% of the dry matter in prepartum and postpartum diets, respectively), whole linola (WLO; 4.82% and 7.59% of the dry matter in prepartum and postpartum diets, respectively) or a source of calcium salts of palm oil (MEG; 1.07% and 2.57% of the dry matter in prepartum and postpartum diets, respectively). Diets of WFL and WLO had higher ether extract. Cow fed MEG had greater milk production and higher daily lactose yield. Cows fed WLO had higher milk enterolactone. Dietary fat had no effect on plasmatic non-esterified fatty acids, β-hydroxybutyrate and glucose. Furthermore, there was no effect of dietary fat on hepatic glycogen, superoxide dismutase and glutathione peroxidase. Liver lipids and triglyceride tended to be higher for cows fed WFL and WLO on week 4 after calving. Cows fed WFL had greater catalase on week 4 after calving. In conclusion, the effects of different lipid sources on hepatic fat metabolism were lower than expected, probably due to a similar fatty acid profile reaching liver.

INTRODUCTION

The transition period in dairy cows lasts from 3 weeks before calving to 3 weeks after calving and has great importance on cow's lactation performance (Grummer et al., 1995). Fatty liver is a metabolic disease that has its major incidence during the first four weeks after calving, period in which 50% of cows have some hepatic lipids accumulation (Jorritsma et al., 2000). According to Grummer (2008), fatty liver occurs in periods of elevated concentration of plasmatic NEFA. The increase of plasmatic NEFA may be a response to negative energy balance (**NEB**) or to hormonal changes related to calving. During normal periods of a cows' life, the circulating NEFA is absorbed by the liver and part is re-esterified to triglyceride (**TG**) before being secreted in blood as very-low-density lipoprotein (**VLDL**; Herdt et al., 1988). However, when plasmatic NEFA rapidly increases due to intense lipolysis, there is an enhanced liver NEFA intake response. Ruminants have a poor rate of VLDL production compared to TG synthesis, thus TG accumulates in liver, resulting in aberrant function of the liver, decreased milk production, decreased DMI and lower reproductive performance of dairy cattle (Herdt et al., 1988).

Flaxseed contains 35% of ether extract, of which 48% is α-linolenic FA (Petit and Gagnon, 2009). Dietary fat supplementation of α-linolenic acid decreased liver TG and enhanced hepatic FA oxidation rates in rodents compared to a high saturated fatty acid diet (Morise et al., 2006). Petit et al. (2007) reported that feeding whole flaxseed six weeks before calving was a useful strategy to increase hepatic concentrations of glycogen and decrease TG of multiparous cows. Mashek et al. (2005) infused flaxseed oil intravenously in dairy cows and related decreased plasma concentrations of NEFA and BHBA, and a tendency of hepatic TG decrease, when compared to tallow. During the transition period, the addition of 250 g/d of fish oil, a rich source of n-3 FA, or an

Energy Booster (Milk Specialties Co., Dundee, IL) tended to increase plasma glucose and decrease NEFA and BHBA during postpartum (Ballou et al., 2009). Furthermore, fish oil supplementation decreased hepatic total saturated FA and increased n-3 and long chain FA.

Despite the positive effects of α-linoleic FA on lipid metabolism, flaxseed has high antioxidant contents which are concentrated on the outer fibre-containing layers (Adlercreutz and Mazur, 1997). According to Kiso (2004), some antioxidants may increase the gene expression involved in β-oxidation of rats. Besides, Rajesha et al. (2006) found that flaxseed antioxidants enhance the expression of genes encoding the enzymes such as superoxide dismutase (**SOD**), catalase (**CAT**) and glutathione peroxidase (**GPx**). Thus, the effects of enhanced β-oxidation and greater hepatic antioxidative capacity could lead to fatty liver prevention in dairy cows.

The hypothesis of the present work was that dietary FA affects the hepatic lipid metabolism in dairy cows. Therefore, the objectives of the present experiment were to determine the effects of feeding whole flaxseed, a rich source of n-3 FA, whole linola, a rich source of n-6 FA, and calcium salts of palm oil, on the hepatic lipid metabolism in dairy cows from week 4 before calving to week 12 after calving.

MATERIALS AND METHODS

Animals and Treatments

The experiment was conducted at the Dairy and Swine Research and Development Centre (QC, Canada), from December 2009 to May 2010, using 29 multiparous Holstein dry cows averaging 777 kg of body weight (**BW**; SE = 18 kg). There were four cows that data was not used because of serious health problems: one cow had a sudden death before entering the project, one cow had a severe milk fever

after calving, one cow had to go on surgery due to displaced abomasum and another cow had problems during liver biopsy. The experiment was carried out from week 4 before parturition to week 12 of lactation. Four weeks before calving cows were stratified in groups of three animals, for similar expected calving dates. Cows within groups were assigned randomly to one of the three isonitrogenous and isoenergetic total mixed rations (**TMR**). The three prepartum TMR consisted of fat supplements based on either: unsaturated lipids supplied with 4.82% of whole flaxseed (**WFL**) rich in n-3 FA; unsaturated lipids supplied with 4.82% of whole linola (**WLO**) rich in n-6 FA; or calcium salts of palm oil supplied with 1.07% of Megalac® (**MEG;** Church and Dwight Company, Inc., Princeton, NJ, USA; Table 1). Prepartum diets were formulated to meet requirements of dry cows that averaged 635 kg of BW (NRC, 2001). After calving, diets (WFL, WLO and MEG) were formulated to meet requirements for cows that averaged 635 kg of BW and produced 40kg/d of milk with 3.90% of fat (NRC, 2001; Table 2). On a DM basis, postpartum diets WFL and WLO contained 7.59% of whole flaxseed and whole linola, respectively; and MEG 2.57% of Megalac®. Cows were housed in tie stalls and milked twice a day at 0730 and 1900 h. Milk production was recorded at every milking. Cows were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Sampling

Milk samples were obtained from each cow on week 1, 2, 3, 4, 6, 10 and 12 in 2 consecutive milking and pooled on a yield basis. Milk samples were stored at 4°C with a preservative (bronopol-B2) and then analyzed for concentrations of fat, protein, lactose and SCC. Milk samples without preservative were frozen at -20°C until analysis

of milk FA profile and enterolactone. Body weight of cows was determined on week 4 and 1 prepartum, at calving and on week 1, 2, 3, 4, 8, and 12 postpartum.

Dry matter of silage was analyzed weekly for DM adjustment of the TMR. Cows were fed individually twice a day at 0800 and 1500 h for 10% refusals, and feed consumption was recorded daily. Samples of TMR were taken weekly, frozen, and composed on a 4-wk basis for the prepartum period and a 6-wk basis for the postpartum period. Samples of hay, Megalac®, whole linola and whole flaxseed were taken on a 4-wk basis. Composited samples were mixed thoroughly and sub sampled for chemical analyses. Diets and feed ingredients were dried at 55˚C and ground through a 1-mm sieve in a Wiley mill before analysis of nitrogen, ether extract (**EE**), ADF and αNDF. Postpartum energy balance was calculated weekly as the difference between the energy consumed and required, in which NE_L required postpartum = NE_L for maintenance plus NE_L for milk. Energy required for maintenance (Mcal/d) = 0.08 x kg of $BW^{0.75}$, and for lactation = kg of milk x [(0.0929 x milk fat %) + (0.0547 x milk protein %) + 0.192] (NRC, 2001).

Blood was sampled on week 2 and 1 prepartum, at calving and on week 1, 2, 4, 6 and 8 postpartum. Blood was collected after feed refusals were removed and before the morning feeding, as described by Janovicket al. (2011). Blood was withdrawn from the coccygeal vessels into vacutainer tubes (Becton, Dickinson and Co., Rutherford, NJ, USA) containing EDTA, for NEFA, glucose and BHBA analysis. Tubes were immediately placed on ice and centrifuged for 30 min at 4˚C for 12 min at 3,000 *g*. The plasma was separated and frozen at -20˚C for subsequent analysis.

Samples of liver were collected from each cow on week 2 prepartum and on week 2 and 4 postpartum. Liver samples were obtained by puncture biopsy under local anaesthesia through an incision on the right side of the cow at the 10^{th}intercostals space,

where it crossed a line from mid-humerus to tuber coxae. An incision of approximately 1 cm long was made, and about 2.5g of liver tissue were collected at each biopsy. Upon collection, the liver samples were rinsed with saline to remove excess blood and immediately placed into liquid nitrogen. Samples were stored at -80ºC until analyzed for TG, glycogen and total lipids.

Chemical Analysis

Dry matter of TMR, hay, whole linola and whole flaxseed were determined in a forced-air oven at 105˚C for 48 h (AOAC, 1990; method 934.01). Total nitrogen and EE concentrations in TMR and feed ingredients were done according to the 990.03 and 920.39 of AOAC (1990) method, respectively. The concentration of αNDF in TMR was determined with sodium sulfite and heat stable α-amylase (Van Soest et al., 1991) and the ADF content was determined according to the AOAC (1990; method 973.18). The procedures for αNDF and ADF analysis were adapted to be used in an Ankom^{200} Fiber Analyzer (Ankom Technology Corp., Fairport, NY).

Nitrogen, fat and lactose concentrations and SCC in milk were determined with a Foss MilkoScan4000 instrument (Foss Electric, Hillerød, Denmark) combined with a Bentley 2000 instrument (Bentley Instruments, Chaska, MN, USA). All milk composition analysis were conducted at the Québec Dairy Production Centre of Expertise (Ste-Anne-de-Bellevue, QC, Canada). Milk enterolactone was determined with an enzyme immunoassay kit (kit 500520, Cayman Chemical Company, Ann Arbor, MI, USA) as described by Petit and Gagnon (2009). Plasma samples were analyzed for concentrations of NEFA (NEFA C-kit, Wako Chemicals USA, Richmond, VA, USA) as described by McCutcheon and Bauman (1986), glucose by glucose oxidase method (kit 144448668, Roche/Hitachi, Indianapolis, IN, USA) as described by

Trinder (1969); and BHBA by enzymatic kinetic method (kit 310-A, Sigma Diagnostic Inc., St. Louis, MO, USA) based on oxidation of β-hydroxybutyrate to acetone by acetoacetate decarboxylase, according to Williamson et al. (1962). Frozen liver (80 mg) was homogenized in PBS and a portion was used to determine glycogen according to the modified procedure of Andersen et al. (2002) using a colorimetric method (kit 1060, Stanbio, Boerne, TX, USA). Liver was analyzed for concentrations of total lipids and TG using the methodology described by Douglas et al. (2004). Liver catalase, superoxide dismutase and glutathione peroxidase activity were measured with enzyme immunoassay kit (kit 707002, 706002, 703102, respectively; Cayman Chemical Company, Ann Arbor, MI, USA).

Total lipids in milk were extracted as described by Côrtes et al. (2010). Fatty acids were methylated according to the method described by Chouinard et al. (1997). Transesterification was performed on whole flaxseed, whole linola and TMR according to Park and Goins (1994); and on milk as described by Côrtes et al. (2010). Composition analyses of the fatty acids were carried out with a gas chromatograph (HP 5890A Series II; Hewlett-Packard, Palo Alto, CA) equipped with a 100-m CP-Sil 88 capillary column (0.25 μm i.d., 0.20 μm film thickness; Chrompack, Middleburg, the Netherlands) and a flame ionization detector. At the time of sample injection, the column temperature was 80°C for 1 min, and increased 2°C/min to 215°C and maintained for 30 min. Inlet and detector temperatures were 220 and 230°C, respectively. The split ratio was 100:1. The flow rate for H_2 carrier gas was 1 mL/min. Most fatty acid peaks were identified and quantified using either a quantitative mixture or pure methyl ester standards (Larodan Fine Chemicals; Sigma-Aldrich Canada Ltd.; Matreya LLC, Pleasant Gap, PA; Nu-Chek Prep, Elysian, MN; Naturia, Sherbrooke, QC, Canada).

Statistical Analysis

Measurements of DMI and milk yield were reduced to weekly means before statistical analysis. The model included the fixed effects of treatment, block, week, treatment by week interaction and the residual error. Data on DMI and BW were analyzed separately for the prepartum and postpartum periods. Data on DMI, milk production and composition were analyzed as repeated measurements using PROC MIXED (SAS Institute, 2000) and the compound symmetry was used as the covariance structure. When differences ($P<0.10$) due to interactions or dietary treatments were detected, means separation was conducted using the Tukey's adjustment for the probability. Differential temporal responses to dietary treatments were further examined using the SLICE option of the MIXED procedure. Significance was declared at $P\leq0.05$ and a trend at $0.05<P\leq0.10$ unless otherwise stated. Residuals were plotted to detect assumptions of normality and homogeneity of variance. Data on SCC were transformed (log) because of lack of variance homogeneity.

RESULTS

The compositions of the diets were generally similar during the prepartum period (Table 1). The ether extract was higher for WLO and WFL, compared to MEG, as expected, but there was no difference between WFL and WLO. The quantity of the dietary C16:0 was higher for MEG than for WFL and WLO. During the postpartum period (Table 2), the postpartum dietary ether extract was higher for WLO and WFL, compared to MEG. The NDF content was smaller for MEG, but was similar for WFL and WLO. Whole flaxseed had 23.70% CP, 40.85% EE, 17.84% NDF, 10.36% ADF, 16.30% of *cis-9, cis-12* 18:2 and 54.94% of *cis-9, cis-12, cis-15* 18:3 as percentage of

the total identified FA and whole linola had 21.95% CP, 40.85% EE, 21.00% NDF, 9.78% ADF, 72.77% of *cis-9, cis-12* 18:2 and 1.66% of *cis-9, cis-12, cis-15* 18:3 as percentage of total identified FA.

In general, there was high incidence of metabolic disease (Table 3). The highest incidence of ketosis was in WLO treatment, with six cases; followed by MEG, with five cases; and WFL had the lowest incidence, two cases. Cows from the treatment MEG had the higher incidence of mastitis, five in total. Health problems were numerically higher for WLO and lower for WFL.

Prepartum DMI was similar among treatments, expressed in kilograms per day or as a percentage of BW (Table 4). Body weight was similar before calving. On week 4 before calving cows had similar body condition score (3.85). There were no differences on the postpartum DMI, expressed in kilograms per day or as a percentage of BW (Table 4) and BW. There was an energy balance difference, expressed as mega calories per day, in which WFL was higher, and WLO was similar to MEG.

Milk yield was higher (Table 4) for cows of the MEG treatment, but was similar for WFL and WLO. From week 7 until the last week of the experiment (week 12) there was an interaction between treatment and week of lactation ($P = 0.0154$). There was no difference for protein, fat, lactose and total solids when expressed as percentage. However, when lactose yield was expressed as kilogram per day, MEG was higher than WLO and WFL was similar for both treatments. There was no difference for SCC when expressed as logarithm. Milk enterolactone was higher for WLO compared to WFL and MEG.

Data on relative percentages of milk FA were expressed as percentage of total identified FA, which did not include the unknown relative percentages in milk fat. The short chains C10:0, C12:0 and C14:0 were higher for WFL and similar for WLO and

MEG, although the C16:0 was higher for MEG and similar between WFL and WLO. The C18:0 was higher for WFL and WLO compared to MEG. There was no difference for the *cis*-9 18:1. *Cis*-9, *trans*-11 18:2 was higher for WLO. *Cis*-9, *cis*-12 18:2 was higher for WLO compared to WFL, but similar for MEG. And *cis*-9, *cis*-12, *cis*-15 18:3 were higher for WFL compared to WLO and MEG.

There was no difference for NEFA, BHBA and glucose on plasma (Table 6). There was a 2 fold increase on NEFA from week 2 to week 1 before parturition and a new 2 fold increase from parturition to week 1 after parturition, where it peaked, and then it decreased (data now shown). Plasma glucose remained stable during the 10 weeks of experiment. Plasma BHBA was stable from week 2 before parturition to parturition, and then it increased 2 folds from parturition to week 1 and then remained stable.

No differences were found for glycogen, TG, total lipids, CAT, GPx and SOD on liver (Table 7). Liver glycogen decreased by one third from week 2 before parturition to week 2 after parturition, and then it increased by half at week 4 after parturition. Liver TG increased almost 18 folds from week 2 before parturition to week 2 after parturition, and then it decreased by one quarter from week 2 to week 4 after parturition. There was a treatment and week interaction tendency ($P = 0.0834$) on week 4 after parturition, in which liver TG was higher for MEG (9.60 % of wet weight) compared to WLO (5.38% of wet weight) and WFL (4.56% of wet weight). Total liver lipids increased 2.5 folds from week 2 before parturition to week 2 after parturition for WFL and WLO, and 3.5 folds for MEG, but there was no statistical difference of treatment. From week 2 to week 4 after parturition, total liver lipids decreased by approximately 20% for WFL and WLO, and decreased by approximately 10% for MEG. There was a treatment and week interaction tendency ($P = 0.0961$) on week 4

after parturition, in which liver TG was higher for MEG (13.04 % of wet weight) when compared to WLO (8.66 % of wet weight) and WFL (8.31 % of wet weight). The decrease pattern of liver superoxide dismutase and GPx were similar, as it had a slight drop from week 2 before parturition to week 2 after parturition, and then it remained constant. Liver CAT had a slight increase from week 2 before calving to week 2 after calving. Moreover, from week 2 to week 4 after calving, liver CAT had another slight increase, for WLO and MEG, and 20% increase for WFL, when a significant effect of treatment and week interaction was observed ($P = 0.0214$).

DISCUSSION

There was no effect of dietary fat sources on prepartum and postpartum DMI, expressed either as kg per day or as a percentage of BW. Ward et al. (2002) fed diets containing 8.32% of whole ground flax, linola or canola and found no effect on DMI. Also, Petit and Benchaar (2007) fed 5.9% of whole flaxseed or 2.7% of Megalac® prepartum, and 10.8% whole flaxseed or 4.9% Megalac® postpartum and found no effect on DMI. According to Petit (2010), the effects of level and type of fat supplement on DMI are negligible when total dietary fat concentration is below 6% of DM.

Cows supplemented with MEG had higher milk yield ($P = 0.0044$). This result is in contrast to Petit (2002), which found higher milk yield for cows fed whole flaxseed compared to Megalac®, and is different from Petit and Benchaar (2007), who found no effect in milk production for cows supplemented with whole flaxseed, Megalac® or micronized soybean. Since DMI was similar for all the treatments, probably, the higher milk yield by MEG treatment can be explained by a greater utilization of body reserves to produce milk. Cows from MEG experienced the lowest energy balance. Petit and Côrtes (2010) supplemented cows from calving to week 28 of lactation, with 7.2% of

whole flaxseed or 2.1% of Megalac® and found no effect on milk productivity and DMI. Maybe, milk yield could equalize if this experiment had a longer duration, since it lasted half of the experiment of Petit and Côrtes (2010).

Milk protein, fat, lactose and total solids, expressed as percentage, were similar for all the treatments ($P>0.05$). These results are in agreement with Petit and Benchaar (2007). When expressed as daily production, lactose yield was higher for MEG (1.90 kg/d) than for WLO (1.76 kg/d) and similar for WFL (1.82 kg/d). Lactose is the milk component that has the lowest variation and since milk yield was higher for MEG, a higher lactose yield was expected.

Milk enterolactone was higher for WLO cows, which could be explained by the higher intake of secoisolariciresinol diglucoside (**SDG)** from whole linola. According to Spence et al. (2003), depending on the cultivation of linola, SDG in seeds can range from 9.0 to 15.0 g/kg. Petit and Gagnon (2009) reported that whole flaxseed contains 6.30 g/kg of SDG. Secoisolariciresinol diglucoside are plant antioxidants and are converted into mammalian antioxidants, the enterolactone, in the rumen of cows (Petit and Gagnon, 2009). Petit and Gagnon (2009) found an increase in milk enterolactone according to SDG intake. So a higher SDG intake of WLO cows explains the higher WLO milk enterolactone content. Although SDG was not measured on the diets on the present experiment, a two fold increase of dietary SDG content could be expected. Thus, the two fold increase of SDG content could account for the two fold increase in milk enterolactone.

Milk C16:0 can be derived from the diet or can be synthesized in the mammary gland (Grummer, 1991). The higher content of C16:0 in milk of cows fed Megalac® probably is due to higher dietary C16:0 of MEG diet. Lower contents of milk C16:0 is desirable for human health due to its association to hypercholesterolemic effects. Milk

FA's C10:0, C18:0, and *cis*3- 18:3 were higher ($P<0.05$) for cows fed WFL than for those fed MEG. This is in agreement with the results of Petit et al. (2001) and Petit (2002), which compared, respectively, formaldehyde-treated flaxseed with Megalac® and untreated whole flaxseed with Megalac®. Milk *cis*-9 *trans*-11 18:2 (**CLA**) was two times higher for WLO compared to WFL and MEG. The higher percentage of CLA in WLO can be explained because the CLA is derived in the rumen and mammary gland from incomplete bio-hydrogenation of C18:2 (Harfoot and Hazelwood, 1988). These results are in agreement with Ward et a. (2002), that supplemented dairy cows with whole flaxseed or whole linola with 8.32% of DM, and found that milk CLA was higher ($P = 0.03$) for the treatment with linola (1.49 % of total milk fatty acids) compared to flaxseed (1.16 % of total milk fatty acids).

Plasmatic NEFA, BHBA and glucose were similar among treatments ($P>0.05$). This is in agreement with Castañeda-Gutiérrez et al. (2009), Petit and Benchaar (2007), and Douglas et al. (2006) who found no differences for plasma NEFA, BHBA and glucose. Lack of differences in these variables is likely explained by the similar DMI among treatments, which means similar NE_L intake.

There was no difference for liver parameters, but hepatic total lipids and TG tended ($P = 0.0834$; $P = 0.0961$, respectively) to be higher in MEG compared to WLO and WFL on week 4. The modulation of hepatic fat is difficult in dairy cows. To efficiently decrease hepatic TG, Mashek et al. (2002) treated 8 to 12 mg dry weight of bovine hepatocytes with one of the following medium: 2 m*M* palmitic acid; 1 m*M* palmitic acid plus either 1 m*M* oleic (C18:1); or 1 m*M* linoleic (C18:2); or 1 m*M* linolenic(C18:3). The medium with linoleic and linolenic FA decreased incorporation of FA into cellular TG almost by half compared to other treatments, suggesting a capacity of n-3 to modulate liver fat metabolism. Hayirli et al. (2011) supplemented cows during

the prepartum period with 8.00% of DM of canola, linola and flaxseed, and found no effect on supplementing different fat sources on hepatic lipid metabolism. They suggested that the lack of effect was because the amount of FA that reached the liver was similar among treatments. Indeed, the long chain FA bio-hydrogenation in the rumen can nullify most of dietary PUFA because FA that passes through rumen are similarly saturated, and are unable to modulate hepatic fat metabolism. Marsman et al. (2010) reported that when steatosis induced rats were supplemented with n-3 FA, they presented reduction on the hepatic fat of 58.8% when compared to a control diet (i.e. baseline measurements), from wk 1 to wk 2 after the supplementation of n-3 FA. A greater reduction was observed when the hepatic fat was compared to rats supplemented with mainly saturated FA. Also, rats supplemented with the omega-3 FA had an enhanced antioxidative capacity. Probably, to efficiently decrease hepatic TG concentration, the n-3 FA content must be 2 folds higher than the total FA reaching the liver. According to Jump et al. (1999), n-3 FA coordinately regulates the expression of several enzymes involved in carbohydrate and lipid metabolism.

The overall high content of liver lipids can be explained by the elevated BW of cows on the beginning of the experiment (777 kg) and by the elevated body condition score (3.85). Obese cows with body condition score higher than four, have increased lipolysis of the adipose tissue during challenging situations (Rukkwamsuk et al., 1998), decreased DMI and more severe negative energy balance (Stockdale, 2001). Thus, an enhanced lipolysis results in higher plasmatic NEFA, which leads to an increase in liver NEFA uptake. Cows from the present experiment had higher liver lipids content than normal cows. In the experiment of Petit et al. (2007), liver triglycerides as percentage of wet weight were approximately 4.0% on the week 2 postpartum for cows fed 11% with whole flaxseed on postpartum. In our experiment, on week 2 postpartum cows fed WFL

had hepatic triglycerides content of 7.35% of wet weight and MEG had 11.23% of wet weight, the former is categorized as a severe fatty liver according to Bobe et al. (2004). We expected a better health status on WFL cows, especially lower hepatic triglyceride and total lipids and higher glycogen; and lower non-esterified and β-hydroxybutyrate and higher glucose in plasma, as seen in another research project by our team. Probably, for better health status, a higher quantity of n-3 FA reaching liver is required.

In general, cows had greater disease incidence. Cows fed WLO had more ketosis incidence than WFL, but had similar performance throughout the lactation. Furthermore, WLO cows had greater incidence of diseases but there was no effect on DMI, milk yield, plasmatic metabolites and liver parameters, compared to WFL. Probably there was an enhanced antioxidant function, since SDG intake of WLO was higher compared to the other treatments. Catalase activity was higher on week 4 for WFL treatment. Rajesha et al. (2006) found a crescent CAT activity when rodents were fed 5% and 10% flaxseed for 14 days and had a challenge with 2.0 g kg^{-1}BW CCl_4 as toxin flaxseed. The dietary antioxidant may have enhanced the immune status of these cows, and maybe attenuated health disorders.

CONCLUSIONS

Cows fed whole flaxseed, whole linola or calcium salts of palm oil during the transition period had similar DMI, milk composition, blood metabolites and liver parameters. Cows supplemented with calcium salts of palm oil had higher milk production and higher milk lactose yield, but tended to have greater hepatic lipids and triglycerides on week 4 after calving. Cows supplemented with whole linola had greater milk enterolactone. Cows supplemented with whole flaxseed had greater energy balance and higher hepatic CAT on week 4 after calving. There were some mild effects on the

hepatic lipid metabolism of all treatments. In general, the lack of effect on overall lactation performance is probably due to the composition of fatty acids that reached the liver, which could not modulate hepatic fat metabolism in order to improve health status during the transition period.

Table 1. Ingredient and chemical composition of the precalving experimental diets[1,2]

Item	WFL	WLO	MEG	SE
Ingredient, % of DM				
Corn silage	26.53	26.53	26.41	-
Chopped hay	51.88	51.88	50.91	-
Cracked corn	1.86	1.86	5.24	-
Soybean meal (48% CP)	12.77	12.77	14.38	-
Calcium carbonate (34% Ca)	0.88	0.88	0.74	-
Whole linola	-	4.82	-	-
Whole flaxseed	4.82	-	-	-
Megalac Low®	-	-	1.07	-
Mineral Iode[3]	1.26	1.26	1.26	-
Chemical				
DM, %	67.95	67.81	67.77	0.23
CP, % of DM	13.47	13.94	13.84	0.44
Ether extract, % of DM	3.68[a]	3.81[a]	2.20[b]	0.11
NDF, % of DM	45.09	44.93	73.70	0.40
ADF, % of DM	27.87	27.59	26.65	0.33
NE_L, Mcal/kg[4]	1.35	1.35	1.35	0.00
TMR fatty acids, % of total				
C14:0	0.13	0.12	0.68	
C16:0	9.32	9.30	28.50	
C16:1 cis-9	0.14	0.12	0.23	
C18:0	3.28	3.29	3.42	
C18:1 *cis*-9	19.88	18.74	26.86	
C18:1 *cis*-11	0.78	0.74	0.90	
C18:2 *cis*-9, *cis*-12	34.78	56.40	35.43	
C18:3 *cis*-9, *cis*-12,*cis*-15	31.39	10.99	3.56	
C20:0	0.29	0.28	0.42	

[a–c]Means within rows with different superscripts differ ($P<0.05$).

[1]WFL= supplement with whole flaxseed; WLO = supplement with whole linola and MEG = supplement with Megalac®.

[2]Mean of 4 weekly samples prepared by compositing weekly samples.

[3]Contained 6.27 % Ca; 6.00 % P; 20.00 % Mg; 1.50 % S; 5.60 % Na; 1.26 % K; 198 mg/kg I; 2846 mg/kg Fe; 1560 mg/kg Cu; 6698 mg/kg Mn; 7300 mg/kg Zn; 128 mg/kg Co; 332 mg/kg Fl; 1080287 UI/kg vitamin A; 189951 UI/kg vitamin D; and 12644 UI/kg vitamin E.

[4]Calculated using published values (NRC, 2001).

Table 2. Ingredient and chemical composition of the postcalving experimental diets[1,2]

Item	WFL	WLO	MEG	SE
Ingredient, % of DM				
Grass silage	28.39	28.39	28.23	-
Corn silage	28.40	28.40	28.17	-
Cracked corn	2.38	2.38	19.53	-
Soybean meal (48% CP)	5.11	5.11	13.07	-
Beet Pulp (19.48% CF)	4.12	4.12	3.31	-
Calcium carbonate (34% Ca)	0.66	0.66	0.17	-
Gluten[3]	7.83	7.83	-	-
Wheat[4]	10.96	10.96	-	-
Whole linola	-	7.59	-	-
Whole flaxseed	7.59	-	-	-
Megalac Low®	-	-	2.57	-
Protein supplement[5]	1.96	1.96	1.83	-
Mineral Iode[6]	1.53	1.53	1.92	-
Chemical				
DM, %	45.50	45.16	45.44	0.99
CP, % of DM	16.44	16.78	16.34	0.39
Ether extract, % of DM	5.71[a]	6.00[a]	4.07[b]	0.16
NDF, % of DM	36.59[a]	36.45[a]	29.87[b]	0.92
ADF, % of DM	21.41	21.54	19.62	0.58
NE_L, Mcal/kg[7]	1.68	1.68	1.67	0.00
Fatty acids, % of total				
C14:0	0.16	0.18	0.75	
C16:0	10.76	10.83	30.02	
C16:1 cis-9	0.17	0.15	0.26	
C18:0	2.87	2.90	3.18	
C18:1 *cis*-9	18.96	17.44	26.63	
C18:1 *cis*-11	0.80	0.67	0.86	
C18:2 *cis*-9, *cis*-12	29.89	59.62	29.74	
C18:3 *cis*-9, *cis*-12,*cis*-15	36.04	7.88	8.08	
C20:0	0.35	0.34	0.47	

[a–c]Means within rows with different superscripts differ ($P<0.05$).

[1]WFL= supplement with whole flaxseed; WLO = supplement with whole linola and MEG = supplement with Megalac®.

[2]Mean of 6 weekly samples prepared by compositing weekly samples.

[3]Contained 21.2 % CP; 1.54 Mcal/kg EN_L; 3.1 % fat; 0.06 % Ca; 0.89 % P; and 0.37 % Mg.

[4] Contained 16.6 % CP; 1.49 Mcal/kg EN_L; 4.6 % fat; 0.11 % Ca; 0.81 % P; and 0.25 % Mg.

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

[6]Contained 9.2 % Ca; 4.79 % P; 4.78 % Mg; 1.52 % S; 13.72 % Na; 1.37 % K; 19.5 mg/kg Se; 23 mg/kg I; 2013 mg/kg Fe; 1068 mg/kg Cu; 1796 mg/kg Mn; 2657 mg/kg Zn; 57 mg/kg Co; 265 mg/kg Fl; 442000 UI/kg vitamin A; 56670 UI/kg vitamin D; and 2630 UI/kg vitamin E.

[7]Calculated using published values (NRC, 2001).

Table 3. Postpartum diseases of Holstein cows fed supplements basedon whole flaxseed (WFL), whole linola (WLO) or Megalac® (MEG)

Disease	Treatment		
	WFL (n = 9)	WLO (n = 14)	MEG (n = 11)
Ketosis	2	6	5
Pneumonia	1	1	-
Mastitis	2	3	5
Retained placenta	1	1	-
Metritis	1	2	-
Milk fever	1	1	1
Diarrhea	1	-	-

Table 4. Feed intake, BW, milk production and energy balance of Holstein cows fed supplements based on whole flaxseed (WFL), whole linola (WLO) or Megalac® (MEG)

Item	WFL	WLO	MEG	SE
Precalving DMI, kg/d	11.77	11.32	12.34	0.49
Precalving DMI, % of BW	1.53	1.50	1.54	0.07
BW, kg				
4 wk before calving	774	762	795	-
1 wk before calving	795	783	824	-
Body condition score				
4 wk before calving	3.84	3.91	3.79	-
1 wk before calving	4.09	3.77	3.93	-
Postcalving DMI, kg/d	22.16	21.12	20.72	0.61
Postcalving DMI, % of BW	3.22	3.17	2.93	0.11
BW, kg				
1 wk after calving	701	674	729	-
12 wk after calving	683	670	709	-
Body condition score				
1 wk after calving	3.48	3.34	3.59	-
12 wk after calving	3.25	3.12	3.30	-
Energy balance, Mcal/d	0.67[a]	-1.05[b]	-3.78[b]	1.05
Milk yield, kg/d	39.19[b]	38.16[b]	41.62[a]	0.93
Milk composition, %				
Protein	2.95	2.95	2.92	0.04
Fat	3.52	3.59	3.45	0.10
Lactose	4.64	4.58	4.60	0.04
Total Solids	12.09	12.09	11.93	0.12
SCC[1]	1.96	2.92	1.94	0.47
Milk enterolactone nM/l	123.92[b]	222.60[a]	64.98[b]	18.16
Milk yield, kg/d				
Protein	1.16	1.13	1.20	0.03
Fat	1.38	1.36	1.41	0.05
Lactose	1.82[ab]	1.76[b]	1.90[a]	0.05
Total Solids	4.75	4.61	4.90	0.13

[1]Somatic cell score = $\log_{10}$SCC.

Table 5. Average milk fatty acid (FA) composition of Holstein cows fed supplements based on flaxseed whole (WFL), whole linola (WLO) or Megalac® (MEG)

Item	Treatment			SE
	WFL	WLO	MEG	
Fatty acids, % of total				
C10:0	3.51[a]	2.89[b]	2.76[b]	0.16
C12:0	3.62[a]	2.99[b]	2.93[b]	0.17
C14:0	11.28[a]	9.89[b]	9.99[b]	0.30
Cis-9 14:1	0.70	0.82	0.79	0.06
C16:0	25.82[b]	23.43[b]	30.51[a]	0.83
Cis-9 16:1	1.11[ab]	0.95[b]	1.29[a]	0.08
C18:0	12.91[a]	13.67[a]	10.00[b]	0.54
Cis-11 18:1	0.56[b]	0.54[b]	0.76[a]	0.06
Cis-9 18:1	19.29	21.40	20.88	0.82
Trans-11 18:1	1.27[b]	2.20[a]	1.15[b]	0.14
Cis-9 *Cis*-12 18:2	2.01[b]	2.75[a]	2.44[ab]	0.12
Cis-9 *Trans*-11 18:2	0.47[b]	0.83[a]	0.51[b]	0.06
Cis-9 *Cis*-12 *Cis*-15 18:3	0.68[a]	0.40[b]	0.42[b]	0.04
Cis-8 *Cis*-11 *Cis*-14 20:3	0.08[b]	0.09[ab]	0.11[a]	0.01
Cis-5 *Cis*-8 *Cis*-11 *Cis*-14 20:4	0.10[b]	0.11[ab]	0.12[a]	0.01
Cis-5 *Cis*-8 *Cis*-11 *Cis*-14 *Cis*-17 20:5	0.06[a]	0.03[b]	0.04[b]	0.00
Others fatty acids	16.98	17.83	15.81	-

[a-b]Means within rows with different superscripts differ ($P<0.05$)

Table 6. Blood parameters of Holstein cows fed supplements based on whole flaxseed (WFL), whole linola (WLO) or Megalac® (MEG)

Item	WFL	WLO	MEG	SE
NEFA, µEq/L	416.63	472.54	411.03	48.09
BHBA, m*M*/L	0.86	1.03	0.87	0.12
Glucose, mMol/L	3.35	3.28	3.28	0.08

Table 7. Liver parameters of Holstein cows fed supplements based on whole flaxseed (WFL), whole linola (WLO) or Megalac® (MEG)

Item	WFL	WLO	MEG	SE
Glycogen, mMol of glucose/ g of liver	0.09	0.09	0.09	0.01
Total lipids, % wet weight	7.80	7.96	10.60	1.11
Triglycerides, % wet weight	4.12	4.38	7.11	1.10
SOD, U / mg of protein	82.39	84.72	81.53	5.65
CAT, U / mg of protein	4304.02	3965.76	3681.21	232.29
GPX, U / mg of protein	129.31	120.98	132.69	7.94

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