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Flaxseed meal feeding to dairy cows as a strategy to improve milk enterolactone concentration: a literature review

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ABSTRACT: Flaxseed *(Linum usitatissimum)* is the richest source of the plant lignan secoisolariciresinol diglucoside (SDG). In mammals, including bovine, SDG is converted to the mammalian lignans enterolactone (EL) and enterodiol (ED) by the action of gastrointestinal microbes. There is a great deal of interest in promoting increased intakes of lignans in humans' diet due to the potential health benefits of mammalian lignans, especially in the prevention of cardiovascular diseases, hypercholesterolaemia, breast and prostate cancers, and osteoporosis. Consumption of milk and dairy products enriched in EL could be an excellent strategy to increase the intake of lignans by humans. This literature review will focus on presenting feeding strategies capable to improve milk enterolactone concentration. Research has demonstrated the potential of flaxseed meal (FM) feeding to dairy cows as a strategy to improve milk EL concentration, therefore enhancing milk nutraceutical proprieties. A considerable number of studies have demonstrated that feeding vegetable lignans-rich sources, such as FM, to dairy cows improves EL in milk. Additionally, it has been reported that changes in the carbohydrate profile of FM-based diets fed to dairy cows can alter the output of milk EL. The application of animal nutrition as a tool to increase nutraceutical properties of milk (i.e. increased EL concentration) is a valuable strategy for promoting the association of milk with humans' health benefits and is of great interest in contemporary society.
Keywords: milk nutraceutical proprieties; bioactive compounds; lignans; disease risk reduction; dairy cattle production.

O farelo de linhaça na dieta de vacas leiteiras como estratégia para aumentar a concentração de enterolactona no leite: revisão de literatura

RESUMO: A linhaça (*Linum usitatissimum*) é a principal fonte da lignana vegetal secoisolariciresinol diglucosídeo (SDG). Em mamíferos, incluindo bovinos, SDG é precursor para a síntese das lignanas de mamíferos enterolactona (EL) e enterodiol (ED) pelos microrganismos gastrointestinais. Existe um grande interesse em promover o aumento da ingestão de lignanas na dieta humana devido aos potenciais benefícios da EL a saúde, incluindo principalmente a prevenção de doenças cardiovasculares, hipercolesterolemia, câncer de mama e de próstata e osteoporose. Assim, objetivou-se fazer uma revisão de literatura sobre estratégias de alimentação capazes de melhorar a concentração de enterolactona no leite, melhorando assim a atividade biológica e os benefícios do leite para a saúde humana. A alimentação de vacas leiteiras com fontes ricas em lignanas vegetais, como o farelo de linhaça (FM), aumenta a concentração de EL no leite. Além disso, estudos têm demonstrado que mudanças no perfil de carboidratos de dietas à base de FM fornecidas a vacas leiteiras alteram a concentração de EL do leite. A aplicação da nutrição animal como ferramenta para aumentar as propriedades nutracêuticas do leite (ex. aumentar a concentração de EL) é uma estratégia valiosa para promover a associação do leite com benefícios à saúde humana e é de grande interesse na sociedade moderna.
Palavras-chave: propriedades nutracêuticas do leite; compostos bioativos; lignanas; redução do risco de doenças; bovinocultura leiteira.

1. INTRODUCTION

Flaxseed has been consumed by humans since ancient times and it has establishing importance as a functional food mainly due to the presence of three main bioactive compounds: α-linolenic acid (ALA), dietary fiber, and lignans (TOURE; XUEMING, 2010; KAJLA et al., 2015). In dairy cows diets, flaxseed can be fed as a source of both energy and protein (PETIT, 2011). Whole flaxseed and flaxseed oil are excellent sources of ALA and the outer fiber-containing layers of flaxseed is the richest source of lignans, which are polyphenolic compounds known as phytoestrogens (THOMPSON et al., 1991; KAJLA et al., 2015).

Feeding flaxseed to dairy cows contributes to favorable changes in milk composition for better human health by enhancing milk nutraceutical compounds. For instance, improved amounts of polyunsaturated fatty acids (PUFA) in milk of dairy cows have been reported with feeding extruded

flaxseed (ZACHUT et al., 2010) and flaxseed oil (CAROPRESE et al., 2010) to dairy cows.

Additionally, supplementation with flaxseed oil has been shown to increase the concentration of conjugated linoleic acid in milk of dairy cows (GLASSER et al., 2008). Whereas, improved concentration of mammalian lignans in milk has been reported with feeding flaxseed meal (FM), a lignan-rich source, to dairy cows (PETIT; GAGNON, 2009; BRITO et al., 2015; LIMA et al., 2016). Mammalian lignans are bioactive compounds with a wide range of biological activities, including: antioxidant, antitumor, and weakly estrogen-linked proprieties. There is great deal of interest in promoting the inclusion of lignan-rich foods in humans' diets due to the potential humans' health benefits of mamalians lignans, including prevention of cardiovascular diseases, hypercholesterolemia, breast and prostate cancers, menopausal symptoms, and osteoporosis (MURKIES et al., 1998; ADLERCREUTZ, 2002).

Secoisolariciresinol diglucoside (SDG) is the major lignan in flaxseed, accounting for more than 95% of the total lignans. It is mostly concentrated in the outer fiber-containing layers of the seed (ADLERCREUTZ; MAZUR, 1997), thus resulting in greater concentration of SDG in hulls compared to seeds. Secoisolariciresinol diglycoside is the major precursor for synthesis of the mammalian lignans enterolactone (EL) and enterodiol (ED) by the gut microbiota in humans (THOMPSON et al., 1991) and ruminants (CÔRTES et al., 2008; GAGNON et al., 2009a). In dairy cows, EL is the predominant mammalian lignan found in the rumen and physiological fluids, including plasma, urine, and milk (CÔRTES et al., 2008; GAGNON et al., 2009a).

Saarinen et al. (2002) reported that rats fed pure EL had a 5-fold increase in urinary excretion of EL compared with those fed plant lignans. These findings indicate that prior absorption, plant lignans must be converted to EL by microbes in the colon, whereas deconjugated EL may be passively absorbed along the intestine of mammals . The concentration of EL in milk of dairy cows can be modulated by dietary changes and EL-enriched milk or dairy products could be used as a source of EL for humans (PETIT; GAGNON, 2009; BRITO et al., 2015). Humans rely on gut microbes to convert plant lignans to mammalian lignans (THOMPSON et al., 1991). Therefore, the intake of EL-enriched milk or dairy products may be more efficient in providing EL for humans, than the intake of plant lignans.

In this sense, there is a growing interest in improving milk EL concentration and dietary strategies have been investigated in order to produce EL-enriched milk. Feeding SDG-rich sources to dairy cows is one of the dietary strategies that can be applied to improve milk EL concentration. Indeed, supplementation with FM (PETIT; GAGNON, 2009; PETIT et al., 2009b; BRITO et al., 2015; LIMA et al., 2016) and flaxseed hulls (GAGNON et al., 2009a; PETIT et al., 2009a) have been reported to improve the concentration of EL in milk of dairy cows. It has also been reported that changes in the carbohydrate profile of FM-based diets fed to dairy cows can potentially alter the output of milk EL (BRITO et al., 2015).

This literature review will focus on presenting feeding strategies capable to improve milk enterolactone concentration. Additionally, this literature review will describe the chemical composition of flaxseed, specifically regarding lignans content while discussing the metabolism of mammalian lignans in both non-ruminant and ruminant animals. Biological activities and potential human health benefits of mammalian lignans will be briefly addressed.

2. LITERATURE REVIEW

2.1 Flaxseed

Flaxseed is a blue flowering annual herb that belongs to the family Lineaceae (RUBILAR et al., 2010; SIGH et al., 2011). Flaxseed is cultivated for fiber, medicinal purposes, and as nutritional product in more than 50 countries (SIGH et al., 2011). Currently, Canada is the largest producer in the world and India, China, United States, and Ethiopia can also be cited as important flaxseed growing countries (SIGH et al., 2011; KAJLA et al., 2015).

The chemical composition of whole-grain flaxseed is detailed on Table 1. Flaxseed chemical composition varies upon growing environment, genetics, processing conditions, and method of analysis (MORRIS, 2007). The major component of flaxseed is its oil. It has around 40% fat found manly as triglycerides (98%) with lower contents of phospholipids (0.9 %) and free fatty acids (0.1%) (MUELLER et al., 2010). Flaxseed is a rich source of n-3 fatty acids, especially ALA which can constitute up to 55% of the total fatty acids in flaxseed (MUSTAFA et al. 2003; MUELLER et al., 2010). The content of neutral detergent fibre (NDF) of flaxseed is around 30% (CHUNG et al., 2005). Flaxseed is also a good protein source. It contains around 20% of crude protein (CP), mainly globulin (26–58%) and albumin (20–42%). Regarding amino acid profile, flaxseed is rich in arginine, aspartic acid, and glutamic acid, and limiting in lysine (CHUNG et al., 2005).

Table 1. Nutritional composition of whole flaxseed
Tabela 1. Composição nutricional da semente de linhaça

Nutrients	Amount[1]
Moisture, g	6.5
Protein, g	20.3
Fat, g	37.1
Minerals, g	2.4
Crude fiber, g	4.8
Total dietary fiber, g	24.5
Carbohydrates, g	28.9
Energy, kcal	530.0
Potassium, mg	750.0
Calcium, mg	170.0
Phosphorus, mg	370.0
Iron, mg	2.7
Vitamin A, μg	30
Vitamin E, mg	0.6
Thiamine (B1), mg	0.23
Riboflavin (B2), mg	0.07
Niacin, mg	1.0
Pyridoxine, mg	0.61
Pantothenic acid, μg	0.57
Biotin, μg	0.6
Folic acid, μg	112

Source: (Kajda et al., 2015)
[1] Amount per 100 g of edible Flaxseed
Fonte: (Kajda et al., 2015)
[1] Quantidade por 100 g de linhaça

Flaxseed can be fed to dairy cows as whole flaxseed, flaxseed oil, and FM. Flaxseed meal is the residue remaining after flaxseed oil extraction. Compared to whole flaxseed, FM is greater in fiber and protein and lower in crude fat

(GAGNON et al., 2009). Therefore, FM is fed to dairy cows mainly as a protein source. Chemical composition of FM and other protein supplements fed to dairy cows are detailed in Table 2.

Table 2. Chemical composition of flaxseed meal, canola meal, soybean meal and cottonseed meal. Value are expressed as % DM except amino acids, which are expressed as % crude protein
Tabela 2. Composição química do farelo de linhaça, farelo de canola, farelo de soja e farelo de algodão. Os valores são expressos como% DM exceto aminoácidos, que são expressos como % proteína bruta

Item	Protein Supplements			
	Flaxseed meal	Canola meal	Soybean meal	Cottonseed meal
DM	91.6	90.1	88.6	90.2
CP	34.3	40.0	48.8	35.0
EE	1.32	1.32	1.71	1.38
NDF	25.0	30.7	14.6	28.5
ADF	16.4	21.77	9.86	28.8
Lysine	3.85	2.36	2.82	1.45
Methionine	1.86	0.83	0.63	0.62
Cysteine	1.56	1.02	0.66	0.51
Threonine	3.65	1.67	1.8	1.27
Tryptophan	1.66	0.48	0.67	0.55
Phenylalanine	4.93	1.56	2.34	2.00
Leucine	6.00	2.69	3.62	2.21
Isoleucine	4.18	1.41	2.07	1.11
Valine	4.99	1.64	2.16	1.64
Histidine	2.15	1.00	1.16	1.00
Arginine	9.10	3.94	5.5	3.94
Tyrosine	2.71	1.21	1.5	1.21
Alanine	4.50	1.64	2.06	1.64
Aspartate	9.14	3.17	5.50	3.17
Glutamate	18.3	7.10	8.70	7.10
Glycine	5.84	1.64	1.97	1.64
Serine	3.88	1.66	2.47	1.66

Source: Valadares Filho et al. (2006)
Fonte: Valadares Filho et al. (2006)

2.1.1 Flaxseed lignans

One of the most interesting characteristics of flaxseed is its content of complex phenols, known as phytoestrogens, primarily lignans. Phytoestrogens are a diverse group of compounds found naturally in many edible plants and their seeds that have a phenolic group shared with estrogenic steroids (WANG et al., 2002). Phytoestrogens are known as plant compounds with estrogen-like biological activity and are classified according to their chemical structure in three major categories: isoflavones, cousmestans, and lignans (ADLERCREUTZ; MAZUR, 1997).

Plant lignans are defined as diphenolic compounds produced by the coupling of 2 coniferyl alcohol residues existing in cell wall of high plants (TOUREAND; XUEMING, 2010). They are found in fibrous rich plants: cereals (barley, wheat and oats), vegetables (broccoli, garlic, asparagus and carrots), legumes (bean, lentil and soybean), fruits, berries, tea, and alcoholic beverages (WANG et al., 2002).

Flaxseed is the richest source of plant lignans. Flaxseed lignans are mostly concentrated in the outer fiber-containing layers of the seed (ADLERCREUTZ; MAZUR, 1997). The concentration of SDG in flaxseed hulls has been reported to be 3.4-times greater than in whole flaxseeds (CORTES et al., 2012). The chemical structure of flaxseed lignans is detailed in Figure 1. Secoisolariciresinol diglycoside is the major lignan of flaxseed, representing over 95% of the total lignans (DAUN et al., 2003; LIU et al., 2006). Minor concentrations of other lignans including matairesinol, pinoresinol, lariciresinol, and isolariciresinol are also present in flaxseed (RAFFAELLI et al., 2002). Table 3 details the concentration of lignans in whole-grain flaxseed. According to Johnsson et al. (2000) SDG concentration in flaxseed ranges from 1.7 to 2.4 % in defatted flour and 0.6 to 1.3 % in whole flaxseed flour. Flaxseed meal SDG concentration were 1.66% and 1.64% of DM in the studies done by Brito et al. (2015) and Petit and Gagnon (2009), respectively.

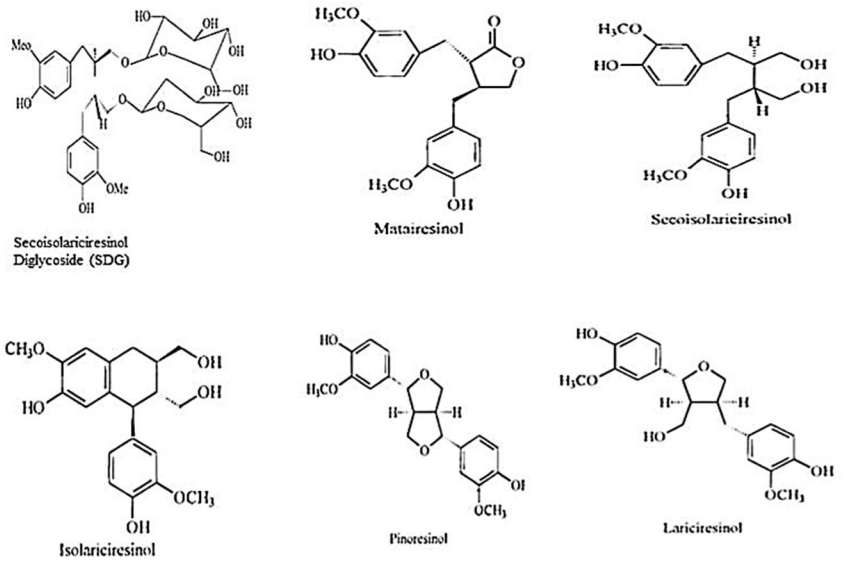

Figure 1. Chemical structure of flaxseed lignans (Adapted from Landete, 2012 and Kajla et al., 2015).
Figura 1. Estrutura química das lignanas de linhaça (adaptado de Landete, 2012 e Kajla et al., 2015).

Table 3. Lignan content of flaxseed
Tabela 3. Conteúdo de Lignanas da linhaça

Serving Size	SDG[1]	MAT[2]	LAR[2]	PINO[2]	SECO[2]	Total[2]
mg[3]	82-2600	0.15	2.8	0.7	375	379
mg[4]	11-286	0.02	0.3	0.1	41	42
mg[5]	8-208	0.01	0.2	0.1	30	30

Abreviations: LAR: lariciresinol; MAT: matairesinol; PINO: pinoresinol.
[1] data adapted from Muir, 2006; [2] data adapted from Thompson et al., 2006. The values for total lignans were calculated by summing the values for MAT, LAR, PINO and SECO. [3] 100 g; [4] one tbsp. (11g) of whole seed; [5] one tbsp. of milled flax (8 g).
Source: adapted from Muir, (2006) and Thompson et al. (2006).
Abreviações: LAR: lariciresinol; MAT: matairesinol; PINO: pinoresinol.
1 dados adaptados de Muir, 2006; 2 dados adaptados de Thompson et al., 2006. Os valores para lignanas totais foram calculados somando os valores de MAT, LAR, PINO e SECO. [3] 100 g; 4 uma colher de sopa. (11g) de semente inteira; 5 uma colher de sopa. de linho moído (8 g).
Fonte: adaptado de Muir, (2006) e Thompson et al. (2006)

2.2 Lignans metabolism in non-ruminant and ruminant animals

2.2.1 Non-ruminant animals

In mammals, plant lignans are metabolized by the gastrointestinal microbiota to the mammalian lignans: EL and ED (THOMPSON et al., 1991; CHEN et al., 2007; CÔRTES et al., 2008; GAGNON et al., 2009) (Figure 2). Figure 3 shows the pathways for mammalian lignans synthesis from different plant lignans. The pathway for conversion of SDG, the major lignan found inflaxseed, to mammalian lignans is detailed in Figure 4. The conversion of plant SGD to mammalian lignans involves basically 3 steps that take place in the gut of non-ruminant animals:

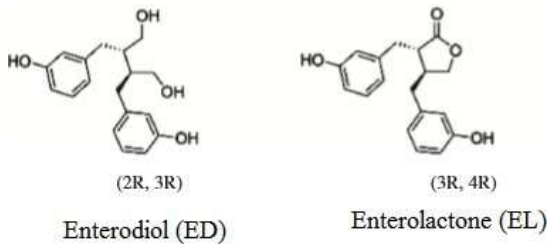

Figure 2. Chemical structure of the mammalian lignans
Fonte: Landete, 2012.
Figura 2. Estrutura química dos lignanos mamíferos
Fonte: Landete, 2012.

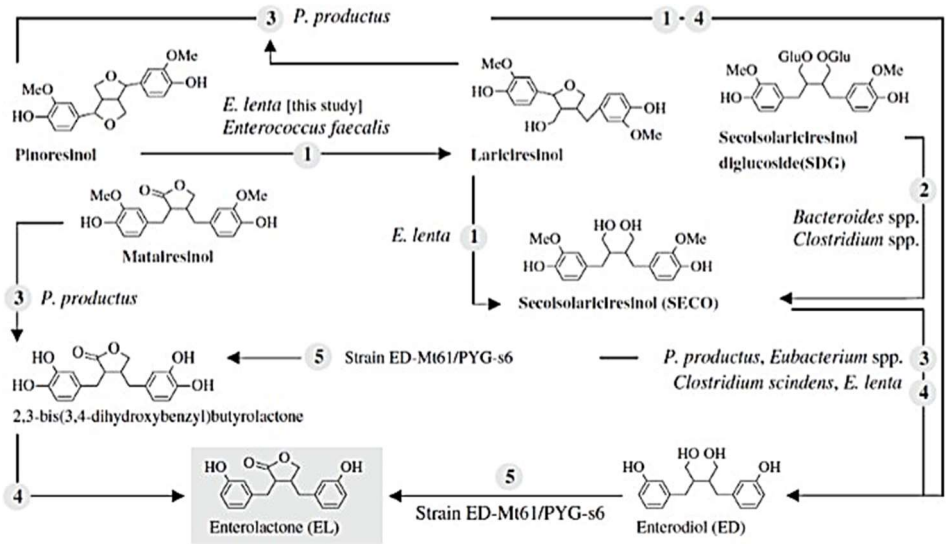

Figure 3. Pathways for mammalian lignans synthesis from plant lignans. Numbers indicate the reactions catalyzed by intestinal bacteria in humans: (1) indicates reduction reaction, (2) deglycosylation, (3) demethylation, (4) dihydroxylation, and (5) dehydrogenation (adapted from
Source: Clavel et al. (2006).
Figura 3. Vias para a síntese de lignanas de mamíferos a partir de lignanas de plantas. Os números indicam as reações catalisadas por bactérias intestinais em humanos: (1) indica reação de redução, (2) desglicosilação, (3) desmetilação, (4) dihidroxilação e (5) desidrogenação (adaptado de
Fonte: Clavel et al. (2006).

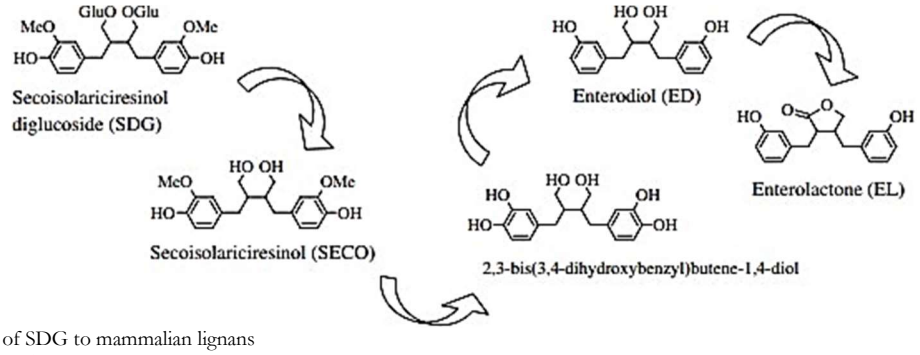

Figure 4. Metabolism of SDG to mammalian lignans
Source: Clavel et al. (2006).
Figura 4. Metabolismo de SDG para lignanos de mamíferos
Fonte: Clavel et al. (2006).

(1) first, SDG is hydrolyzed under the action of intestinal glycosidases to SECO, which is the non-sugar moiety of SGD (CLAVEL et al., 2006; CHEN et al., 2007). *Bacteroides* and *Clostridia* have been reported to release the glucosyl moieties from SDG to yield SECO (CLAVEL et al. 2006). (2) Further, colonic microbes convert SECO to ED by demethylation and dehydrogenation. (3) Finally, ED can be converted to EL by dihydroxylation (CLAVEL et al., 2006; CHEN et al., 2007). *Peptostreptococcus productus*, *Eubacterium callanderi*, *Eubacterium limosum*, and *Bacteroides methylotrophicum* have been identified as the major bacteria responsible for demethylation reactions, whereas dehydrogenation reactions are carried out mainly by *Eubacterium lentum* (WANG et al., 2000; CLAVEL et al., 2007). Several *Clostridia* and *Ruminococcus spp*. have been cited as the major microorganisms responsible for converting ED to EL by dihydroxylation as cited above (CLAVEL et al., 2007).

Mammalian lignans formed from plant lignans by the gut microbiota have three metabolic fates: (1) they are directly excreted in feces, (2) they are taken up by epithelial cells lining of the colon and conjugated with glucuronic acid or sulfate. After conjugation, EL and ED enter the circulation and can eventually be excreted in feces, and (3) they can be absorbed from the gut in their deconjugated form and reach the liver, where free forms are conjugated and released into the bloodstream. Eventually, the conjugated mammalian lignans are excreted into physiological fluids (e.g., plasma and urine) or return to the intestine via enterohepatic circulation (WANG, 2002; LANDETE, 2012). The conjugated forms of mammalian lignans that reach the intestine via enterohepatic circulation are poorly absorbed. The microbial enzyme β-glucuronidase converts mammalians lignans to their deconjugated forms, allowing them to be reabsorbed in the intestine (RAFFAELI et al., 2002). The activity of β-glucuronidase in humans has been attributed to intestinal-dominant bacteria belonging to *Bacteroides, Bifidobacterium, Eubacterium,* and *Ruminococcus* genera (AKAO et al., 2000).

2.2.2 Ruminant animals

The metabolism of lignans in ruminant aminals is not completely elucidated. It is known that plant lignans are metabolized to mammalian lignans by both ruminal and fecal microbiota (CORTES et al., 2008). Recent studies have demonstrated that lignans metabolism in ruminants occurs mainly into the rumen, where plant lignans are converted to mammalian lignans by ruminal microbes (CORTES et al., 2008; GAGNON et al., 2009a).

The study done by Gagnon et al. (2009a) was the first *in vivo* study to investigate the role of ruminal microorganisms in flaxseed lignans metabolism in lactating dairy cows. Ruminally-cannulated dairy cows were assigned to the following experimental treatments: (1) Flaxseed oil and flaxseed hulls administration into the rumen and water infusion in the abomasum; (2) oil and hulls administration into the abomasum; oil infusion in the abomasum and hulls placed in the rumen; or (4) oil placed in the rumen and hulls administered in the abomasum. Enterolactone concentration on milk and urine were 12 and 16 times greater, respectively, with flaxseed hulls administration in the rumen compared to administration of flaxseed hulls in the abomasum. Similarly, plasma EL concentration was three times higher in cows receiving flaxseed hulls in the rumen than those receiving flaxseed hulls in the abomasum. These results demonstrated that ruminal microbiota plays an important role in converting flaxseed lignans to mammalian lignans in dairy cows.

Despite the importance of ruminal microbes on lignans metabolism in dairy cows, just few studies have investigated lignans metabolism in the rumen and how dietary changes could impact this process. Recently, Schogor et al. (2014) studied lignans metabolism using selected pure cultures of ruminal bacteria incubated in vitro with SDG. It was reported that 11 ruminal bacteria, mainly *Prevotella spp.* were able to convert SDG to SECO, which is formed as an intermediate in the ruminal metabolim of SDG to EL. These findings suggest that *Prevotella spp*. may play an important role in lignan metabolism in dairy cows (SCHOGOR et al., 2014). Enterolactone has been identified as the major lignan metabolite present in ruminal fluid, urine, plasma, and milk of dairy cows (PETIT; GAGNON, 2009; GAGNON et al., 2009a; BRITO et al., 2015). Gagnon et al. (2009b) investigated the length of time to obtain peak EL concentration in milk of dairy cows fed FM (20% of diet DM). The length of time need for EL to return to baseline level was also evaluated in this study. It was reported that the conversion of SDG to EL and the transfer of EL to the mammary gland are established after one week of FM supplementation, whereas milk concentration of EL returned to baseline level after one week of FM deprivation.

Studies have investigated β- glucuronidase activity in lignans metabolism in dairy cows. Petit et al. (2009) evaluated the effect of feeding monensin and flaxseed hulls on β-glucuronidase activity in rumen fluid and feces. Monensin is known to decrease the growth of Gram-positive bacteria and could potentially impact β- glucuronidase activity, considering that strains of ruminal bacteria with β-glucuronidase activity (e.g. Ruminococcus and Eubacterium) are Gram positive bacteria (JENAB; THOMPSON, 1996). Indeed, the activity of β- glucuronidase in ruminal fluid tended to decrease when cows received monensin. Flaxseed hulls supplementation decreased β- glucuronidase activity in both ruminal fluid and feces (PETIT et al.,2009).

Similar results were reported with ruminal infusion of flaxseed oil in the study done by Gagnon et al. (2009a) and could be explained by the high content of omega-3 FA o these flax products which can negatively affect the growth of ruminal bacteria (MAIA et al. 2007). A subsequent study, (LIMA et al., 2016) investigated the effects of dietary FM and abomasal infusion of flaxseed oil and their interaction on activity of β-glucuronidase. It was reported that abomasal infusion of flaxseed oil, which is a PUFA rich source, had no effect on β-glucuronidase activity. These results suggest that polyunsaturated fatty acids do not interfere with the absorption of mammalian lignans in ruminants. No effect of FM supplementation was reported on activity of β-glucuronidase. The author also reported higher activity of β-glucoronidase in feces than in ruminal fluid suggesting that the deconjugation reactions may be more important in the large intestine than in the rumen of ruminant animals (LIMA et al., 2016).

2.3 Biological activities and potential human health benefits of mammalian lignans

There is a growing interest in promoting the inclusion of lignans-rich sources in human diets due to the potential health benefits of mammalian lignans. Flaxseed lignans and the mammalian lignans ED and EL are biologically active

substances that elicit a wide range of biological activities including weak estrogenic and cardioprotective effects, as well as antiestrogenic, antioxidant, anti-inflammatory, and anticarcinogenic properties (ADOLPHE et al., 2010; HÖGGER, 2013; IMRAN et al., 2015; LANDETE, 2012).

Lignans and mammalian lignans are known to be strong antioxidants and their antioxidant proprieties are presumably the main reason for the anticancer activity of these components in humans (PRASAD, 2000; LANDETE, 2012). Prasad (2000) studied the antioxidant activity of SECO, ED, and EL using chemiluminescence of zymosan-activated polymorphonuclear leukocytes. SDG and vitamin E were used for comparison. The highest antioxidant activity was reported with SECO and ED, whereas, vitamin E resulted in the lowest antioxidant activity. The antioxidant potency of SECO, ED, EL, and SDG was 4.86, 5.02, 4.35, and 1.27 respectively, compared to vitamin E (PRASAD, 2000). Phytoestrogens, including lignans exhibit both in vitro and in vivo weak estrogenic and antiestrogenic actions (LANDETE, 2012). Mammalian lignans have an aromatic structure similar to the endogenous estrogen, estradiol (MORRIS, 2007). It is believed that mammalian lignans act by binding to estrogen receptors (ER) on cell membranes (LANDETE, 2012; MORRIS, 2007).

Enterolactone can function as weak estrogen, in this circumstance EL binds to ER and mimic the action of endogenous estrogen working as an agonist (LANDETE, 2012). For example, EL can stimulate growth of breast cancer cells as reported by Wang and Kurzer (1997). Anti-estrogenic proprieties of EL have also been reported. In cell-based studies EL binds the ER inhibiting breast cancer cells growth (BUCK et al., 2010). In this situation, EL blocks the action of endogenous estrogen, working as antagonists (LANDETE, 2012).

In addition to the estrogen-like activity of mammalian lignans, studies have also reported that mammalian lignans exhibit effects on hormone metabolism and availability. For example, EL has been shown to stimulate the synthesis of sex hormone binding globulin, which binds sex hormones and reduce their circulation in blood, thus decreasing their biological activity (THOMPSON et al., 1996). Furthermore, mammalian lignans are believed to influence enzyme activity. For instance, EL inhibit the activity of aromatase, an enzyme involved in the production of estrogens (LANDETE, 2012, ADLERCREUTZ et al., 1993).

Research has reported the important role of mammalian lignans in preventing various types of cancer specially the hormone sensitive ones, primarily breast and prostate cancer. In vitro studies have reported that mammalian lignans are possibly responsible for growth inhibition of human prostate cancer (WESTCOTT; MUIR, 2003) and breast cancer cells (BUCK et al., 2010). Additionally, epidemiological studies have linked high lignan intake to lower cancer risk.

For instance, Touilland et al. (2007) conducted a seven years long study with 58,049 female participants and reported that high dietary intake of plant lignans were associated with reduced risks of postmenopausal breast cancer. Buck et al. (2010) conducted a meta-analysis to investigate the association between lignans and breast cancer risk. The meta-analysis investigated a total of 21 studies, in which high lignans intake was associated with a significant reduction in breast cancer risk in postmenopausal women (BUCK et al., 2010). Despite the potential human health benefits of mammalian lignans, intake of phytoestrogens may also have adverse health effects, particularly in critical stages of infant development (SETCHELl, 1998; ZUNG et al., 2008; LANDETE, 2012) and timing of exposure is crucial to maximize potential health benefits while minimizing adverse health effects.

2.4 Improving enterolactone concentration in milk

The concentration of EL in milk of dairy cows can be modulated by dietary changes. Improved concentrations of EL have been reported with feeding SDG-rich sources, such as, FM and flaxseed hulls to dairy cows. Petit et al. (2009b), conducted a study to determine the effects of feeding FM and whole flaxseed (both fed at 10% of DM) on concentrations of ED and EL in milk of Holstein cows. The mammalian lignan ED was not detected in milk. Milk enterolactone concentration was higher in cows receiving FM (0.713 mg/d of EL in milk) than those fed whole flaxseed (0.505 mg/d) and the control diet (no flaxseed products, 0.231 mg/d). Feeding the two flaxseed products resulted in different intakes of SDG: 15280 mg/d in cows receiving FM and 8050 mg/d in cows receiving whole flaxseed which is explain by the fact that lignans are concentrated in the outer fibre containing layers of grains (ADLERCREUTZ; MAZUR, 1997) which leads to higher SDG concentration in flax products with lower concentrations of oil. Lower SDG intake in cows receiving whole flaxseed explains the lower EL concentration in milk reported in this study in cows fed whole flaxseed at 10% of the DM (PETIT et al., 2009b). Petit and Gagnon (2009b) fed Holstein cows with incremental amounts of FM: 0, 5, 10 and 15% of DM and reported that the concentration of EL in milk increased linearly. Concentration of EL in milk, expressed as mg/d, was 0.175, 0.312, 0.393 and 0.535 for cows receiving 0, 5, 10 and 15% of FM, respectively. Concentration of ED in milk was below detection level. Similarly, Petit and Gagnon (2011) reported linear increase on milk EL concentration with feeding increasing levels of flaxseed hulls (0, 5, 10, 15, 20% of DM) to Holstein cows (PETIT; GAGNON, 2011).

Lima et al. (2016) investigated the effects of dietary FM and abomasal infusion of flaxseed oil and their interaction on milk enterolactone concentration in rumen-fistulated Holstein cows. Cows received four different diets: (1) control diet with no FM (CON); (2) diet containing 12.4 % of FM in the dry matter; (3) no FM and 250 g of flaxseed oil/day infused in the abomasum; and (4) 12.4% of FM and 250 g flaxseed oil/day infused in the abomasum.

Dietary FM increased concentrations of EL in milk. Milk EL concentration were 2.11 mg/d and 2.61 mg/d in cows fed 12.4% of FM and no oil (diet 2) and those fed 12.4% of FM and flaxseed oil infusion in the abomasum (diet 4), respectively (LIMA et al., 2016).

Recently, it was reported that the concentration of EL in milk can be modified by the type of NSC source supplemented to dairy cows fed diets containing FM, with LM resulting in greater milk EL than GRC (BRITO et al., 2015). In this study, Jersey cows were fed mixed mostly grass hay and one of the following 4 concentrate blends: (1) GRC (12% of DM) plus a protein mix containing soybean meal (11% of DM) and sunflower meal (5% of DM); (2) GRC (12% of DM) plus flaxseed meal (16% of DM); (3) LM (12% of DM) plus the same protein mix of diet 1; or LM (12% of DM) plus flaxseed meal (16% of DM). Milk EL concentration were: 0.37, 1.68, 0.75 and 2.40 mg/d in cows fed diets 1, 2, 3 e 4, respectively. Increased milk EL

concentration was observed when FM was fed to the cows. Additionally, it was reported that cows fed LM and FM had higher concentration and yield of milk EL than those fed GRC and FM. This finding suggests that LM, which is a sucrose source, may be more efficient in selecting for ruminal microbes with higher capacity to metabolize plant lignans to mammalian lignans in the rumen than GRC, which is a starch source (BRITO et al., 2015).

A subsequent study was carried to better understand how changes in diet NSC profile could impact EL output in milk of dairy cows fed FM (GHEDINI et al., 2017). This study investigated the effects of replacing GRC with incremental amounts of LM on milk enterolactone concentration in Jersey cows fed FM (15% DM) and low-starch diets (GRC-to-LM dietary ratio were: 12:0, 8:4, 4:8, and 0:12, dry matter basis). The concentration of EL in milk tended to respond cubically with replacing GRC by incremental amounts of LM in FM-based diets. Based on their results, the authors suggest that further studies are needed to fully elucidate how FM-SDG is affected by changes in ruminal microbiome and diet composition (GHEDINI et al., 2017).

Considering that *Prevotella spp*. may play a role in lignan metabolism in dairy cows (SCHOGOR et al., 2014) as described previosly, dietary changes that favor or result in a greater prevalence of *Prevotella spp.* in the rumen could improve EL secretion on milk. Li et al. (2015) reported that when steers were fed diets containing 4% flaxseed oil the genus *Prevotella* dominated the ruminal bacterial community, suggesting that PUFA supplementation favors *Prevotella spp.* growth in the rumen. The effect of feeding sucrose and FO alone or in association (sucrose + FO) on milk EL concentration of dairy cows fed 15% FM was investigated by Ghedini et al (2017). Holstein cows were fed four different treatments: 1) 8% soybean meal (control); (2) 5% sucrose + 15% FM; (3) 3% FO + 15% FM; and (4) 5% sucrose + 3% FO + 15% FM. The authors hypothesized that sucrose and FO could synergistically interact to increase the concentration of EL in milk as both,sugars and FO, have been shown to promote growth of Prevotella spp. The average concentration of EL in milk increased 4-fold in cows fed 15% FM compared with the control diet (diet with no FM). However, no differences in milk EL concentration were observed among the treatments containing FM supplemented with sucrose or FO or their association (GHEDINI, 2017). Despite these findings, the effect of PUFA on SDG metabolism and subsequent milk EL concentration has not been fully investigated.

3. CONCLUSIONS

Milk concentration of EL, a nutraceutical component with humans' health benefits can be modulated by altering diets of dairy cows. Feeding FM improves milk EL concentration as it is a lignan-rich source. In ruminants, plant lignans (SDG) are converted to mammalian lignans (EL and ED) mainly in the rumen by the action of rumen microbes. Increased concentration of EL with FM feeding to dairy cows demonstrates that EL produced in the rumen reaches the mammary gland and is transferred to milk. Therefore, FM feeding to dairy cows improves the capacity of milk to favor humans´ health. Research has also demonstrated that changing carbohydrate profile of FM-based diets fed to dairy cows can potentially alter the output of milk EL. Considering the importance of rumen microbes on lignans metabolism, changes in diversity and function of the ruminal microbiome have the potential to alter the concentration of EL in milk.

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