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Dietary flax meal and abomasal infusion of flax oil on microbial β-glucuronidase activity and concentration of enterolactone in ruminal fluid, plasma, urine and milk of dairy cows

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ABSTRACT

This study was performed to evaluate the effects of dietary flax meal (FM) and abomasal infusion of flax oil (FO) and their interaction on activity of β-glucuronidase in ruminal fluid and feces, and concentration of the mammalian lignan enterolactone (EL) in ruminal fluid, plasma, urine, and milk. Rumen fermentation characteristics and the pH in ruminal fluid and feces were also investigated to determine any potential interference with β-glucuronidase activity and EL concentration. Eight rumen fistulated lactating Holstein cows were assigned to a double 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments: (1) control diet with no FM (CON); (2) diet containing 124 g/kg FM (FMD) in the dry matter (DM); (3) CON and 250 g FO/day infused in the abomasum; and (4) FMD and 250 g FO/day infused in the abomasum. Abomasal infusion of FO had no effect on β-glucuronidase activity in the gastrointestinal tract and EL concentration of ruminal fluid, plasma, urine, and milk. Dietary FM and abomasal infusion of FO had no effect on fecal pH and microbial activity of β-glucuronidase in ruminal fluid and feces. Dietary FM increased concentrations of EL in ruminal fluid, plasma, urine, and milk. Molar proportion of propionate was increased and that of isovalerate was decreased with FM supplementation. Abomasal infusion of FO had no effect on ruminal concentration of ammonia N. Dietary FM had no effect on ruminal pH. As abomasal infusion of FO had no effect on β-glucuronidase activity in the gastro-intestinal tract and EL concentration, our results suggest that polyunsaturated fatty acids such as n-3 do not interfere with the absorption of mammalian lignans. Moreover, feeding 124 g/kg DM FM with a source of FA rich in n-3 bypassing the rumen increases EL concentration in physiological fluids of dairy cows, especially milk, which can be used as a strategy to optimize the concentration of mammalian lignans in milk and their potential beneficial effects on human health.

Abbreviations: DM, dry matter; DMI, dry matter intake; EL, enterolactone; FA, fatty acids; FM, flax meal; FMD, flax meal diet; FO, flax oil; VFA, volatile fatty acids.

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1. Introduction

Lignans are fiber-related polyphenols (Begum et al., 2004) found in many plants or plant-derived food (Milder et al., 2005; Penalvo et al., 2008) such as flax (*Linum usitatissimum*) products (seeds, meal and hulls), which are the richest sources (Landete, 2012). When consumed by mammals, plant lignans are metabolized by the gastrointestinal microbiota into enterolignans or mammalian lignans (Ford et al., 2001; Saarinen and Thompson, 2010; Toure and Xu, 2010). Previous studies (Gagnon et al., 2009a; Petit and Gagnon, 2009) have shown that the main mammalian lignan present in physiological fluids (*i.e.*, plasma, urine, and milk) of cows fed flax products is enterolactone (EL). As Prasad (2000) has shown that EL has greater antioxidant activity than vitamin E, feeding strategies to increase EL concentration in milk could then add a plus value to milk and dairy products.

Rumen microbes have been reported to play an important role in the conversion of plant into mammalian lignans (Côrtes et al., 2008; Gagnon et al., 2009a). Although the main site of transformation of lignans into EL is the rumen, the intestine may play an important role in the absorption of mammalian lignans. Indeed, mammalian lignans are absorbed in the intestine and under the action of specific enzymes they are conjugated as sulphate and glucuronide in the intestinal wall and liver (Barnes et al., 1996). Conjugated mammalian lignans are transferred into physiological fluids, excreted in urine or in the intestinal lumen via enterohepatic circulation (Hoikkala et al., 2003). The conjugated forms of mammalian lignans are poorly absorbed by the intestine and deconjugation is required to increase the hydrophobicity of lignans and allow their reabsorption (Raffaelli et al., 2002). Microbial β-glucuronidase is the enzyme responsible for deconjugation of mammalian lignans. As variation in intestinal microflora plays an important role in the inter-individual variation metabolism of plan lignans (Raffaelli et al., 2002), any change in microbial β-glucuronidase activity may then affect EL absorption in the intestine and concentration in milk.

Dietary supplementation with sources of fat and oil has become a common practice to potentially improve the energy balance of high-yielding cows. However, fatty acid (FA) addition to the diet may affect the metabolism and absorption of mammalian lignans. Indeed, the presence of n-3 FA in ruminal fluid has been shown to decrease the activity of β-glucuronidase in the rumen (Gagnon et al., 2009a) although n-3 FA infused in the abomasum had no effect on enzyme activity in the intestine (Côrtes et al., 2013) of dairy cows fed flax hulls. However, as flax hulls contain both oil and lignans, it is impossible to differentiate the exact role of each component of flax (*i.e.*, oil and lignan) on the activity of β-glucuronidase and EL concentration in the previous experiments (Gagnon et al., 2009a; Côrtes et al., 2013). Thus, further studies to dissociate the effects of lignans and oil may improve our knowledge on the metabolism of lignans in dairy cows.

Flax meal (FM) is a rich source of flax lignans owing to oil extraction. When fed to dairy cows without oil supplementation, FM has been shown to increase the concentration of EL in blood, urine and milk of dairy cows (Gagnon et al., 2009b; Petit et al., 2009). However, there is no information on the interaction between FM with n-3 FA on β-glucuronidase activity and EL concentration in physiological fluids of dairy cows. We hypothesized that dietary FM increases EL concentration in biological fluids of dairy cows supplemented with a source of rumen bypass n-3 FA in the form of flax oil (FO) and that a source of rumen bypass n-3 FA has no effect on β-glucuronidase activity. Therefore, the present study was performed to evaluate the effects of dietary FM and abomasal infusion of FO and their interaction on activity of β-glucuronidase in ruminal fluid and feces, and the concentration of EL in ruminal fluid, plasma, urine, and milk. Rumen fermentation characteristics and the pH in ruminal fluid and feces were also investigated to determine any potential interference with β-glucuronidase activity and EL concentration.

2. Material and methods

2.1. Cows, experimental design, and diets

The guidelines of the Canadian Council on Animal Care (2009) were followed and all experimental procedures were approved by the local Animal Care Committee.

Details of the experimental design have been published previously (Lima et al., 2014). Briefly, eight multiparous lactating Holstein cows (averaging at the beginning of the experiment, 108 ± 39 days in milk, 32.6 ± 5.6 kg of milk/day, and 759 ± 44 kg of body weight) fitted with ruminal cannulas were used in a replicated 4 × 4 Williams Latin square with four 21-day periods. The experimental treatments were: (1) control diet with no FM (CON); (2) diet containing 124 g/kg FM (FMD) in the dry matter (DM); (3) CON and 250 g FO/day infused in the abomasum; and (4) FMD and 250 g FO/day infused in the abomasum. Diets (Table 1) were formulated according to NRC (2001) guidelines. Flax oil contained, expressed as g/kg of total FA, 65 g/kg of 16:0, 48 g/kg of 18:0, 258 g/kg of *cis*9-18:1, 169 g/kg of *cis*9,*cis*12-18:2, 447 g/kg of *cis*9,*cis*12,*cis*15-18:3, and 13 g/kg of others. Flax meal was added to the total mixed diet and contained, on DM basis, 363 g/kg of crude protein, 256 g/kg of neutral-detergent fiber, 177 g/kg of acid-detergent fiber, and 12.9 g/kg of ether extract. Cows were housed in a tie stall barn with free access to water, fed twice a day (08:00 and 19:00 h) and milked twice a day (06:30 and 19:30 h).

As described by Gressley et al. (2006), abomasal infusions were performed using an infusion line which was inserted through the ruminal cannula and the sulcus omasi. Plastisol discs (12 cm in diameter and 9 mm in height) were used to anchor the infusion line in the abomasum, and placement of infusion lines in the abomasum was monitored daily to ensure postruminal delivery. Flax oil was delivered in the abomasum using variable-speed peristaltic pumps