Animal Feed Science and Technology 220 (2016) 143–150

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of duodenal infusion of sunflower oil on β-glucuronidase activity and enterolactone concentration in dairy cows fed flax meal

F.E. De Marchi[a], M.F. Palin[b], G.T. Santos[a], C. Benchaar[b], H.V. Petit[b,*]

[a] Departamento de Zootecnia, Universidade Estadual de Maringá, Maringá, PR 87020-900, Brazil
[b] Sherbrooke Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC J1M 0C8, Canada

ARTICLE INFO

Article history:
Received 24 March 2016
Received in revised form 28 July 2016
Accepted 1 August 2016

Keywords:
β-glucuronidase
Cow
Fatty acids
Lignans
Flax
Sunflower oil

ABSTRACT

Microbial β-glucuronidase activity is important for the absorption of enterolignans. This study was performed to evaluate the effects of dietary flax meal and abomasal infusion of sunflower oil (SO) on activity of β-glucuronidase in ruminal fluid and feces, and concentration of enterolactone (EL) in ruminal fluid, plasma, urine and milk. 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 (CO); (2) diet containing 137 g/kg flax meal (FM); (3) CO and infusion of 250 g SO/d in the abomasum; and (4) FM and infusion of 250 g SO/d in the abomasum. Activity of β-glucuronidase in ruminal fluid was similar among diets. Cows fed the FM diets had lower fecal β-glucuronidase activity than those fed the CO diets. Infusion of SO lowered fecal β-glucuronidase activity. Supplementation with FM increased EL concentration in plasma, urine and prefeeding ruminal fluid, and SO reduced EL concentration in ruminal fluid before feeding. Dietary FM and SO infusion had no effect on milk EL concentration. Overall, the metabolism of flax lignans and the absorption of enterolignans were not affected by the presence of SO, a rich source of n-6 fatty acids, in the intestine as indicated by similar concentrations of EL in ruminal fluid (pool of 2, 4 and 6 h postfeeding), plasma and urine. These results suggest that the effect of fat on the metabolism of lignans may differ between results observed in the present experiment with ruminant animals and those obtained in previous studies with non-ruminant animals.

1. Introduction

In recent years, emphasis has been put on improvement of quality of milk by increasing the amount of nutraceutical compounds such as polyunsaturated fatty acids (FA), conjugated linoleic acids, phytoestrogens, and antioxidants while promoting health of cows (Zened et al., 2013; Xu et al., 2014). Many feedstuffs contain phytoestrogens, mainly isoflavones, coumestans, and lignans, which are transferred to milk after digestion (Hojer et al., 2012; Njåstad et al., 2014).

Abbreviations: CO, control diet; EL, enterolactone; FA, fatty acids; FM, flax meal; SDG, secoisolariciresinol diglucoside; SO, sunflower oil; VFA, volatile fatty acids.

* Corresponding author.
E-mail address: helene.petit@agr.gc.ca (H.V. Petit).

http://dx.doi.org/10.1016/j.anifeedsci.2016.08.002
0377-8401/

Lignans, which are polyphenolic compounds found in grains, vegetables, seeds and fruits, are converted by the intestinal microflora into enterolignans, also known as mammalian lignans, with phytoestrogens properties and strong antioxidant capacity (Landete, 2012). Flaxseed (*Linum usitatissimum*) is the richest source of plant lignans, and secoisolariciresinol diglucoside (SDG) and matairesinol are the major lignans present in flax, with SDG representing more than 95% of all lignans (Meagher and Beecher, 2000; Schogor et al., 2014). As SDG is concentrated in the outer fiber-containing layers of flax, extraction of oil results in flax meal being a good source of plant lignans with around 1.64 g SDG/kg dry matter (Petit and Gagnon, 2009b). Conversely, soy products have only around 0.02 mg/100 g fresh material (Milder et al., 2005).

Enterolactone (EL) and enterodiol are the main enterolignan metabolites present in ruminal fluid, urine, and milk of cows fed flax products (Gagnon et al., 2009a; Petit et al., 2009). In ruminants, the metabolism of plant lignans and other phytoestrogens occurs in the rumen by microbes that hydrolyze the glycosides SDG and matairesinol mainly into enterodiol and EL (Gagnon et al., 2009a; Njåstad et al., 2014). Studies have shown that the absorption of EL and enterodiol occurs either in the rumen or the small intestine, where enterodiol may be further converted to EL by colon microbes (Côrtes et al., 2008; Njåstad et al., 2014). It is believed that after absorption, similar to what happens in non-ruminant, lignans are conjugated as sulphates or glucuronides and transferred into physiological fluids, excreted in milk and urine or in the intestinal lumen via enterohepatic circulation where they may be deconjugated by microbial β-glucuronidases and reabsorbed (Gagnon et al., 2009a; Njåstad et al., 2014). Microbial β-glucuronidase is the enzyme responsible for deconjugation of enterolignans in non-ruminant animals (Jenab and Thompson, 1996), thus it is important to ensure that diet does not affect its activity.

Concentration of EL in milk of dairy cows increases with supplementation of flax hulls (Côrtes et al., 2013) while that of isoflavones increases in urine of dairy cows fed soybeans (Flachowsky et al., 2011). Altogether, these results suggest that microbial β-glucuronidase activity contributes to increase the amount of phytoestrogens in milk as a result of greater gastrointestinal absorption in ruminant animals. A recent study has shown that ruminal activity of microbial β-glucuronidase is decreased by the presence of n-3 FA (Gagnon et al., 2009a) although n-3 FA in the intestine did not interfere with the absorption of the enterolignan EL (Côrtes et al., 2013). However, the metabolism of flax lignans in ruminants is not well understood and there is no information on the effect of n-6 FA on microbial 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 n-6 FA (sunflower oil; SO) bypassing the rumen and that the presence of n-6 FA in the small intestine has no effect on β-glucuronidase activity. Sunflower was selected as the source of n-6 FA as lignans are present in the seeds but not in the oil (Milder et al., 2005). Therefore, the present study was performed to evaluate the effects of dietary flax meal and abomasal infusion of SO 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.

2. Material and methods

2.1. Animals and experimental treatments

Eight multiparous Holstein cows fitted with ruminal cannula (10 cm; Bar Diamond Inc., Parma, ID) were used in a double 4 × 4 Latin square design balanced for residual effects with a 2 × 2factorial arrangement of treatments with four 21 d periods. Cows averaged 56 ± 21.5 d in milk, 45 ± 9.6 kg of milk/d, and 707 ± 38.8 kg of body weight at the start of the experiment. Cows were kept in individual stalls with free access to water and they were fed for *ad libitum* intake (100 g/kg, as-is basis refusals) twice a day (09:00 and 19:00 h) and milked twice daily at 07:15 and 19:15 h. Milk production and feed intake were recorded daily throughout the experiment. The diets (Table 1) were formulated to meet requirements for cows producing 45 kg/day of milk with 37 g/kg of fat (NRC, 2001). The experimental protocol complied with guidelines of the Canadian Council on Animal Care (CCAC, 2009).

The four treatments were: 1) control diet with no flax meal (CO); 2) diet containing 137 g/kg flax meal (FM); 3) CO and infusion of 250 g SO/d in the abomasum; and 4) FM and infusion of 250 g SO/d in the abomasum. The infusion of SO in the abomasum was conducted with 100% of the experimental dose of oil over a 23-h period (250 g/d) from day 8–21 of each period at a rate of 10.86 g/h. To perform abomasal infusions, an infusion line was inserted through the rumen cannula as described by Gressley et al. (2006). Placement of the infusion lines was monitored daily to ensure postruminal delivery. Oil was pumped into the abomasum using a peristaltic pump (Masterflex L/S; Cole-Parmer Canada Inc., Montreal, QC, Canada). The SO (Brenntag Canada, Inc.) contained, expressed per kg of total FA, 81 g of 16:0, 50 g of 18:0, 295 g of *cis*9-18:1, 532 g of *cis*9,*cis*-12-18:2, 4.3 g of *cis*9,*cis*12,*cis*15-18:3 and 33 g of others (method 996.06; AOAC, 2005). Flax meal contained, expressed per kg of dry matter (DM), 354 g of crude protein, 266 g of neutral-detergent fiber, 148 g of acid-detergent fiber and 9.2 g of fat.

2.2. Experimental procedures

Samples of the total mixed diets were taken daily from day 15–21 and pooled by period and treatment, and one sample of FM was taken on the third week of each period. All samples were frozen at −20 °C for subsequent drying at 55 °C for 72 h. On day 19, a total of 600 ml of ruminal contents were collected 0, 2, 4, and 6 h after the morning meal from five different locations within the rumen. Ruminal pH was monitored immediately after sample collection with a portable pH meter (OAKTON; Eutech Instruments, Singapore). The ruminal contents were then strained through four layers of cheesecloth.