Livestock Science 176 (2015) 196–204

Contents lists available at ScienceDirect

Livestock Science

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

Effects of supplementation of flax meal and flax oil on mammary gene expression and activity of antioxidant enzymes in mammary tissue, plasma and erythrocytes of dairy cows

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ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form
16 February 2015
Accepted 17 March 2015

Keywords:
Antioxidants
Lignans
Flaxseed
Mrna abundance
Omega-3

ABSTRACT

The effects of antioxidants from flax meal (FM) and abomasal infusion of flax oil (FO) on the activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase (GPX)) in blood and mammary tissue and the mRNA abundance of antioxidant and lipogenic-related genes in mammary tissue of dairy cows were determined. Eight ruminally 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/d infused in the abomasum; (4) FMD and 250 g FO/d infused in the abomasum. Catalase activity in erythrocytes tended to increase when cows were fed FMD. Abomasal infusion of FO had no effect on activity and gene expression of antioxidant enzymes and gene expression of lipogenic-genes in mammary tissue, except for an increase in GPX1 expression in the absence of FM. The results suggest that feeding 124 g/kg FM and infusing 250 g of FO/d in the abomasum of dairy cows does not induce significant changes in the activity of antioxidant enzymes in blood and mammary tissue, and expression of antioxidant and lipogenic-genes in mammary tissue. However, more studies are required to determine any beneficial effects of natural antioxidants such as FM on the oxidative status of cows supplemented with polyunsaturated fatty acids, which could lead to feeding strategies to prevent diseases affecting the health status of dairy cattle.

Abbreviations: ACACA, acetyl-coenzyme A carboxylase alpha; ACTB, actin beta; CAT, catalase; CD36, cluster of differentiation 36; CON, control diet; DM, dry matter; FASN, fatty acid synthase; FM, flax meal; FMD, flax meal diet; FO, flax oil; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPX, glutathione peroxidase; LPL, lipoprotein lipase; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PPIA, peptidylprolyl isomerase A; PPARG1, peroxisome proliferator-activated receptor-γ1; PPARG2, peroxisome proliferator-activated receptor-γ2; PPARα, peroxisome proliferator-activated receptor alpha; PUFA, polyunsaturated fatty acids; RG, reference gene; ROS, reactive oxygen species; SCD, stearoyl-coA desaturase; SOD, superoxide dismutase; SREBP1, sterol regulatory element binding transcription factor 1; UBQ, ubiquitin

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http://dx.doi.org/10.1016/j.livsci.2015.03.015
1871-1413/

1. Introduction

Fat has been considered an important constituent contributing to the organoleptic, processing and physical properties of ruminant milk. Therefore, understanding the regulation of milk fat synthesis is central to the development of nutritional strategies to enhance the nutritional value of milk and improve the energy balance of lactating ruminants (Shingfield et al., 2010). Supplying rumen bypass vegetable oils to dairy cows changes milk composition and enhances the concentration of polyunsaturated fatty acids (FA) in milk fat (Litherland et al., 2005; Kazama et al., 2010; Côrtes et al., 2011). However, supplemental fat rich in polyunsaturated FA and oilseeds impact cellular processes affecting many physiological systems. For example, Côrtes et al. (2012) reported that abomasal infusion of flax oil (FO) reduces mRNA abundance of catalase (CAT), glutathione peroxidases (GPX1 and GPX3) and superoxide dismutase (SOD3) in mammary tissue. Moreover, Jacobs et al. (2013) observed that C18:2*cis*-9,*cis*-12 and C18:3*cis*-9,*cis*-12,*cis*-15 FA reduced mRNA abundance of acetyl-coenzyme A (CoA) carboxylase (ACACA), fatty acid synthase (FASN; trend at $P=0.07$) and stearoyl-CoA 1 (SCD1), as well as the transcription factor sterol regulatory element binding factor (SREBP1) in bovine mammary epithelial cells. Other studies also have suggested that polyunsaturated FA increase free radical load in ruminants (Vázquez-Añón and Jenkins, 2007; Vazquez-Anon et al., 2008; Wang et al., 2010) as shown by higher lipoperoxidation susceptibility of plasma in sheep (Gladine et al., 2007) and dairy cows (Gobert et al., 2009) supplemented with omega-3-rich diets. Although the physiological basis for this response to lipid supplements is not totally understood yet, failure of the endogenous antioxidant system in preventing lipoperoxidation may be involved as shown by reduced activity of GPX in plasma of dairy cows infused with FO (Côrtes et al., 2012). Moreover, Mach et al. (2011) have reported that unsaturated FA supplementation down-regulates genes associated with the nuclear factor kappa-light-chain-enhancer of activated B cells in mammary tissue, which is a transcription regulator of genes encoding cytokines, cytokine receptors, cell adhesion molecules and antioxidant enzymes that drive immune, inflammatory and antioxidant responses (Sigal, 2006; Abarikwu et al., 2013). Indeed, abomasal infusion of FO decreases mRNA abundance of catalase, GPX1, GPX3 and superoxide dismutase (SOD2 and SOD3) in mammary tissue (Côrtes et al., 2012).

Recently, much attention has been focused on antioxidant supplementation in order to develop nutritional strategies to reduce oxidative stress in dairy cows (Abuelo et al., 2013; Lean et al., 2013; Rizzo et al., 2013). For example, flax products are rich in antioxidants (Toure and Xu, 2010), and flax hull supplementation has been shown to increase mRNA abundance of CAT, GPX1 and SOD1 genes in mammary tissue of dairy cows, which can contribute to protect against damage of oxidative stress occurring in the mammary gland and other tissues. Antioxidants in flax products are present as lignans, which have stronger antioxidant activity than vitamin E (Prasad, 2000). Furthermore, it is thought that flax lignans modulate both antioxidant and lipogenic-related genes. Indeed, flax hulls increase the abundance of antioxidant genes (Côrtes et al., 2012) and some lipogenic-related genes (Palin et al., 2014) in mammary tissue of dairy cows. Flax meal is the richest source of flax lignans owing to oil extraction and Schogor et al. (2013) reported that flax meal improves the oxidative status of Holstein cows as suggested by decreased TBARS production in ruminal fluid 2 h post-feeding and increased NFE2L2/nuclear factor-E2-related factor 2 (Nrf2) mRNA abundance in mammary tissue. Although FM supplementation contributes to improve the oxidative status of cows, there is no information on the potential of FM to prevent oxidative conditions brought by polyunsaturated FA supplementation. We hypothesized that supplementation with a source of omega-3 FA bypassing the rumen down-regulates lipogenic-related genes and that FM supplementation increases the activity of enzymes involved in free-radical scavenging and the expression of antioxidant-related genes in mammary tissue. Therefore, the present study was conducted on lactating cows with the objectives of evaluating the distinct effects of dietary FM and FO abomasal infusion and their interaction on: (1) the activity of antioxidant enzymes (SOD, CAT and GPX) in blood and mammary tissue together with the mammary mRNA abundance of antioxidant genes and transcription factors known to affect the transcription of genes encoding for many antioxidant enzymes and (2) the mRNA abundance of lipogenic genes and transcription factors known to play roles in the regulation of lipogenic-related genes.

2. Materials and methods

2.1. Animal, diets and experimental treatments

Eight multiparous lactating Holstein cows fitted with ruminal cannulas (10 cm, Bar Diamond Inc.) were assigned to a replicated 4×4 Latin square with a 2×2 factorial arrangement of treatments and four 21 d periods balanced for residual effect. 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/d infused in the abomasum; (4) FMD and 250 g FO/d infused in the abomasum. Experimental doses of FM and FO were based on previous studies (Petit et al., 2009; Côrtes et al., 2011) performed by our team where both FM and FO were evaluated in dairy cows diets. The amount of FO infused was based on our previous results (Côrtes et al., 2011) showing that abomasal infusion of 250 g/d FO effectively increased polyunsaturated FA proportion in milk, which likely also increased polyunsaturated FA proportion in blood. As higher concentration of polyunsaturated FA in blood increases plasma peroxidizability index, maximal conjugated diene production and conjugated diene peroxidation rate (Gobert et al., 2009), abomasal infusion of 250 g FO/d was judged sufficient to generate oxidative stress in dairy cows. Indeed, our recent results (Lima et al., 2014) showed that infusing 250 g/d FO made lactating dairy cows highly susceptible to lipoperoxidation. The CON and FMD were designed to have similar protein and energy contents (Table 1). Flax oil (Brenntag Canada, Inc.) contained,