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Gastrointestinal metabolism of phytoestrogens in lactating dairy cows fed silages with different botanical composition

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ABSTRACT

Dietary phytoestrogens are metabolized or converted in the gastrointestinal tract of ruminants, only limited knowledge exists on the extent and location of this conversion in vivo. The objective of this study was to quantify the gastro-intestinal metabolism of phytoestrogens in lactating dairy cows fed silages with different botanical composition. Four lactating rumen cannulated Norwegian Red cattle were assigned to a 4 × 4 Latin square with 1 cow per treatment period of 3 wk. The 4 treatment silages were prepared from grasslands with different botanical compositions: organically managed short-term timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) ley (2 yr old: ORG-SG); organically managed long-term grassland with a high proportion of unsown species (6 yr old; ORG-LG); conventionally managed perennial ryegrass (*Lolium perenne* L.) ley (CON-PR); and conventionally managed timothy ley (CON-TI). The herbages were cut, wilted, and preserved with additive in round bales, fed as a mix of the first and third cut at 90% of ad libitum intake, and contributed to 70% of the total dry matter intake. Milk, feed, omasal digesta, urine, and feces were collected at the end of each period and analyzed for the concentrations of phytoestrogens by using a liquid chromatography–tandem mass spectrometry technique. Concentration of total isoflavones was highest in ORG-SG and lowest in CON-TI silage, whereas the content of total lignans was highest in the grass silages. The isoflavones were extensively metabolized in the rumen on all diets, and the recovery of formononetin and daidzein in omasum, mainly as equol, averaged 0.11 mg/mg. The apparent intestinal metabolism was less severe as, on average, 0.29 mg/mg of the omasal flow was recovered in feces. The plant lignans were also strongly degraded in the rumen. However, the flow of lignans to omasum and excretion in feces were, on average, 7.2- and 5.2-fold higher, respectively, than the intake of the plant lignans matairesinol and secoisolariciresinol, known as precursors of mammalian lignans. Thus, excretion to milk could not be directly related to intake, implying that plant lignans other than matairesinol and secoisolariciresinol in forage are precursors for enterolactone production in the rumen and for its content in milk. Equol followed mainly the flow of large particles out of the rumen, whereas the mammalian lignans were distributed between phases proportional to dry matter flow. The main metabolism of phytoestrogens occurred in the rumen and the main route of excretion was through feces and urine, with only a small part being excreted in milk. The concentration of phytoestrogens in milk can be manipulated through intake but the intermediate transfer capacity to milk appears to be limited by saturation.

Key words: isoflavone, lignan, silage, recovery

INTRODUCTION

Phytoestrogens are a large group of plant-derived nonsteroidal compounds, with structural similarities to mammalian estrogen (17-β estradiol). Phytoestrogens can bind to estrogen receptors and provide a weak estrogenic or antiestrogenic effect (Tham et al., 1998; Sirtori et al., 2005). Based on in vitro and in vivo studies the estrogenic potency of phytoestrogens has been estimated and is ranked as follows: estradiol > coumestrol > genistein and equol > glycitein > daidzein > formononetin, biochanin A (COT, 2003). A physiological effect of phytoestrogens was first shown in the 1940s when Bennetts et al. (1946) reported that sheep grazing on pastures containing red clover had fertility problems. The interest in phytoestrogens is currently linked to their possible favorable influence in human health, as related to cardiovascular disease, hormone-related cancers, osteoporosis, and menopausal

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symptoms, but also possible adverse health effects on infant development and processes influenced by sex steroids (Zung et al., 2001; Mendez et al., 2002; Tuohy, 2003), as endocrine-disrupting chemicals when lost to the wider environment from livestock urine and feces (Tucker et al., 2010), and on hormone-dependent cancers, depending on the timing of exposure (Bouker and Hilakivi-Clarke, 2000; De Assis and Hilakivi-Clarke, 2006).

Many ruminant feedstuffs may contain phytoestrogens, mainly isoflavones, coumestans, and lignans, which can be transferred to milk after digestion. Soybean [*Glycine max* (L.) Merrill] in concentrate supplements and grassland legumes, such as red clover (*Trifolium pratense* L.), are the most important sources (quantitatively) of isoflavones in ruminant feedstuffs (Mustonen et al., 2009; Třináctý et al., 2009). Lignans are primarily found in the cell walls of seeds from grain, legumes, and linseed (*Linum usitatissimum* L.) used in concentrate (Axelson et al., 1982; Cassidy et al., 2000; Petit et al., 2009). The third group, coumestans, of which coumestrol is the most important, are mainly found in legume seeds and sprouts of lucerne (*Medicago sativa* L.; Mazur, 1998).

Antignac et al. (2004) and Hoikkala et al. (2007) found 191 and 411 μg/L of equol in organically produced retail milk, respectively, whereas comparable milk from conventional production contained 36 and 62 μg of equol/L in the same studies, respectively. Concentrations of phytoestrogens in milk can be modulated by the concentration of phytoestrogens and their precursors in pasture (Andersen et al., 2009a; Adler et al., 2014), silage (Steinshamn et al., 2008; Andersen et al., 2009b; Mustonen et al., 2009), and concentrate (Petit et al., 2009; Třináctý et al., 2009). The mammalian isoflavonoid equol is the dominating phytoestrogen in milk from diets containing red clover (Steinshamn et al., 2008; Mustonen et al., 2009; Höjer et al., 2012), where concentrations in milk of up to 1,494 μg/kg have been reported (Höjer et al., 2012), and in diets with soybeans (Třináctý et al., 2009; 53 μg/kg of milk). Enterolactone is quantitatively the most important lignan in milk from diets containing white clover (*Trifolium repens* L.), lucerne or birdsfoot trefoil (*Lotus corniculatus* L., 226 μg/kg of milk; Höjer et al., 2012) in studies were legume species have been compared (Steinshamn et al., 2008; Andersen et al., 2009b; Höjer et al., 2012) and where linseed has been included in the diet (Petit and Gagnon, 2009; 24 μg/kg of milk).

Phytoestrogens are mainly present in plant material as glycosides, which are not active estrogenically (Miksicek, 1995). The major metabolic transformation of isoflavones in ruminants most likely occurs in the rumen by microbes that hydrolyze glycosides to aglycones (Nilsson et al., 1967; Dickinson et al., 1988). Biochanin A is demethylated to genistein and further metabolized via ring cleavage to *p*-ethyl phenol and organic acids without estrogenic effect (Nilsson et al., 1967; Batterham et al., 1971). Formononetin is mainly demethylated to daidzein (Lindner, 1967; Nilsson et al., 1967) and further to equol via hydrogenation and ring fission (Nilsson et al., 1967; Shutt and Braden, 1968; Batterham et al., 1971). Coumestrol does not seem to be metabolized in the rumen but provides an estrogenic effect itself. Isoflavones are mainly absorbed as aglycones in the rumen but also in the intestine, and are conjugated with glucuronic acid in the rumen gastrointestinal epithelium (Lundh, 1990). The major lignans found in plant food material are matairesinol and secoisolariciresinol (Axelson et al., 1982). The main site of metabolism of lignans in ruminants is, as for isoflavones, the rumen (Gagnon et al., 2009a). Microbes convert glycosides, as secoisolariciresinol-diglucoside, to aglycones that are further converted to mammalian lignans, enterodiol and enterolactone (Borriello et al., 1985). Absorption to portal blood occurs either in rumen or in the small intestine, where enterodiol may be further converted to enterolactone by colon microbes (Côrtes et al., 2008; Gagnon et al., 2009a). After absorption to the portal blood, the lignans are reconjugated with sulfate or glucuronic acid in the liver and excreted through the bile duct, where they may be deconjugated by bacterial enzymes and reabsorbed (Tham et al., 1998; Gagnon et al., 2009a). Some lignans reach the kidney and are excreted in the urine.

Although it is generally accepted that the main metabolism of phytoestrogens occurs in the rumen by rumen microbes, to our knowledge, no studies have examined and quantified the metabolism of phytoestrogens in lactating dairy cows in vivo. This knowledge is important if milk content of phytoestrogens is to be manipulated. Therefore, the objectives of the present study were to quantify the conversion of plant phytoestrogens into mammalian phytoestrogens in dairy cows fed different diets, and to evaluate their transfer to urine, feces, and milk.

MATERIALS AND METHODS

Experimental Design and Animals

The experiment was carried out at the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (Ås, Norway) with 4 rumen-cannulated lactating multiparous dairy cows. Adler et al. (2013) described the experiment in detail. In brief, the cows weighed on average (±SD) 631 ± 35.5 kg, were 118 ± 40.9 d in lactation, and yielded 22.5 ± 2.7