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Phyto-oestrogens and their metabolites in milk produced on two pastures with different botanical compositions

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ABSTRACT

Phyto-oestrogens are a group of secondary plant metabolites that may bind to oestrogen receptors and exert oestrogenic or anti-oestrogenic effects in humans and can protect against cancer diseases. When ingested by dairy cows, phyto-oestrogens can be metabolised and transferred to the milk. The objective of this study was to assess the effects of grazing a recently established pasture containing red clover (*Trifolium pratense* L.) and an older pasture containing a variety of sown and unsown plant species on milk concentrations of phyto-oestrogens. Sixteen Norwegian Red dairy cows [mean (standard deviation); body weight 599 (45.1) kg, stage of lactation 73 (15.0) d in milk, milk yield 29.9 (2.90) kg/d at the start of the experiment] were divided into two groups and grazed either a short-term pasture (SP) or a long-term pasture (LP). The SP was representative of organically managed leys in Norway, which are frequently, approximately every third year, renewed by soil tillage and seeding, whereas LP was representative of organically managed grasslands that are less frequently renewed. The SP contained meadow fescue (*Festuca pratensis* Huds.) (mean 34%), timothy (*Phleum pratense* L.) (mean 19%), red clover (mean 28%), shepherd's-purse (*Capsella bursa-pastoris* (L.) Medik.) (mean 6%), pineappleweed (*Matricaria matricarioides* Porter ex Britton) (mean 5%) and scentless mayweed (*Tripleurospermum perforatum* (Mérat) Laínz) (mean 4%), and LP contained mainly white clover (*Trifolium repens* L.) (mean 21%), smooth meadowgrass (*Poa pratensis* L.) (mean 19%), timothy (mean 17%), meadow fescue (mean 15%), perennial ryegrass (*Lolium perenne* L.) (mean 6%), tufted hairgrass (*Deschampsia cespitosa* (L.) P. Beauv.) (mean 5%), northern dock (*Rumex longifolius* DC.) (mean 4%), common couch (*Elytrigia repens* (L.) Desv. Ex Nevski) (mean 4%), red clover (mean 3%) and dandelion (*Taraxacum* spp.) (mean 3%). In addition to a daily pasture allowance of 20 kg dry matter per cow, supplements of 3.0 kg barley (*Hordeum vulgare* L.) concentrate were fed. Herbage, concentrates and milk was sampled during the last week of three experimental periods and analysed for phyto-oestrogens using LC-MS/MS technology. Herbage from SP had 19 times higher concentration of isoflavones than herbage from LP, whereas only small differences were found for lignans. Milk produced on SP had 14 times higher concentrations of the mammalian isoflavonoid equol, and the concentrations of equol were higher than found in most other studies. This study confirms that grazing pastures containing red clover increases

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concentrations of isoflavones and especially equol in bovine milk compared to grazing pastures with other botanical composition. The higher milk concentrations of the lignan metabolite enterodiol in milk from SP compared to LP could not be related to differences in intake of the analysed lignans and may therefore be related to unidentified lignans.

1. Introduction

Phyto-oestrogens are secondary plant metabolites with the ability to induce or inhibit the response of steroid hormone receptors due to their structure similar to endogenous steroids. Phyto-oestrogens are divided into the groups of isoflavones, lignans, coumestans and others. Isoflavones are phenolic compounds and include formononetin, daidzein, biochanin A, genistein and prunetin. They have various functions in plants, as for example attraction of pollinators and defence against pathogens (Taiz and Zeiger, 2010). In the diet of dairy cows phyto-oestrogens are found in concentrates and forages. Soybean (*Glycine max* (L.) Merr.) has high concentrations of genistein and daidzein (Mazur and Adlercreutz, 1998). In forages phyto-oestrogen concentrations depend mainly on botanical composition (Andersen et al., 2009a), but they also depend on other factors like stage of development (Tsao et al., 2006), season (Booth et al., 2006), part of plant (Tsao et al., 2006) and preservation method (Sivesind and Seguin, 2005). Red clover (*Trifolium pratense* L.) has high concentrations of formononetin and biochanin A (Sivesind and Seguin, 2005), whereas the concentrations of isoflavonoids in other forage plants, e.g. white clover (*Trifolium repens* L.), timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.) are low (Andersen et al., 2009a; Kallela et al., 1987; Mustonen et al., 2009). Höjer et al. (2012) recently reported higher concentrations of the lignan secoisolariciresinol in silage produced on long-term grassland containing a variety of sown and unsown plant species compared to silage from short-term grassland containing red clover and timothy. Coumestans are found in small concentrations in lucerne (*Medicago sativa* L.) (Andersen et al., 2009b) and white clover and elevated concentrations are found in diseased plants (Wong et al., 1971).

Phyto-oestrogens are partly metabolised in the rumen, absorbed by the intestine and a small part is transferred to milk (Gagnon et al., 2009, Tucker et al., 2010). Formononetin and daidzein are converted to the isoflavone equol (Batterham et al., 1965), and biochanin A is converted to genistein, which may be degraded further to non-oestrogenic substances (Pfitscher et al., 2008). The lignan secoisolaricinol is converted to enterolactone via enterodiol or mataresinol (Heinonen et al., 2001).

In ewes, phyto-oestrogens have been reported to reduce reproductive performance (Bennetts et al., 1946), whereas effects in cattle are inconsistent (Austin et al., 1982; Kallela et al., 1984). Equol has shown much higher oestrogen activity than daidzein (Hwang et al., 2006). Equol in milk and dairy products is transferred to humans when consumed (Frankenfeld, 2011), but the health effects of intake of equol have not been elucidated. Several human intestinal bacteria can convert daidzein from sources like soybean or milk into equol (Kim et al., 2008). In humans, phyto-oestrogens can protect against certain cancers (Cotterchio et al., 2008) or prevent osteoporosis (Setchell and Lydeking-Olsen, 2003), but they may also have negative health effects; for example, they may interfere with steroid hormone metabolism (Ososki and Kennelly, 2003).

Feeding red clover-grass silage results in higher isoflavone and lower lignan concentrations in milk than feeding white clover-grass silage (Steinshamn et al., 2008). Milk isoflavone concentrations are also higher for cows grazing red clover-grass compared to white clover-grass, whereas red clover results in lower concentrations of enterolactone in milk than white clover (Andersen et al., 2009a).

Silage produced from short-term grassland containing red clover that are renewed frequently, approximately every third year and usually cultivated in rotation with arable crops also results in milk with more isoflavones than silage from long-term grassland containing white clover and a variety of grass and dicotyledon species that are renewed less frequently (Höjer et al., 2012). In the latter study, feeding silage produced from short-term grassland resulted in milk that contained less lignans, enterodiol and enterolactone than silage from long-term grassland.

The objective of the present experiment was to assess the effects of grazing a newly established pasture, containing red clover, or a long-term pasture containing a variety of sown and unsown plant species on concentrations of phyto-oestrogens in bovine milk. We hypothesised that grazing a short-term pasture increases milk concentrations of isoflavones and decreases lignans compared to grazing a long-term pasture.

2. Materials and methods

2.1. Cows, feeds, experimental design, herbage botanical composition and feed sampling

Cows, feeds, experimental design and feed sampling have been described by Adler et al. (2013). In brief, 16 multiparous Norwegian Red dairy cows in mid-lactation cows [mean (standard deviation); body weight 599 (45.1) kg, body condition score 2.7 (0.39) points, stage of lactation 73 (15.0) d in milk, milk yield 29.9 (2.90) kg/d at the start of the experiment] participated in a grazing experiment in Ås, Norway, at the Animal Production Experimental Centre (59.67°N, 10.75°E; 50 m a.s.l.), Norwegian University of Life Sciences. On the basis of pre-experimental milk yields, days in milk and sire, the cows were blocked and allocated randomly to two groups of