Equol and enterolactone – two mammalian phytoestrogens with estrogenic potency found in organically produced milk

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# Implications

Cows fed high proportions of red clover yield milk with high concentrations of the isoflavandiol equol, a phytoestrogen with high estrogen potency that may have beneficial effects in prevention of osteoporosis and breast cancer. Soy products have much higher content of isoflavones than milk, but not all humans are able to produce equol from isoflavones. In cows many plant lignans can be metabolised to enterolactone, and milk concentrations tend to be higher during grazing season than indoor feeding season. Therefore, milk produced on feed containing red clover and milk produced on pasture may be considered as functional food. However, more documentations on possible effects in humans, including unwanted side effects are needed.

# Background and objectives

Phytoestrogens are a group of non-steroidal plant compounds with structural similarities to mammalian estrogen (17-β estradiol). Phytoestrogens and their mammalian metabolites can bind to estrogen receptors in animals and humans, and provide a weak estrogenic or anti-estrogenic effect. In sheep, intake of phytoestrogens may impair fertility, whereas effects in cattle are not consistent. In humans, favourable effects of phytoestrogens have been reported related to osteoporosis, different types of cancer and cardiovascular disease. Isoflavones and lignans are metabolised to equol and enterolactone in the rumen. Human gut microbiota can metabolise plant lignans to enterolactone in the intestines, but only 30-50% are able to produce equol. Equol has a higher estrogenic potency than its precursors, whereas that of enterolactone is weak, but it may also exert biological effects by non-estrogenic mechanisms. The objective of this paper is to present the relationship between intake of forage legumes and milk content of equol and enterolactone in organically managed dairy cows.

# Key results and discussion

Organic dairy farms with short-term grassland in mid Norway produced milk with higher concentrations of equol (241 µg/kg) than organic farms with long-term grassland (65 µg/kg) (Adler et al., 2015b). Grasslands on the farms with short-term grasslands contained on average 17% red clover at first cut and the farms with long-term grassland 9%. Pastures had only small amounts of red clover, and equol concentrations in milk from organic short-term grassland farms were lower in the grazing period than in the indoor feeding period. Enterolactone concentrations did not differ between the two farm types, but the concentrations were highest during the grazing period.
In a grazing experiment, red clover-grass pasture (28% red clover) was compared with a botanically diverse pasture (Adler et al., 2015a). In milk, concentrations of equol (1,199 vs. 86 µg/kg) and enterolactone (165 vs. 120 µg/kg) were higher when red clover-grass rather than botanically diverse pasture was grazed.
In two silage feeding experiments red clover-grass silages (31-34% red clover in the dry matter) were compared to silages of birdsfoot trefoil-grass (16% birdsfoot trefoil) or botanically diverse silage (Höjer et al., 2012). Milk equol concentrations were higher for the red clover treatments (716-1,494 µg/kg) than for the other treatments (84-145 µg/kg). For enterolactone the effect was opposite, birdsfoot trefoil-grass silage (226 µg/kg of milk) and botanical diverse silage (133 µg/kg) resulted in milk with higher concentrations than the red clover-grass silages (51-108 µg/kg of milk). However, huge individual differences were observed between cows. Steinshamn et al. (2008) found that red clover silage yielded milk with higher equol concentrations than white clover silage. Concentrate intake decreased isoflavone and increased lignan levels in milk. In a metabolism study with red clover-grass and botanically diverse silage, isoflavones and lignans were extensively metabolised in the rumen to mammalian phytoestrogens (Njåstad et al., 2014). The phytoestrogens and their mammalian metabolites were further metabolised in the intestine, but to a lesser extent than in the rumen. For lignans the authors suggested that a high amount of precursors has not been detected as the amount of mammalian lignans that escaped the rumen was larger than the intake of known precursors in the feed intake.
This paper confirms the findings of Antignac et al. (2003) and Mustonen et al. (2009), who found elevated levels of equol in milk produced organically or with high red clover proportion in the diet. The following equation was found: Equol concentration in milk (µg/kg) = 2.56 × red clover proportion in total feed intake (g/kg of dry matter) + 76.61 (R2 = 0.52). For white clover no correlation was found in the present study. Birdsfoot trefoil was only included in one feeding experiment, but resulted in milk with the highest concentration of enterolactone in milk in the present study. Average enterolactone levels in the 6 studies were 150 µg/kg of milk (SD = 22.5) in grazing and 68 µg/kg milk (SD = 56.4) in indoor feeding situations. Compared to soy products, such as raw tofu containing roughly 0.2 g of total isoflavones per kg, milk produced on red clover rich diets has a low content of equol. However, considering the high estrogenic potency of equol and that not all humans are able to produce equol, further studies on possible health effects of consuming milk based on red clover rich feed are needed. Accordingly, enterolactone in milk produced on pasture may have positive health implications. Further studies must also focus on unwanted side effects.

# How work was carried out?

Forage intake, red clover proportion, milk yield, phytoestrogen content in feed and milk from 6 feeding experiments from Norway with dairy cows in organic farming were included in this study.

## References

Adler SA, Purup S, Hansen-Møller J, Thuen E, Gustavsson A-M & Steinshamn H 2015a. Phyto-oestrogens and their metabolites in milk produced on two pastures with different botanical compositions. Livest. Sci. 163: 62-68.

Adler SA, Purup S, Hansen-Møller J, Thuen E & Steinshamn H 2015b. Phytoestrogens and their metabolites in bulk-tank milk: effects of farm management and season. PLoS ONE 10(5): e0127187.

Antignac JP, Cariou R, Le Bizec B, Cravedi JP & Andre F 2003. Identification of phytoestrogens in bovine milk using liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun. Mass. Spectrom. 17: 1256-1264.

Höjer A, Adler SA, Purup S, Hansen-Møller J, Martinsson K, Steinshamn & H Gustavsson A-M 2012. Effects of feeding dairy cows different legume-grass silages on milk phytoestrogen concentration. J. Dairy Sci. 95:4526-4540.

Mustonen EA, Tuori M, Saastamoinen I, Taponen J, Wähälä K, Saloniemi H & Vanhatalo A 2009. Equol in milk of dairy cows is derived from forage legumes such as red clover. Br. J. Nutr. 102: 1552-1556.

Njåstad KM, Adler SA, Hansen-Møller J, Thuen E, Gustavsson A-M & Steinshamn H 2014. Gastrointestinal metabolism of phytoestrogens in lactating dairy cows fed silages with different botanical composition. J. Dairy Sci. 97:1-16.

Steinshamn H, Purup S, Thuen E & Hansen-Møller J 2008. Effects of clover-grass silages and concentrate supplementation on the content of phytoestrogens in dairy cow milk. J. Dairy Sci. 91: 2715-2725.