Effect of pasture botanical composition on milk composition in organic production

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Abstract

Milk samples from sixteen Norwegian Red dairy cows grazing mixed swards of either grass-red clover (GR) or mixed swards of sown and unsown species of grass, clover and other herbs (GCH) were collected during four periods. Both pastures were organically managed. Pasture botanical composition had no effect on milk fat, protein or vitamin concentration and only minor effects on fatty acid composition. Milk from GR had higher concentrations of the phytoestrogens equol, genistein and biochanin A than the milk from GCH. Concentrations of equol in milk from GR were higher than concentrations reported from experiments with red clover silage. The oxidative stability of the milk lipids was not affected by pasture type.

Keywords: Dairy production, grazing, botanical composition, fatty acids, vitamins, phytoestrogens, oxidative stability

Introduction

Forages with high proportions of legumes tend to increase the proportion of polyunsaturated fatty acids (PUFA) in milk fat (Dewhurst *et al.*, 2003). Red clover (*Trifolium pratense* L.)-grass silage also increases the milk proportion of PUFA, in particular α-linolenic acid (ALA), compared to white clover (*Trifolium repens* L.)-grass silage (Steinshamn and Thuen, 2008). However, increased proportion of PUFA in milk fat may increase the susceptibility of milk lipids to photo-oxidation (Havemose *et al.*, 2004). Red clover has also shown to yield high concentrations of phytoestrogens in milk (Mustonen *et al.*, 2009). The aim of this study was to examine if milk composition of fatty acids (FA), vitamins and phytoestrogens, and the susceptibility of milk to photo-oxidation are affected by pasture botanical composition.

Material and methods

A continuous grazing experiment was conducted in Ås, Norway, with sixteen Norwegian Red dairy cows (80 ± 15.0 days in milk (d.i.m.)) with three 3-week periods of measurements: in June, July and September 2008. The cows were blocked by genetic line, d.i.m. and milk yield and were allocated randomly to the two treatments: a grass-red clover pasture (GR) in the first production year, and a five-year-old pasture including sown and unsown species of grass, clover and other herbs (GCH) with a daily dry matter (DM) allowance of approximately 20 kg cow^{-1} $d^{-1}$and supplemented with 3.0 kg d^{-1} of barley pellets, including minerals. Both pastures were organically managed. A period with indoor silage feeding before grazing was used as a baseline period. Both groups were grazing together between the three3-periods on pastures similar to GCH. GR contained 54% grasses, 28% red clover, 1% white clover and 17% other herbs, and GCH contained 66% grasses, 3% red clover, 21% white clover and 10% other

herbs as estimated with the dry-weight-rank method (Mannetje and Haydock, 1963). Pasture intake was estimated as net energy requirement for lactation and maintenance minus net energy in concentrates divided by net energy concentration in pasture samples. Individual pooled samples from four consecutive milkings in each period were analysed for the content of fat, protein, free FA, vitamins, phytoestrogens, FA composition and milk oxidative stability. Milk oxidative stability was analysed in a light exposure experiment (three replicates were exposed for light for 0, 24 or 48 hours at 4 ºC) by determination of lipid hydroperoxides as described by Østdal *et al.* (2000) and front face fluorescence spectroscopy as described by Veberg *et al.* (2007). Milk composition variables were analysed using the mixed model procedure by SAS 9.2 with treatment, period and treatment-period interaction as fixed effects and cow within treatment, and block as random effects accounting for repeated measurement on cow. The statistical model for hydroperoxides included also fixed effects of light exposure and treatment-light exposure interaction. For milk yield and milk chemical composition the baseline data were used as a covariate. The fluorescence emission spectra were analysed in a principal component analysis (Unscrambler).

Table 1. Pasture dry matter intake, milk yield and milk composition from cows grazing swards of grass-red clover (GR) or sown and unsown species of grass, clover and herbs (GCH)

	GR	GCH	SED	P^a
Pasture DM intake (est.), kg day^{-1}	15.4	15.3	0.76	NS
Milk yield, kg d^{-1}	24.6	24.9	0.74	NS
Fat, g kg^{-1}	37.7	37.2	1.04	NS
Protein, g kg^{-1}	33.6	33.2	0.82	NS
Free FA, meq L^{-1}	0.49	0.62	0.122	NS
Total saturated FA, g per 100 g FAME[b]	66.72	68.37	1.032	NS
Total monounsaturated FA, g per 100 g FAME	28.35	26.93	0.820	NS
Total polyunsaturated FA, g per 100 g FAME	4.95	4.70	0.314	NS
n-6:n-3 FA ratio	2.00	1.89	0.120	NS
β-caroten, mg L^{-1}	0.25	0.24	0.022	NS
α-tocopherol, mg L^{-1}	1.51	1.32	0.082	NS
Retinol, mg L^{-1}	0.52	0.45	0.041	NS
Enterolactone, µg L^{-1}	172.3	120.9	28.54	(*)
Formononetin, µg L^{-1}	49.4	5.5	16.01	(*)
Equol, µg L^{-1}	1230.8	88.0	220.40	*
Genistein, µg L^{-1}	15.5	2.6	4.11	*
Biochanin A, µg L^{-1}	16.2	1.2	5.12	*
Hydroperoxides, after 48 h of light exposure	0.47	0.46	0.041	NS

[a]*P*-value: NS $P > 0.10$, (*) $P < 0.10$, *$P < 0.05$; [b]Fatty acid methyl esters

Results and discussion

There was no significant effect of pasture botanical composition on pasture intake, milk yield, concentration of fat, protein and vitamins and composition of most FA in milk (Table 1). The elevating effect of red clover diets on milk content of ALA (Dewhurst *et al.*, 2003; Steinshamn and Thuen, 2008) is due to the activity of polyphenol oxidase (PPO) that inhibits lipolysis (Lee *et al.*, 2009). The activation of the enzyme requires the presence of oxygen and occurs during mastication of fresh red clover, but it is limited by the anaerobe condition in the rumen (Lee *et al.* 2009). Thus, the lack of effect in the present experiment might be due to the short period of time the red clover is exposed to oxygen during the grazing and mastication process. Concentrations of the phytoestrogens equol, genistein and biochanin A were significantly higher in milk from GR than GCH. This is in accordance with other experiments where red clover has been compared with white clover containing diets (Steinshamn *et al.*,

2008; Andersen *et al.*, 2009). The milk concentrations of equol, enterolactone and formononetin from GR fed cows in the present experiment were several times higher than found in other experiments with red clover based diets, both from silage (Steinshamn *et al.*, 2008; Mustonen *et al.*, 2009) and pasture (Andersen *et al.*, 2009). Pasture type did not affect concentration of hydroperoxides. In the principal component analysis of the fluorescence spectra principal component (PC) 1 explained 94% and PC2 6% of the total variation. PC1 grouped the samples by light exposure time which was correlated with reduction of the photosensitiser riboflavin. PC2 grouped the pasture typed to some degree but the correlated tops in the fluorescence spectra are of unknown substances and it is not sure that these are oxidation products since differences were consistent even for samples not exposed to light.

Conclusions

Pasture with red clover did not affect FA composition in milk to an extent susceptible to a higher risk of milk fat oxidation compared to pasture including several grass species, white clover and herbs. Grazing GR compared to GCH increased the concentrations of phytoestrogens in milk.

Acknowledgements

This work was part of the CORE Pilot project 'PhytoMilk' funded by the CORE Organic Funding Body Network.

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