Variations an α-Tocopherol and β-Carotene Concentrations in Forage Legumes and Grasses Harvested at Different Sites and Maturity Stages

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Variations in α-tocopherol and β-carotene concentrations in forage legumes and grasses harvested at different sites and maturity stages

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Abstract. Forage is a major source of natural α-tocopherol and β-carotene for dairy cows. This study examined vitamin concentrations of birdsfoot trefoil (Bft), red clover (Rc), timothy (Ti) and meadow fescue (Mf) at different sites, years and cutting dates. Mixtures of Bft+Ti, Rc+Ti and Rc+Mf were established at Skara (58°21'N; 13°08'E) and Umeå (63°45'N; 20°17'E) in Sweden. First-year leys were cut on three occasions in spring (Umeå 2005, Skara 2005 and 2007); one week before heading of timothy, at heading and one week after heading. Birdsfoot trefoil had higher α-tocopherol concentration at Skara than at Umeå in the first two cuts in 2005 (66 vs. 27 and 50 vs. 36 mg/kg DM, respectively) and had generally higher concentration than Rc. α-Tocopherol concentrations of Bft and Rc were 32 vs. 17 and 50 vs. 25 mg/kg DM at Umeå and Skara 2005, respectively, averaged over cuts. At Skara, α-tocopherol concentration decreased with later cutting date of Bft and grasses ($P<0.01$). Birdsfoot trefoil had higher β-carotene concentration at Skara than at Umeå in 2005 (70 vs. 55 mg/kg DM) and higher concentration than Rc at Skara, when averaged over cuts (70 vs. 46 mg/kg DM in 2005 and 82 vs. 52 in 2007; $P = 0.037$). Grasses had higher α-tocopherol and β-carotene concentrations at Skara than at Umeå in 2005, when averaged over cuts (56 vs. 33 and 46 vs. 23 mg/kg DM; $P<0.001$). Interactions between site, species and cutting date affected α-tocopherol and β-carotene concentrations in forages.

Keywords: Vitamins, forage crops, latitude, maturity.

Introduction

α-Tocopherol (vitamin E) and β-carotene (pro-vitamin A) are antioxidants and they play important roles in the immune system of animals (McDowell 2000). Forage is often the major source of natural α-tocopherol and β-carotene for dairy cows (Jensen *et al*., 1999; Lindqvist *et al*. 2011). Vitamin concentrations in forages are affected by species (Brown, 1953; Lynch *et al*. 2001; Lindqvist *et al*. 2012), harvest time (Lynch *et al*. 2001), maturity (Brown 1953), photoperiod, temperature and growth rate (Olsson *et al*. 1955; Hjarde *et al*. 1963). Leaves contain more α-tocopherol and β-carotene than stems and, therefore, species with high leaf/stem ratio usually provide higher concentrations of vitamins than species with low leaf/stem ratio (Brown 1953). Vitamin concentrations in plants usually decrease as plants mature, mainly because of a decreasing leaf/stem ratio (Brown 1953).

Latitude and seasonal changes, such as weather conditions and photoperiod affect α-tocopherol and β-carotene concentrations in forages (Olsson *et al*. 1955; Hjarde *et al*. 1963). Shorter photoperiod has been shown to increase α-tocopherol and β-carotene concentrations of forage. Olsson *et al*. (1955) and Hjarde *et al*. (1963) found higher α-tocopherol and β-carotene concentrations in shaded stands of forage compared with un-shaded stands. Cold and rainy weather is beneficial for α-tocopherol and β-carotene production, which might be related to decreased growth rate of the plants and decreased solar radiation during such weather conditions (Olsson *et al*. 1955; Hjarde *et al*. 1963). The aim of this study was to investigate the effects of site, year and cutting date on α-tocopherol and β-carotene concentrate-ions in red clover (*Trifolium pratense* L.), birdsfoot trefoil (*Lotus corniculatus* L.), timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.) during spring.

Methods

Forages and harvests

The field experiment was carried out at two sites in Sweden; Lanna Research Station (58º21´N; 13º08´E, 75 m above sea level, silty clay loam with 3% organic matter) at Skara and Röbäcksdalen Research Centre (63°45'N; 20°17'E, 5 m above sea level, silt loam with 5% organic matter) at Umeå. Three mixtures, birdsfoot trefoil + timothy (Bft+Ti), tetraploid red clover + timothy (Rc+Ti) and tetraploid red clover + meadow fescue (Rc+Mf) were harvested as first-year leys in 2005 at both sites and in 2007 at Skara. The same cultivars of birdsfoot trefoil (cv. Oberhaunstaedter), timothy (cv. Grindstad) and meadow fescue (cv. Kasper) were used at both sites but the red clover cultivars were Sara at Skara and Betty at Umeå. Plot size was approximately 1.5 x 13 m^2 = 19.5 m^2. Seed rates in kg/ha of germinative seeds were 10 + 10, 7 + 15 and 7 + 20 for Bft+Ti, Rc+Ti and Rc+Mf, respectively. The plots at both sites were fertilised with 25 to 40 t slurry/ha before sowing in 2004 and with 20 t slurry/ha before sowing at Skara in 2006.

The aim was to harvest the three forage mixtures at three different stages of maturity in spring, one week before expected heading (early), at heading (mid) and one week after heading of timothy (late). Cutting dates in 2005 were 20 June, 28 June and 3 July at Umeå and 9 June, 14 June and 22 June at Skara. The cutting dates at Skara in 2007 were 28 May, 5 June and 11 June. Representative samples of forage from each plot were cut by hand between 10.00 and 12.00 hours at a stubble height of ca 5 cm. One sample was sorted into sown legumes, unsown legumes, sown grasses, weeds and dead plant materials for determination of botanical composition on a dry matter (DM) basis and each species was sorted to calculate leaf blade proportions on a DM basis. Samples of each species also were frozen at -20 ºC for subsequent analysis of vitamins and other nutrients. Each species was sorted into phenology classes according to the scale devised by Zadoks *et al*. (1974) and Gustavsson (2011). Data on precipitation, temperature and solar radiation at Lanna Research Station, Skara and Röbäcksdalen, Umeå, during the growing season were obtained from the Swedish Meteorological and Hydrological Institute.

Analysis of vitamins

Analyses of α-tocopherol and β-carotene were performed at Aarhus University, Department of Animal Science, Research Centre Foulum, Denmark. Herbage was freeze dried before analysis. The concentrations of α-tocopherol and β-carotene were determined by high-pressure liquid chromatography after saponification and extraction into heptane according to Jensen *et al*. (1998).

Statistical analysis

Data were analysed as a split-split-plot design using the mixed linear model procedure (PROC MIXED) of SAS (2001). Grasses and legumes were analysed separately. The effects of site (Skara 2005 *vs*.Umeå 2005) and year (Skara 2005 *vs*. Skara 2007) were named trial. In the analysis of α-tocopherol and β-carotene, trial (n = 3) was treated as the main plot, species (n = 3) as the sub-plot and cutting date (n = 3) as the sub-sub-plot. Mixtures were replicated three times in the field. When a significant F - value was detected, pair-wise comparisons between LSMEANS were analysed with Tukey's test at $P<0.05$.

Results and Discussion

Botanical composition, maturity and yield

Mixtures at Skara contained more legumes than mixtures at Umeå, and mixtures with red clover had higher legume proportions than mixtures with birdsfoot trefoil at both sites. Variations in maturity stages of birdsfoot trefoil were larger at Skara (leaf stage to early bloom) than Umeå (extended internode to bud stage), whereas red clover seemed to differ more in maturity stages at Umeå (leaf stage to early bloom) than at Skara (most in extended internode stage) with only small differences in maturity stages of legumes between years at Skara. Variations in maturity stages between grass species were more inconsistent than between legume species but, generally, grasses at Umeå were more developed than grasses at Skara, with a larger proportion of grasses at full heading stage. Forages at Skara 2005 yielded more than the forages at Umeå 2005 and Skara 2007, when averaged over mixtures and cuts in spring growth (5700 *vs*. 4400 and 5000 kg DM/ha, respectively; $P<0.01$). Red clover mixtures yielded more than mixtures with birdsfoot trefoil, when averaged over trials and cuts (5300 *vs*. 4500 kg DM/ha; $P<0.0001$). Averaged over trials and mixtures, DM yield increased with later cutting date in spring growth (3800, 5100 and 6200 kg DM/ha for early, mid and late cut, respectively; $P<0.0001$).

Vitamin concentrations and leaf blade proportions of legumes

Birdsfoot trefoil had higher concentrations of α-tocopherol and β-carotene at Skara than at Umeå in 2005, whereas the vitamin concentrations of red clover were similar between sites (Table 1). Differences in vitamin concentrations of birdsfoot trefoil between the sites could be caused by differences in latitude and photoperiod (Olsson *et al*. 1995). Lack of differences in vitamin concentrations of red clover could be a cultivar effect as different red clover cultivars were used at the sites. α-Tocopherol concentrations of legumes grown at Skara were similar between years. Birdsfoot trefoil contained more α-tocopherol than red clover at each cut, when averaged over trials ($P<0.01$), which is consistent with our earlier findings (Lindqvist *et al*. 2012). The α-tocopherol concentration of birdsfoot trefoil decreased with later cutting date at Skara in both years (from 61 to 41 mg/kg DM), whereas there was no effect of cutting date on the α-tocopherol concentration of birdsfoot trefoil at Umeå (32 mg/kg DM; $P<0.01$). The α-tocopherol concentration of red clover was not affected by cutting date in any of the sites (22.3 mg/kg DM, $P<0.01$). Birdsfoot trefoil and red clover grown with meadow fescue at Skara 2007 had higher β-carotene concentrate-ions than the corresponding species at Skara 2005, when averaged over cuts (Table 1). At Skara, birdsfoot trefoil had a higher β-carotene concentration than red clover, whereas birdsfoot trefoil only differed from red clover grown with timothy at Umeå. Averaged over species,

Table 1. Concentrations of α-tocopherol and β-carotene (mg/kg DM) and leaf blade proportion (DM basis) of birdsfoot trefoil (Bft) and red clover (Rc) in mixtures with timothy (Ti) and meadow fescue (Mf) from three trials in Sweden (Umeå 2005, Skara 2005 and 2007). Forages were cut on three occasions (early, mid and late) in spring of first-year leys

	Species			Cut			Species x Trial		Cut x Trial	
	Bft+ Ti	**Rc+ Ti**	**Rc+ Mf**	Early	Mid	Late	*P* - value	SEM $LSD_{0.05}$	*P* - value	SEM $LSD_{0.05}$
α-Tocopherol										
Umeå 2005	31.7	16.9	17.4	19.7	23.4	22.9	NS	2.49	NS	2.39
Skara 2005	50.4	24.7	24.4	35.1	32.6	31.8				
Skara 2007	51.4	26.0	24.6	36.8	34.2	31.0				
β-Carotene										
Umeå 2005	54.9	38.6	46.3	45.1	45.0	49.8	<0.05	4.12	<0.001	4.12
Skara 2005	69.6	48.5	42.9	48.8	68.3	43.9		11.58		11.58
Skara 2007	82.1	49.9	54.8	69.4	61.1	56.2				
Leaf blade proportion										
Umeå 2005	0.54	0.43	0.46	0.55	0.46	0.42	NS	0.013	NS	0.011
Skara 2005	0.38	0.30	0.31	0.37	0.34	0.28				
Skara 2007	0.43	0.37	0.37	0.45	0.39	0.34				

Table 2. Concentrations of α-tocopherol and β-carotene (mg/kg DM) and leaf blade proportion (DM basis) of timothy (Ti) and meadow fescue (Mf) in mixtures with birdsfoot trefoil (Bft) and red clover (Rc) from three trials in Sweden (Umeå 2005, Skara 2005 and 2007). Forages were cut on three occasions (early, mid and late) in spring of first-year leys

	Species			Cut			Species x Trial		Cut x Trial	
	Bft+ Ti	**Rc+ Ti**	**Rc+ Mf**	Early	Mid	Late	*P* - value	SEM $LSD_{0.05}$	*P* - value	SEM $LSD_{0.05}$
α-Tocopherol										
Umeå 2005	34.3	27.6	35.8	34.3	33.4	30.0	<0.0001	2.57	<0.001	2.57
Skara 2005	50.7	43.1	73.5	61.0	55.6	50.6		7.40		7.40
Skara 2007	46.5	39.0	48.6	59.8	42.2	32.0				
β-Carotene										
Umeå 2005	22.7	22.0	23.4	23.9	23.2	21.1	<0.01	1.77	<0.01	1.76
Skara 2005	43.0	42.9	52.8	46.6	53.5	38.6		5.29		5.05
Skara 2007	23.2	33.8	36.2	35.3	34.0	23.9				
Leaf blade proportion										
Umeå 2005	0.26	0.26	0.29	0.36	0.25	0.19	<0.001	0.028	NS	0.022
Skara 2005	0.27	0.33	0.60	0.51	0.40	0.30		0.085		
Skara 2007	0.37	0.38	0.41	0.49	0.38	0.28				

legumes harvested in the early cut had higher β-carotene concentrations than legumes harvested in the late cut at Skara 2007. At Skara 2005, the highest β-carotene concentrations were found in the mid cut (Table 1). Averaged over trials, birdsfoot trefoil general-ly had a higher leaf blade proportion than red clover within cuts ($P<0.01$), which partly could explain the higher vitamin concentrations in birdsfoot trefoil than in red clover (Brown 1953). The leaf blade proportion decreased with later cutting date for both legumes ($P<0.01$).

Vitamin concentrations and leaf blade proportions of grasses

Timothy and meadow fescue had higher concentrations of α-tocopherol and β-carotene at Skara than at Umeå in 2005, when averaged over cuts (Table 2). Furthermore, grasses grown at Skara had higher concentrations of α-tocopherol (meadow fescue only) and β-carotene in 2005 than in 2007. Lower vitamin concentrations of the grasses at Umeå than at Skara might be related to more developed plants at Umeå (Brown 1953). Meadow fescue contained more α-tocopherol than timothy grown with red clover in all three trials, which partly can be explained by a higher leaf blade proportion in meadow fescue than in timothy ($P<0.05$; Brown 1953). Averaged over species, the α-tocopherol concentration of grasses decreased with later cutting date at Skara, with the largest decrease in 2007. In contrast, no effect of cutting date was found on the α-tocopherol concentration of grasses grown at Umeå in 2005 (Table 2). At Skara, meadow fescue had higher β-carotene concentration than timothy in 2005 and timothy grown with birdsfoot trefoil had the lowest β-carotene concentration at Skara in 2007. The greatest variation in β-carotene concentrations between cuts was observed at Skara in 2005, when averaged across species (Table 2). Meadow fescue had a higher leaf blade proportion at Skara than at Umeå in 2005, when averaged over cuts (Table 2). Meadow fescue grown at Skara had higher leaf blade proportion in 2005 than in 2007, whereas the opposite was true for timothy grown with birdsfoot trefoil. Meadow fescue had a higher leaf blade proportion than timothy at Skara in

2005, when averaged over cuts (Table 2). The leaf blade proportion decreased with later cuts for both grass species, when averaged across trials ($P<0.05$).

Conclusions

Birdsfoot trefoil, timothy and meadow fescue often had higher concentrations of α-tocopherol and β-carotene, when grown in south-west of Sweden (Skara) compared with further north (Umeå). β-Carotene concentrations of both legumes and grasses differed more than α-tocopherol concentrations between years at Skara. Species differences were more obvious in legumes than in grasses, and birdsfoot trefoil had the highest vitamin concentration of the legumes in this experiment. α-Tocopherol and β-carotene concentrations did not always decrease with later cutting date, lower leaf blade proportion or advanced maturity, indicating that there was an interaction between these factors on the vitamin concentration of forages. In addition, other factors, such as differences in weather conditions, influenced vitamin concentrations both directly and indirectly.

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