Seasonal fluctuation in concentrations of α-tocopherol and β-carotene in forage herb and legume species and a grass-clover mixture

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Abstract

Most studies in forages have been carried out with perennial ryegrass and legume species such as white clover. Recently, α-tocopherol and β-carotene in a number of dicotyledonous forage species were compared to a grass-clover mixture. To develop management guidelines, insight into seasonal patterns across the various harvests and years is important. Therefore, four herb species, three legumes and a perennial ryegrass-white clover mixture were investigated in a cutting trial with four harvests (May-Oct) during 2009 and 2010. Concentrations of α-tocopherol and β-carotene were highest in October and lowest in July. Biodiverse pastures could have added benefits in farming practice as various herbs outperformed the grass-clover mixture regarding α-tocopherol concentration, particularly in autumn.

Keywords: herbs, forbs, forage legumes, vitamins, α-tocopherol, β-carotene, seasonal pattern

Introduction

Grasslands provide an important part of the feed used by domestic and wild ruminants and other animals such as horses. Fresh herbage is an important natural source of vitamins in ruminant diets. Concentrations of vitamins in plants depend on factors such as regrowth stage, nitrogen, temperature, day length, and leaf proportion in the harvested herbage (Booth, 1964; Livingston *et al.*, 1968). Most studies on vitamin concentrations in forages have been carried out with agronomically important grass species as perennial ryegrass and legume species such as white clover, but seldom with other grassland forage species. As yield and quality data of broad-leaf herb species grown in a sward are scarce, vitamins in a number of herb and legume species were compared to a grass-clover mixture to get insight into species differences (Elgersma *et al.*, 2012). This study reports seasonal patterns in contents of α-tocopherol and β-carotene across harvests and years.

Materials and methods

The experiment was established in spring 2008 in Foulum, Aarhus University, in the central part of Jutland, Denmark (56°03', N 9°03'E). Pure stands were established with each of four herb species: salad burnet (*Sanguisorba minor)*, caraway (*Carum carvi)*, chicory (*Cichorium intybus)*, and ribwort plantain (*Plantago lanceolata*), and three legumes: yellow sweet clover (*Melilotus officinalis*), lucerne (*Medicago sativa)* and birdsfoot trefoil (*Lotus corniculatus*). Also a mixture with 85% perennial ryegrass (*Lolium perenne*) and 15% white clover (*Trifolium repens*) was sown. Net plot size was 1.5×9 m. Swards were cut with a forage harvester on 29 May, 9 July, 21 August and 23 October 2009 and 31 May, 13 July, 19 August and 21 October 2010. After cutting, samples of the harvested herbage were taken to determine

yield and quality parameters. The concentrations of α-tocopherol and ß-carotene were quantified after high performance liquid chromatography (Jensen et al., 1998). The experimental design was a randomized complete block with two replications. There were eight swards (the four herb and three legume species plus the mixture) and four harvests per year. Analysis of variance procedures were applied using the MIXED procedures of SAS. Yield and vitamin concentrations were evaluated with a model that included fixed main effects of species, harvest date and their interaction. Random effect was assigned to replications, years and their interactions. All tests of significance were made at the 0.05 level of probability.

Results and discussion

All parameters showed significant differences among the species as reported earlier. Yields ranged from 3.9 to 15.4 t DM $ha^{-1}yr^{-1}$ and were lower ($P<0.001$) in yellow sweet clover, salad burnet and caraway than in lucerne and the grass-clover mixture. Concentrations of α-tocopherol were lowest ($P<0.01$) in lucerne and yellow sweet clover, and highest in salad burnet and plantain (*ca.* 22 *versus* 80 mg kg^{-1} DM, respectively); the latter two species outperformed the grass-clover mixture ($P<0.01$). Concentrations of β-carotene ranged between *ca.* 28 and 59 mg kg^{-1} DM and were lower ($P<0.05$) in salad burnet, lucerne and yellow sweet clover than in caraway, birdsfoot trefoil and ribwort plantain. (Elgersma *et al.*, 2012). Interactions between species and cut were not significant for α-tocopherol.

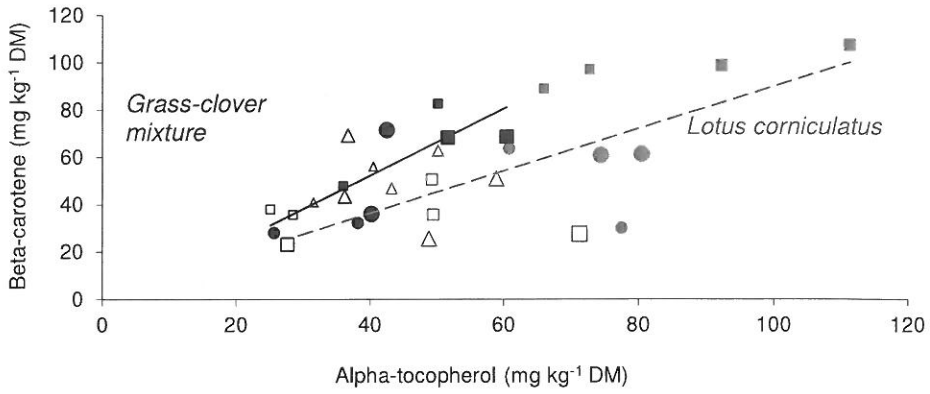

Figure 1. Concentrations of α-tocopherol and of β-carotene during 4 harvests (● late May, □ early July, Δ mid-late August, ■ mid-late October) in 2009 (small symbols) and 2010 (large symbols). Black depicts grass/clover, grey birdsfoot trefoil. Trendlines: $R^2 = 0.60$ and 0.39, respectively.

Large differences between harvests ($P<0.001$) were found for all parameters: yields were lowest in the 4th harvest (not shown), whereas concentrations of α-tocopherol and β-carotene were generally highest in the 4th harvest (illustrated for birdsfoot trefoil and the grass-clover mixture in Figure 1).

Concentrations of α-tocopherol and of β-carotene showed a positive correlation within most species except in salad burnet where no relation was found; trendlines differed ($P<0.05$) among species (e.g., Figure 1). The weather may provide an explanation for the fact that vitamin concentrations in the first harvest were much higher in 2010 than in 2009. First of all, the winter of 2009/2010 was severe and spring growth started late. The average temperature in April 2010 was only 6.5°C whereas in April 2009 it was 9.4°C. Also, in May the air

temperatures in 2010 were lower than in 2009: the 20 days preceding the date of the first cut, average values were 9.6°C and 10.6°C, respectively. Therefore, the effective primary growth period differed. This implies that the forage was probably less mature on 31 May 2010 than on 29 May 2009; however, unfortunately no phenological data were recorded. The 4th harvest showed high vitamin concentrations in both years, but α-tocopherol levels again were higher in 2010 than in 2009. In 2010 there was a cold period after the 3rd cut so again the weather may have caused a delay in regrowth, resulting in physiologically younger forage in the 4th cut in 2010.

In pastures, species are mixed but here pure stands of four herbs and three legumes were studied. Interspecific interaction can affect canopy structure and leaf/stem ratio of species. Brown (1953) found four times more α-tocopherol in grass leaves than in stems, and three times more α-tocopherol in clover leaves than in petioles. Also higher contents of β-carotene have been found in plants containing a higher proportion of leaves (Livingston *et al.*, 1968). Stems in birdsfoot trefoil were small, thin and green. Leaf proportions of species were highest in the 4th harvest, which coincided with generally high concentrations of vitamins.

The high α-tocopherol concentrations of the herbs and birdsfoot trefoil, as found in this study, might offer perspectives for naturally improved contents in milk, particularly in autumn.

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