Vitamin contents in forage herbs

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Summary

Grasslands provide an important part of the feed used by domestic and wild ruminants. Fresh forages are an important natural source of vitamins in ruminant diets. Most studies in forages have been carried out with perennial ryegrass and legume species such as white clover. As yield and quality data of broad-leaf grassland species are scarce, the aim of this study was to obtain novel information on vitamins in a number of herb species compared to a grass-clover mixture and to get insight into species differences and seasonal patterns across the various harvests and years. Seven herb species and a perennial ryegrass-white clover mixture were investigated in a cutting trial with four harvests (May–Oct) during 2009 and 2010. Vitamin concentrations were highest in October. The α-tocopherol concentrations were lowest in lucerne and yellow sweet clover, and highest in salad burnet and plantain. The β-carotene concentrations were lowest in lucerne, salad burnet and yellow sweet clover and highest in caraway, birdsfoot trefoil and plantain. As various herbs outperformed the grass-clover mixture regarding vitamin concentrations, these might provide added benefits in farming practice beyond higher biodiversity *per se*.

Key words: Herbs, forbs, forage legumes, vitamins

Introduction

Grasslands provide an important part of the feed used by domestic and wild ruminants. In grasslands of temperate climate zones, sown plant species comprise three broad functional groups: grasses, forage legumes and forage herbs (forbs). Many grasslands on conventional (non-organic) farms in temperate climate zones are sown with relatively simple grass seed mixtures, sometimes monocultures, whereas on organic farms, grasslands are often sown with seed mixtures comprising multiple functional groups, either as grass-legume mixtures or multi-species mixtures that include forage herbs. In organically managed grasslands, grass-white clover mixtures are commonly used because of their ability to be nitrogen self-sufficient. Grass-clover pastures are an economic source of nutritious, protein-rich forage. There are a number of dicotyledonous plant species that occur naturally in pastures or which may be included in sown mixtures (Foster, 1988; Smidt & Brimer, 2005).

Fresh herbage is an important natural source of vitamins in ruminant diets. Milk from grass-fed cows contains high levels of antioxidants as α-tocopherol (vitamin E) and β-carotene (pro-vitamin

A) (Agabriel *et al*., 2007). The concentrations of vitamins in forage species are thus important for the vitamin concentration as well as the oxidative stability of animal-derived foods such as dairy (Havemose *et al*., 2004) and meat products (Mercier *et al*., 2004).

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 hardly with other grassland forage species. Concentrations of vitamins in plants depend on factors such as regrowth stage, temperature and day length, N fertilisation and leaf proportion in the harvested herbage (Booth, 1964; Livingston *et al*., 1968). As yield and quality data of broad-leaf herb species grown in a sward are scarce, the aim of this study was to obtain novel information on vitamins in a number of herb species compared to a grass-clover mixture and to get insight into species differences and seasonal patterns across the various harvests and years.

Materials and Methods

The experiment was established in spring 2008 at the Research Farm Foulumgaard, Aarhus University, in the central part of Jutland, Denmark at an elevation of 51 m. The loamy-sand soil had a pH of 5.9 and contained 7.7% clay, 10% silt, 48% fine sand, 33% coarse sand, 1.6% carbon, 60 mg kg^{-1} exchangeable K and 21 mg kg^{-1} extractable P in the 0–15 cm layer.

Forage species were undersown in a spring barley crop at a sowing rate of 25 kg ha^{-1} and a sowing depth of 0.5–1 cm. Pure stands were established with each of seven herb species: salad burnet (*Sanguisorba minor*), chicory (*Cichorium intybus*), caraway (*Carum carvi*), birdsfoot trefoil (*Lotus corniculatus*), lucerne (*Medicago sativa*), yellow sweet clover (*Melilotus officinalis*) and ribwort plantain (*Plantago lanceolata*). Also a perennial ryegrass-white clover mixture (*Lolium perenne* + *Trifolium repens*) was sown. Net plot size was 1.5 m × 9 m. Swards were cut with a forage harvester four times per year (May–Oct) during 2009 and 2010.

After cutting, the herbage was weighed and subsamples of the harvested herbage were taken. The botanical composition of the grass-clover mixture was not determined. In the herb plots, unsown species were excluded from the subsamples used for chemical analyses. A subsample of approximately 0.5 kg of the total herbage was taken from the each cut in both years, frozen and later lyophilized and milled with a 1-mm screen. Of this material, 2 g was saponified in alcohol and the vitamins were subsequently extracted into heptane and quantified on HPLC as previous described (Jensen *et al*., 1998).

The experimental design was a randomised complete block with two replications. There were eight ‘species’ (the seven legume and herb 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. All tests of significance were made at the 0.05 level of probability.

Results

All parameters showed significant differences among the species. Generally, interactions between species and cut were relatively unimportant. Large differences between harvests were found for all parameters: yields were lowest in the 4th harvest (late October), whereas vitamin concentrations were lowest in the 2nd (early July) and highest in the 4th harvest (not shown).

Mean values for annual DM yield and concentrations of tocopherol and carotenoids are shown in Table 1. Yields ranged from 3.9–15.4 t DM ha^{-1} yr^{-1} and were lower (P < 0.05) in yellow sweet clover, salad burnet and caraway than in lucerne and the grass-clover mixture. Alpha-tocopherol concentrations averaged over the season ranged from 21–85 mg kg^{-1} DM and were lower in lucerne and yellow sweet clover than in chicory, caraway, birdsfoot trefoil, plantain and salad burnet, and higher in the latter two species than in the grass-clover mixture ($P < 0.01$). In addition, chicory contained a significant amount of γ-tocopherol (19–67 mg kg^{-1} DM, data not shown). Beta-carotene concentrations ranged from 26–61 mg kg^{-1} DM and were lower in lucerne than in chicory, the grass-clover mixture, plantain, caraway and birdsfoot trefoil, and higher in the latter three species than in salad burnet, yellow sweet clover and chicory ($P < 0.05$). Lutein concentrations ranged from 129–219 mg kg^{-1} DM and were lower in salad burnet, lucerne and yellow sweet clover than in caraway, the grass-clover mixture and birdsfoot trefoil; the latter two had higher lutein concentrations than chicory and plantain, while birdfoot trefoil also outperformed caraway ($P < 0.01$).

Table 1. *Dry matter (DM) yield and concentrations of tocopherol and carotenoids for eight 'species' (i.e. seven monocultures and a perennial grass-white clover mixture), averaged over four cuts in 2009 and 2010*

Species		DM Yield (kg ha^{-1} yr^{-1})	α-tocopherol	β-carotene (mg kg^{-1} DM)	Lutein
Herbs:					
Salad burnet		4636^{a1}	85^{d}	30^{ab}	129^{a}
Caraway		5560^{ab}	58^{bcd}	61^{d}	174^{bc}
Chicory		9960^{bc}	55^{bc}	41^{bc}	152^{ab}
Plantain		8416^{abc}	77^{cd}	56^{d}	149^{ab}
Legumes:					
Yellow sweet clover		3904^{a}	23^{a}	33^{ab}	131^{a}
Lucerne		15412^{d}	21^{a}	26^{a}	129^{a}
Birdsfoot trefoil		9460^{abc}	65^{bcd}	59^{d}	219^{d}
Grass-clover:					
Standard mixture		12548^{cd}	39^{ab}	48^{cd}	207^{cd}
Significance	Species	*	**	*	**
	Cut	***	*	***	***
	S × C	NS	NS	***	**
	Year	NS	NS	NS	***

[1]Within a column, least square means without a common superscript are significantly different ($P < 0.05$). Significance of main effects of species and cut, their interaction (S × C) and of year (Y): *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS not significant.

Discussion

Lucerne was the highest yielding species and had the lowest concentrations of α-tocopherol, β-carotene and lutein. As expected, lucerne and the grass-clover mixture outperformed most herbs in terms of yield, although in this experiment the yield of chicory, plantain and birdsfoot trefoil was not different from that of the grass-clover mixture. Chicory and plantain are relatively high yielding compared to other herb species (Labreveux *et al*., 2004) and are used to some extent in commercial forage mixtures. Fisher *et al*. (1996) found in extensively managed swards of herbs sown singly with a standard grass mix that ribwort plantain, salad burnet, birdsfoot trefoil and chicory were among the species that competed well with grasses and produced annual forb herbage yields greater than 2 t DM ha^{-1}.

Caution should be taken when interpreting yield data. Plant species can interact differently with each other when grown in a mixture than when grown in monoculture (Casler *et al*., 1987). In this experiment, pure stands were studied and the yield of salad burnet ranked lowest, in contrast to its relative performance in mixture in the work of Fisher *et al*. (1996). However, this may have been due in part to the absence of fertilisation in the 3-year experiment of Fisher *et al*. (1996). The low productivity of salad burnet could limit its use in practice but a positive aspect is its palatability (Douglas *et al*., 1994) which has been observed with cattle (K Søegaard; R Loges, pers. comm.). Another merit was its high α-tocopherol concentration, which out yielded that of the grass-clover mixture.

Interspecific interaction can affect leaf/stem ratio and the vitamin composition of plant organs. Leaf proportion is the largest factor affecting the α-tocopherol content in forages (Ballet *et al*., 2000). 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). Unfortunately, in this experiment canopy samples were not separated into leaves and stems.

Salad burnet and plantain both form a rosette hence their leaves do not have petioles. Both species had the highest α-tocopherol concentration, out yielding that of the grass-clover mixture. In contrast, the β-carotene content of salad burnet was very low. Lucerne, sweet clover and chicory were stemmy. Caraway had few stems, and stems in birdsfoot trefoil were small, thin and green. Across species means, there was no relation between vitamin concentrations (Table 1) and neutral detergent fibre (NDF) content. Within most species, a negative relation was found between vitamin (α-tocopherol) and NDF concentrations (not shown). The number of stems was very low in the 4th harvest, which coincided with generally high concentrations of vitamins.

Legumes usually contain less α-tocopherol than grasses (Danielsson *et al*., 2008), but this was not observed in our experiment. Birdsfoot trefoil, lucerne and yellow sweet clover all belong to the *Fabaceae* family but contents differed largely within this functional group, as birdsfoot trefoil had a significantly higher α-tocopherol concentration than lucerne and yellow sweet clover, which was also the case for concentrations of β-carotene and lutein. The grass-clover mixture had a numerically intermediate content (Table 1), but no information is available on the grass and clover vitamin contents and yields, hampering a direct comparison with grass. No common feature was found among the four non-leguminous herb species. As species differences within each of the functional groups of herbs and legumes were large, no contrast was found for yield or vitamin contents between these functional groups.

After cutting, wilting often reduces the concentrations of α-tocopherol and β-carotene, especially in sunny weather; both are sensitive to oxidation (Ballet *et al*., 2000). In Sweden, Lindqvist *et al*. (2012) found that a 3-hour outdoor wilting period had no effect on the β-carotene content of birdsfoot trefoil and red clover mixtures with grass, and only a small decreasing effect on the α-tocopherol content of a birdsfoot trefoil-timothy (*Phleum pratense*) mixture. They reported concentrations of α-tocopherol and β-carotene in unwilted mixtures of 57 and 44 mg kg^{-1} DM, respectively, with no differences among mixtures. In this experiment, samples were frozen within 2 h after harvest and wilting in this period was avoided by storage in plastic bags as much as possible, so no effect of wilting is expected. Mean concentrations of α-tocopherol and β-carotene in the grass-clover mixture (39 and 48 mg kg^{-1} DM, respectively) were comparable with those in mixtures of Lindqvist *et al*. (2012).

Various herbs are of relatively high nutritive value and could enhance the nutritional profile of mixed species pastures. For example, according to Pirhofer-Walzl *et al*. (2011), including some specific herb species in forage mixtures is an effective way of increasing mineral concentrations in herbage. These nutritive value benefits may encourage the adoption of these species by farmers, but must, from a management viewpoint, be balanced against the lack of persistence of most herbs. However, in ley systems this would be less of a problem than in permanent pastures.

The high vitamin concentrations of some herbs as found in this study might offer perspectives for naturally improved milk vitamin composition. In a pilot study comparing the transfer efficiency and content in milk of fatty acids and vitamins of cows fed a mixture of herb species, fresh grass-clover forage or a total mixed ration (TMR), no significant differences were observed in the vitamin content of the various milks apart from a higher retinol content with herbs (Petersen *et al.*, 2011). There were, however, increased contents of ω3 and ω6 fatty acids (FA) in milk of herb-fed cows that could be due to an increase in transfer efficiency from feed to milk for ω3 FA compared to both grass-clover and TMR diets. Further studies are underway and effects of herb species on FA will be presented elsewhere.

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