Do Grass and Legume Vitamin Contents Change during Extended Growth in Spring?

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Abstract. Herbage vitamins affect performance of grazing and grass-fed cattle . In silages, knowledge of vitamin contents in herbage is desired to balance supplements in indoor feeding rations. This is of particular interest in organic farming systems. Information on companion species effects in grass-legume mixtures on changes during prolonged growth is scarce, while this is relevant in view of delayed harvests that frequently occur in practice. Perennial ryegrass (*Lolium perenne*) was sown with either red clover (*Trifolium repens*), white clover (*Trifolium pratense*), lucerne (*Medicago sativa*) or birdsfoot trefoil (*Lotus corniculatus*), and white clover with hybrid ryegrass (*Lolium x boucheanum*), meadow fescue (*Festuca pratensis*) or timothy (*Phleum pratense*). Yield and concentrations of alpha-tocopherol, beta-carotene and lutein were studied during two years in Denmark under a silage cutting regime. Data were collected during two harvest years in May in replicated plots. The dynamic development of compounds was investigated during two-week intervals. Herbage samples were hand-separated and individual species were analysed. The contents of alpha-tocopherol, beta-carotene and lutein differed among species, as did rates of decline during 2-week growth intervals. Outcomes are discussed in relation options for designing grassland mixtures for sustainable agricultural systems.

Introduction

In agricultural production systems of temperate climate zones, lucerne, red clover and white clover are the main forage legumes. White clover is used for grazing as well as silage; red clover and lucerne are most suited to silage cropping, often mixed with grasses.
Fat-soluble vitamin nutrition affects animal performance (e.g., Herdt and Stowe 1991). Animal products are an important resource of protein, minerals and fat-soluble vitamins such as Vitamin E and Vitamin A in human consumption patterns: they are part of a healthy and balanced diet. Although synthetic vitamins are cheap and widely used in animal production, there is interest in naturally occurring vitamins, particularly in organic farming systems where use of synthetic or non-organic feed and supplements has to be minimized. Fresh forage contains fat-soluble vitamins with antioxidative properties e.g. α-tocopherol and β-carotene (pro-vitamin A) and antioxidants like lutein. Tocopherols (vitamin E) and carotenoids, such as lutein and β-carotene, can be directly transferred from fresh grass into milk. Vitamins in forage species are thus important for the vitamin concentration as well as the oxidative stability of animal-derived foods such as dairy and meat products.
A field experiment was conducted with seven two-species grass-legume mixtures: perennial ryegrass in mixture with red clover, birdsfoot trefoil, lucerne or white clover, and meadow fescue, timothy or hybrid ryegrass in mixture with white clover. The aim of this study was to obtain novel information on concentrations of α-tocopherol, β-carotene and lutein in grass and legume species in mixtures, and changes during regrowth intervals, in relation to herbage DM yield. We hypothesized that α-tocopherol concentration would be lower, and concentrations of carotenoids would be higher in legumes than in grasses. We also hypothesized that vitamin concentrations would decline at a faster rate in grasses than in legumes during prolonged regrowth periods in spring.

Methods and Study Site

Perennial ryegrass (*Lolium perenne* L.; 'PR') was sown with each of four forage legumes: red clover (*Trifolium pratense* L.; 'RC'), lucerne (*Medicago sativa* L.; 'LU'), and birdsfoot trefoil (*Lotus corniculatus* L.; 'BT'), and white clover (*Trifolium repens* L.; 'WC') was sown with each of four companion grasses: perennial ryegrass, hybrid ryegrass (*Lolium boucheanum* Kunth; 'HR'), meadow fescue (*Festuca pratensis* Huds; 'MF') and timothy (*Phleum pratense* L.; 'TI'). Grass and mixtures were sown in 2006 in a small-plot (1.5 m × 8 m) cutting trial with 4 replications in Denmark. Plots were fertilized with cattle slurry containing 300 kg N ha^{-1} in split applications (100, 80, 60 and 60 kg N ha^{-1}, respectively) and were irrigated at drought stress. Plots were harvested five times in 2007 and four times in 2008 with a Haldrup forage harvester at a residual stubble height of 7 cm. Dry matter yield and botanical composition were determined at each harvest during two years. Results of all nine harvests have been reported earlier (Elgersma and Søegaard 2016).
The dynamic development of compounds was investigated by sampling at the optimal harvest date, i.e. at the onset of stem elongation ± one week (Elgersma and Søegaard 2018). Hand-separated samples were analysed for each species in two replicates; for white clover and perennial ryegrass, samples were taken from the perennial ryegrass – white clover mixture. A second subsample of *ca*. 5 kg fresh material was taken from each cut in both years and brought to the laboratory. Grass and legume components were hand-picked and immediately frozen in a plastic bag at -20 °C, so that the time between cutting and freezing was approximately 1 hour. Two composite samples were made of frozen samples: one of replicates 1 and 4, and one of replicates 2 and 3. Frozen samples were freeze dried and subsequently stored in an air-tight plastic bag at -20 °C until analysis. Samples were then lyophilized and milled with a 1-mm screen. This material was used for chemical analyses, so analyses in individual companion species were thus carried out in two replications.

For vitamin analysis, two g were saponified in alcohol. Then, two extracts were made with heptane and analysed in two separate high performance liquid chromatography (HPLC) runs, first for tocopherols and then for carotenoids. The concentration of α-tocopherol, ß-carotene and lutein was calculated as average from the duplo measurements for each sample.
Species DM yield and concentrations of vitamins were analysed with a linear model with species, year and species × year as fixed factors (n=2). A similar model was used to analyse the values of the slopes of linear regression lines that were calculated for each parameter for each 2-week harvest interval.
For all tests, the MIXED procedure of SAS was used. All tests of significance were made at P=0.05.

Results and Discussion

Vitamin concentrations

Species differed significantly for vitamin concentrations (Table 1). The concentration of α-tocopherol was highest in meadow fescue and lowest in red clover. Legumes usually contain less α-tocopherol than grasses (Danielsson et al. 2008) and this was also observed by Elgersma et al. (2013). Here, however, the α-tocopherol concentration in birdsfoot trefoil was similar to that in timothy, and that in lucerne was similar to that in the ryegrasses. On average, therefore, no significant difference was found between grasses and legumes in α-tocopherol concentration.
The concentrations of β-carotene and of lutein were highest in birdsfoot trefoil, and on average higher in legumes than in grasses as was hypothesized.
Among the legumes, all vitamin concentrations were highest ($P < 0.001$) in birdsfoot trefoil, which agrees with findings of Lindqvist et al. (2014). The concentration of α-tocopherol was lowest in red clover. The concentration of β-carotene was lower in red clover and lucerne than in white clover; birdsfoot trefoil had the highest concentration. Lindqvist et al. (2014) also reported lower β-carotene levels in red clover in May than in white clover and birdsfoot trefoil.
The concentration of lutein was lowest in lucerne ($P < 0.001$).

Among the grasses, meadow fescue had by far the highest α-tocopherol concentration ($P < 0.001$). The α-tocopherol level in timothy was higher than in the ryegrasses. The concentration of β-carotene did not differ among grass species. The lutein concentration was higher in timothy than in hybrid ryegrass.

Weekly change in vitamin concentrations

Table 1 also presents the significance of differences between species for rates of increase or decrease in vitamin concentrations during a 2-week period in May. During growth, herbage yields obviously increased while concentrations of vitamins generally decreased. In individual legumes, rates of change in concentrations of all vitamins differed among the four species. Among grasses, decline rates of carotenoids did not differ.

As hypothesized, concentrations of α-tocopherol and lutein declined at a faster ($P < 0.01$) rate in grasses than in legumes during prolonged growth periods in spring. The decline rate of β-carotene was not different among functional groups, however.

Table 1. Concentrations of α-tocopherol, β-carotene and lutein (mg kg DM $^{-1}$) in May and rates of change (mg kg DM $^{-1}$ week $^{-1}$) during the 2-week interval around this harvest date, in 8 species (S) grown in binary mixture with a companion species (Comp) and in 2 functional groups (G). Average values of 2 replicate plots during 2 years (Y): 2007 and 2008; n=4. Species abbreviations: Legumes (Leg.): white clover (WC), red clover (RC), lucerne (LU), birdsfoot trefoil (BT), and Grasses: perennial ryegrass (PR), hybrid ryegrass (HR), meadow fescue (MF), timothy (TI). SE = standard error.

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		May[1]					
Species	Comp	α-tocopherol	rate[2]	β-carotene	rate	lutein	rate
WC	PR	47 [d3]	-4 [ab]	72 [b]	-7 [b]	290 [ab]	-29 [bc]
RC	PR	34 [e]	-2 [ab]	53 [c]	-6 [b]	257 [bc]	-11 [ab]
LU	PR	62 [c]	-6 [bc]	50 [c]	3 [a]	196 [de]	5 [a]
BT	PR	79 [b]	4 [a]	101 [a]	-7 [b]	335 [a]	-23 [bc]
PR	WC	55 [cd]	-18 [c]	53 [c]	-8 [b]	196 [de]	-45 [c]
HR	WC	53 [cd]	-2 [ab]	56 [c]	-12 [b]	172 [e]	-36 [c]
MF	WC	104 [a]	-18 [c]	53 [c]	-6 [b]	215 [cde]	-36 [c]
TI	WC	73 [b]	-9 [bc]	60 [bc]	-8 [b]	225 [cd]	-24 [bc]
Sign.[4]	S	***	**	***	NS	***	**
	Y	***	NS	**	***	***	***
	S×Y	***	(*)	NS	NS	NS	NS
	SE	3	3	5	3	17	11
Group							
Leg.		56 [a4]	-2 [a]	69 [a]	-4 [a]	270 [a]	-14
Grass		71 [a]	-11 [b]	55 [b]	-9 [a]	203 [b]	-35
Sign.[4]	G	NS	**	*	(*)	***	**
	Y	***	NS	(*)	***	**	***
	G×Y	NS	NS	NS	NS	NS	NS
	SE	6	2	5	1	13	4

Harvest dates: 14 May 2007 and 21 May 2008 (primary growth)

[2] Rate of change in vitamin content (b value of linear regression between concentrations during ± one week harvest interval)

[3] Different letters within a row indicate a significant difference at $P = 0.05$ among 8 species or among 2 functional groups

[4] Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; (*), $P < 0.07$; NS, not significant

Lucerne had the lowest rate of change in concentration of carotenoids, i.e., levels did not decline during the 2-week regrowth period (Table 1). Lucerne and red clover had comparable herbage production levels in mixtures with perennial ryegrass, but red clover had a higher digestibility and protein content, and a lower NDF content than lucerne, while digestibility and protein content declined faster and NDF content increased faster in lucerne during regrowth than in red clover (Elgersma and Søegaard 2018). Results of these studies can be used in practice when designing grass-legume mixtures to find a balance between the aims of high forage yield, nutritive value and vitamin contents, and changes during prolonged growth in spring.

Conclusions

Grasses differed from legumes in having a faster decline rate of α-tocopherol and lutein concentrations during prolonged regrowth in May than legumes. Grasses also had lower concentrations of β-carotene and lutein than legumes. In grass-legume mixtures, the concentration of carotenoids would be higher and that of α-tocopherol and lutein less prone to decline compared with pure grass swards when harvest is delayed.

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