

## Contents of $\alpha$ -tocopherol and $\beta$ -carotene in grasses and legumes harvested at different maturities

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### Abstract

Concentrations of  $\alpha$ -tocopherol and  $\beta$ -carotene in forage species at various maturities were studied in Scandinavia. Red clover (RC)/timothy (TI), RC/meadow fescue (MF), and birdsfoot trefoil (BT)/TI mixtures were grown in Skara and Umeå, Sweden. RC/TI, RC/perennial ryegrass (PR), white clover/PR and BT/TI were grown in Foulum, Denmark. Forages in Sweden were cut one week before heading (BH), at heading and one week after heading of TI. The regrowth was cut six and eight weeks after each harvest in the spring growth cycle. In Denmark, one first harvest and three regrowths were taken. Results from Skara and Foulum are presented. Highest concentrations of  $\alpha$ -tocopherol and  $\beta$ -carotene (mg kg<sup>-1</sup> DM) in legumes were found in BT grown in Skara (49.8 and 69.6 in spring growth cycle, 48.1 and 79.8 in regrowth) and in Foulum (81.3 and 89.2). MF had more  $\alpha$ -tocopherol and  $\beta$ -carotene than TI in the spring growth cycle (73.5 and 54.2 vs. 46.9 and 43.0 mg kg<sup>-1</sup> DM). Highest concentrations of vitamins in the regrowth were found six weeks after BH with 71.8 and 104.8 mg  $\alpha$ -tocopherol and 99.6 and 73.1 mg  $\beta$ -carotene kg<sup>-1</sup> DM in legumes and grasses, respectively.

Keywords: forages, vitamins, maturity stage, season

### Introduction

Forage is the largest natural source of vitamins for ruminants (NRC, 2001), but the knowledge about how forage vitamin content is affected by species, maturity stage and climate is limited. Shortage of vitamins A and E can depress the immune system and cause reproductive disturbances in ruminants (NRC, 2001). The objective of this experiment was to determine the effects of plant species, maturity stage, season and the interactions between plant species and maturity stage and plant species and season on concentrations of  $\alpha$ -tocopherol and  $\beta$ -carotene in forages grown in Sweden and Denmark.

### Materials and methods

Plots with mixtures of grasses and legumes were established in Skara and Umeå, Sweden and in Foulum, Denmark in the spring of 2004. This article includes results from Skara and Foulum. Mixtures grown in Sweden were 1) timothy (TI; *Phleum pratense* L.)/birdsfoot trefoil (BT; *Lotus corniculatus* L.), 2) TI/red clover (RC; *Trifolium pratense* L.) and 3) meadow fescue (MF; *Festuca pratensis* L.)/RC and harvest dates during the spring growth cycle in Skara 2005 were one week before (1:1; June 9) at (1:2; June 15) and one week after (1:3; June 22) heading of timothy. Regrowths were cut six and eight weeks after 1:1 (July 20

and August 3), 1:2 (July 26 and August 8) and 1:3 (August 2 and August 17). Mixtures grown in Foulum were TI/RC, TI/BT, perennial ryegrass (PR; *Lolium perenne* L.)/RC and PR/white clover (WC; *Trifolium repens* L.). Forages were harvested May 30 and regrowths were harvested July 5, August 22 and October 24 at Foulum. Dry-matter (DM) yields were determined at both locations. All mixtures were separated into species before analysis. Alpha-tocopherol and  $\beta$ -carotene in grasses and legumes were analysed at Foulum, Denmark by HPLC, after saponification and extraction into heptane (Jensen *et al.* 1998). The trial in Skara had a split-plot design with mixture treated as the main plot, replicated three times, and with spring growth cycle and regrowth as sub plots. The trial in Foulum had a randomized block design with mixture, replicated four times, and harvest number as fixed factors. Data were analysed for grasses and legumes separately using the general linear model procedure of SAS (ver. 9.1, 2001).

## Results and discussion

In Skara, interactions were detected between legume species and cutting time during the spring growth cycle ( $P = 0.001$ ), where the  $\alpha$ -tocopherol content decreased in BT but increased in RC, grown with TI, with later harvest date. As a result, BT had higher levels of  $\alpha$ -tocopherol in mg kg<sup>-1</sup> DM than RC at 1:1 (63.9 vs. 19.9) and 1:2 (49.6 vs. 24.1) but there was no difference between the legumes at 1:3 (35.9 vs. 31.5). Concentrations of  $\beta$ -carotene in legumes were highest at 1:2, whereas 1:2 only differed from 1:3 in grasses (Table 1). MF had higher levels of  $\alpha$ -tocopherol and  $\beta$ -carotene than TI, when averaged over harvests, which can be related to a larger leaf fraction in MF than in TI (60 vs. 30% of DM) as vitamins primarily are found in the leaves of plants.

Table 1. Alpha-tocopherol and  $\beta$ -carotene, mg kg<sup>-1</sup> DM, in the spring growth cycle of grasses and legumes grown in Skara.

	Mixture			<i>P</i> - value	Harvest time			<i>P</i> - value
	1	2	3		1:1	1:2	1:3	
<i>Grasses</i>	<i>TI</i>	<i>TI</i>	<i>MF</i>					
$\alpha$ -tocopherol	50.6 <sup>b</sup>	43.1 <sup>b</sup>	73.5 <sup>a</sup>	0.001	61.0	55.6	50.6	NS
$\beta$ -carotene	43.0 <sup>b</sup>	42.9 <sup>b</sup>	54.2 <sup>a</sup>	0.028	48.0 <sup>a</sup>	53.4 <sup>a</sup>	38.6 <sup>b</sup>	0.003
<i>Legumes</i>	<i>BT</i>	<i>RC</i>	<i>RC</i>					
$\alpha$ -tocopherol	49.8 <sup>a</sup>	24.7 <sup>b</sup>	24.4 <sup>b</sup>	0.0004	34.6	32.6	31.8	NS
$\beta$ -carotene	69.6 <sup>a</sup>	48.5 <sup>b</sup>	42.9 <sup>b</sup>	0.005	48.8 <sup>b</sup>	68.3 <sup>a</sup>	43.9 <sup>b</sup>	0.002

*TI* = timothy, *MF* = meadow fescue, *BT* = birdsfoot trefoil, *RC* = red clover

1:1 = one week before heading, 1:2 = at heading, 1:3 = one week after heading of timothy in spring growth cycle

<sup>a,b</sup>Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

In the regrowth of grasses and legumes, as a mean over species, the highest levels of both  $\alpha$ -tocopherol and  $\beta$ -carotene were found six weeks after 1:1 (Table 2).

Table 2. Alpha-tocopherol and  $\beta$ -carotene, mg<sup>-1</sup> kg DM, in the regrowth of grass and legume harvested in Skara six and eight weeks after (w a) first cut.

	Regrowth						<i>P</i> - value
	6 w a 1:1	8 w a 1:1	6 w a 1:2	8 w a 1:2	6 w a 1:3	8 w a 1:3	
<i><math>\alpha</math>-tocopherol</i>							
Grass	104.8 <sup>a</sup>	73.7 <sup>b</sup>	68.6 <sup>b</sup>	41.4 <sup>c</sup>	54.0 <sup>c</sup>	54.5 <sup>c</sup>	0.0036
Legume	71.8 <sup>a</sup>	47.5 <sup>b</sup>	37.7 <sup>c</sup>	23.0 <sup>d</sup>	25.0 <sup>d</sup>	38.7 <sup>c</sup>	<0.0001
<i><math>\beta</math>-carotene</i>							
Grass	73.1 <sup>a</sup>	46.7 <sup>c</sup>	34.2 <sup>d</sup>	32.9 <sup>d</sup>	38.7 <sup>c</sup>	60.4 <sup>b</sup>	<0.0001
Legume	99.6 <sup>a</sup>	63.8 <sup>c</sup>	56.0 <sup>c</sup>	43.3 <sup>d</sup>	26.5 <sup>e</sup>	88.0 <sup>b</sup>	<0.0001

1:1 = one week before heading, 1:2 = at heading, 1:3 = one week after heading of timothy in spring growth cycle

<sup>a,b,c,d</sup>Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

On average, MF had a higher level of  $\alpha$ -tocopherol in  $\text{mg kg}^{-1}$  DM than TI in the regrowth after the early cut (1:1) in first harvest (121.2 vs. 74.2) and after the late cut (1:3) in the first harvest (63.5 vs. 49.6;  $P = 0.002$ ). In the average regrowth after 1:2, MF only differed from TI grown with BT (62.8 vs. 47.3  $\text{g kg}^{-1}$  DM). Averaged over 1:1, 1:2 and 1:3, MF had a higher level of  $\alpha$ -tocopherol in  $\text{mg kg}^{-1}$  DM than TI at six (98.6 vs. 64.4) and eight weeks (66.3 vs. 51.6) of regrowth ( $P = 0.02$ ). In legumes, BT had a higher level of  $\alpha$ -tocopherol than RC six weeks after first harvest (51.7 vs. 38.8  $\text{mg kg}^{-1}$  DM), but there was no difference between species eight weeks after first harvest. Average total yield of TI/BT was 8,583 kg DM compared to 10,133 kg DM  $\text{ha}^{-1}$  of TI/RC.

In Denmark, interactions between harvest and species were found in contents of  $\alpha$ -tocopherol and  $\beta$ -carotene of legumes and in  $\beta$ -carotene content of grasses ( $P < 0.001$ ). BT had a higher level of  $\alpha$ -tocopherol in  $\text{mg kg}^{-1}$  DM than the other legumes in second (74.1 vs. 34.8), third (70.6 vs. 20.2) and fourth harvest (118.4 vs. 64.2). High concentrations of vitamins in the fourth harvest indicate that climate, in addition to phenological composition of plants, plays an important role in vitamin content of forage (Table 3). TI contained more  $\alpha$ -tocopherol in  $\text{mg kg}^{-1}$  DM than PR (78.4 vs. 53.3) and BT contained more  $\alpha$ -tocopherol than white and red clover (81.3 vs. 41.6;  $P < 0.0001$ ), when averaged over harvests. The content of  $\beta$ -carotene in BT, WC and RC was 89.2, 74.4 and 52.3  $\text{mg kg}^{-1}$  DM, respectively ( $P < 0.0001$ ). Average total yield of TI/BT was 7,025 kg DM compared to 11,576 kg DM  $\text{ha}^{-1}$  of TI/RC.

Table 3. Alpha-tocopherol and  $\beta$ -carotene,  $\text{mg kg}^{-1}$  DM, in grass and legume harvested in Foulum, Denmark May 30 (1), July 5 (2), August 22 (3) and October 24 (4) 2005.

	Harvest				P - value
	1	2	3	4	
<i><math>\alpha</math>-tocopherol</i>					
Grass	54.4 <sup>b</sup>	41.5 <sup>c</sup>	38.0 <sup>c</sup>	104.3 <sup>a</sup>	< 0.0001
Legume	50.8 <sup>b</sup>	44.6 <sup>bc</sup>	32.8 <sup>c</sup>	77.7 <sup>a</sup>	< 0.0001
<i><math>\beta</math>-carotene</i>					
Grass	30.5 <sup>b</sup>	30.0 <sup>b</sup>	39.7 <sup>b</sup>	152.2 <sup>a</sup>	< 0.0001
Legume	40.7 <sup>b</sup>	46.2 <sup>b</sup>	39.2 <sup>b</sup>	142.0 <sup>a</sup>	< 0.0001

<sup>a,b,c</sup>Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

## Conclusions

BT had a higher content of vitamins than RC and WC but the yield was lower compared to RC. Highest vitamin levels in the regrowth herbage were achieved when cut was taken six weeks after the first early cut (1:1). Higher levels of vitamins in herbage harvested in the autumn, compared to the other harvest dates, indicate that vitamins are affected by climate.

## References

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