α-Tocopherol and β-carotene concentrations in legume-grass mixtures as affected by wilting and ensiling

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Introduction

Fresh or preserved forage is the largest source of natural α-tocopherol and β-carotene for dairy cows. Plant species, wilting and method of preservation can affect the vitamin content of forage and, consequently, have a large impact on the concentrations of these vitamins in plasma of dairy cows (Agabriel *et al.*2007).

The aim of this study was to determine the effects of species mixture, wilting, ensiling and additive on the contents of α-tocopherol and β-carotene in legume-grass forages.

Method

Three legume-grass mixtures; birdsfoot trefoil (*Lotus corniculatus* L.) + timothy (*Phleum pratense* L) (Bft+Ti), red clover (*Trifolium pratense* L.) + timothy (Rc+Ti) and red clover + meadow fescue (*Festuca pratensis* L.) (Rc+Mf) were grown at Lanna Research Station (58°21´N; 13°08´E), Skara, Sweden. The forage mixtures were harvested in the first regrowth on August 11, 2005, eight weeks after the spring growth, and wilted outside for approximately 3 hours in partly cloudy weather before being ensiled in 1.7-L laboratory silos for 100 days.

The experiment had a split-plot design, in which forage mixture, replicated three times in the field, was treated as the main plot and forage treatment (unwilted herbage, wilted herbage and three silage treatments) as the subplot. The additives used were the acid product Proens™ (2/3 formic acid and 1/3 propionic acid, Perstorp Inc., Perstorp, Sweden), applied at 5 L/t herbage and the inoculant Siloferm®Plus (homofermentative *Lactobacillus plantarum* and *Pediococcus acidilactici* (3.2×10^{10} cfu/g), cellulase and hemicellulase (54 000 HEC/g), applied at 768 000 cfu/g herbage. α-Tocopherol and β-carotene concentrations were determined by high-pressure liquid chromatography after saponification and extraction into heptane according to Jensen *et al.* (1998). Other nutrients and fermentation characteristics of the silage were analyzed with conventional methods as described in Lindqvist *et al.* (2012). Data were analyzed in PROC GLM of SAS. When a significant *F*-value was detected, pair-wise comparisons between LSMEANS were analyzed with Tukey´s test at $P<0.05$.

Results and Discussion

The legume proportions of Rc + Mf, Rc + Ti and Bft + Ti were 82, 85 and 65%, respectively. Forage was wilted to a dry-matter (DM) content of 273 g/kg. Wilted herbage contained 163 g crude protein, 441 g NDF and 68 g reducing sugar per kg of DM and the *in vitro* organic matter digestibility was 643 g/kg. The addition of the inoculant and the acid increased sugar (12.4 *vs.* 23.9 and 68.8 g/kg DM, respectively) and decreased acetic acid (23.7 *vs.* 17.2 and 7.5 g/kg DM, respectively), ethanol (6.2 *vs.* 4.0 and 0.9 g/kg DM, respectively), ammonia-N (8.33 *vs.* 7.22 and 4.89% of total N, respectively) and weight losses (3.58 *vs.* 2.43 and 1.40% of DM, respectively) of the control silage. The acid-treated silage had the lowest lactic acid content (39.6 *vs.* 85.9 g/kg DM) whereas the inoculated silage had the lowest pH (4.23 *vs.* 4.41).

Wilting decreased α-tocopherol concentration of Bft+Ti but had no effect in the red clover mixtures (P = 0.015; Table 1). There were no differences in α-tocopherol concentrations between the mixtures before ensiling but an increase in the α-tocopherol concentration of the Bft+Ti during ensiling while no effect of ensiling on the α-tocopherol concentration in the red clover mixtures caused higher concentrations in the untreated Bft+Ti silage than in Rc+Ti and Rc+Mf silages. The increase in α-tocopherol concentration of Bft+Ti during ensiling was further enhanced by use of the inoculant. Use of the acid decreased the α-tocopherol concentrate-ions of the red clover mixtures during ensiling (Table 1). No interactions were found between mixture and treatment for β-carotene concentrations (Table 1). Averaged over treatments, Bft+Ti had higher β-carotene concentration than Rc+Ti and Rc+Mf, which did not differ.

Conclusions

The short wilting period of 3 hours during fair weather conditions caused small effects on the vitamin content of the herbage with a decrease in the α-tocopherol concentration of Bft+Ti but no effect on the β-carotene

Table 1. Alpha-tocopherol and β-carotene concentrations (mg/kg DM) in unwilted herbage, wilted herbage and silages for each forage mixture (n = 3), as a mean over forage mixtures (n = 9) and as a mean over forage treatments (n = 15).

	Treatment					Forage mixture (mean)	Statistics of main effects	
Forage mixture*	Unwilted herbage	Wilted herbage	Control	Inoculant	Acid		SEM	*P*
α-tocopherol								
Bft+Ti	58.8	41.1	56.9	65.2	56.8	55.8 x	2.34	0.014
Rc+Ti	51.1	40.1	30.1	46.2	20.7	37.6 z		
Rc+Mf	59.7	48.6	42.1	54	35.2	47.2 y		
Treatment (mean)	56.5 a	43.3 bc	41.8 b	55.1 a	37.6 c		2.56	<0.0001
β-carotene								
Bft+Ti	56.2	49.5	41.2	42.7	41.2	46.2 x	2.18	0.016
Rc+Ti	39.1	30.3	31.4	32.2	20.5	30.7 y		
Rc+Mf	35.6	35.9	38.5	31.5	27.9	33.9 y		
Treatment (mean)	43.7 a	38.6 ab	37.1 b	35.5 b	29.9 c		2.26	0.005

Bft+Ti = birdsfoot trefoil + timothy, Rc+Ti = red clover + timothy and Rc+Mf = red clover + meadow fescue. Mixture x treatment: α-tocopherol (P = 0.015, SEM = 4.44, $LSD_{0.05}$ = 12.96) and β-carotene (NS, SEM = 3.92). Different superscripts in the same row (a, b, c) or column (x, y, z) differ according to $LSD_{0.05}$ test.

concentrations of the forage mixtures. Both the untreated and treated silages were of good quality and there were no losses of the vitamins during ensiling of the untreated forages. Homofermentative inoculants seem to be appropriate additives to use when the purpose is to preserve the vitamin content of forage. The increased α-tocopherol concentration in the Bft+Ti forage regardless of silage treatment, and the effects of inoculants on α-tocopherol concentration in silage need further investigations.

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