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ORIGINAL PAPER

EFFECT OF A HARVEST DATE AND CUTTING HEIGHT ON THE CONCENTRATIONS OF CAROTENOIDS AND TOCOPHEROLS IN VIRGINIA FANPETALS (*SIDA HERMAPHRODITA*) HERBAGE AND SILAGE*

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Abstract

The aim of this study was to determine the effect of a harvest date and cutting height on the concentrations of carotenoids and tocopherols in Virginia fanpetals (*Sida hermaphrodita* Rusby L.) herbage and silage. First-cut Virginia fanpetals herbage was harvested in the budding stage, on 11, 18 and 25 June 2017. Herbage was cut at 15, 25, 35 and 45 cm above the ground, after which it was chopped and ensiled in vacuum bags. The content of β-carotene and tocopherols (α-, β-, γ-, δ-) was determined in samples of fresh herbage and silage. Herbage cut at a height of 45 cm and silage made from herbage cut at a height of 25 cm had a higher ($P \leq 0,01$) content of β-carotene (113.93 and 44.90 mg kg^{-1} DM, respectively), α-tocopherol (87.20 and 3.50 mg kg^{-1} DM, respectively) and total tocopherols, and a higher value of vitamin E equivalent (Vit. EEq) than the remaining samples. Herbage harvested on the last date (25 June) and silage made from herbage harvested on the first date (11 June) were characterized by higher concentrations of β-carotene (88.30 and 38.27 mg kg^{-1} DM, respectively) and α-tocopherol (70.91 and 2.82 mg kg^{-1} DM, respectively) compared with samples harvested at the remaining dates, but the noted differences were not statistically significant. Positive interactions were found between cutting height (45 cm) vs. β-carotene content and Vit. EEq value in herbage, and cutting height (25 cm) vs. α-tocopherol content and Vit. EEq value in silage. The content of β-carotene and total tocopherols (α-, β-, γ-, δ) and a decrease in their concentrations during ensiling (in silage, average loss of β-carotene and α-tocopherol reached 56% and 96%, respectively) were comparable with the values reported for grasses and legumes. The results of this study indicate that *Sida* herbage and silage can be a valuable source of livestock feed.

Keywords: Virginia fanpetals, herbage, silage, β-carotene, tocopherols.

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INTRODUCTION

Technologies of herbage harvest and preservation affect the quality and chemical composition of forage. In most countries, where biomass is harvested seasonally and preserved as silage, the nutritional value of feed and the content of biologically active compounds, including carotenoids and tocopherols, are regularly monitored and analyzed in view of local production conditions. The results of some studies suggest that provitamins and lipophilic vitamins of natural origin (herbage, silage) are better utilized in metabolic processes in the animal's body, compared with their synthetic counterparts (Yang et al. 2002, Röhrle et al. 2011*a*,*b*, Zhong, Zhou 2013). Fresh and preserved green fodder (hay, silage) represents a cheap source of feed for ruminants. Virginia fanpetals (*Sida hermaphrodita* R.), one of the 13 species of the family *Malvacea*e (Dinda et al. 2015), can produce 5-20 t biomass per ha on average, depending on cultivation conditions, harvest date and technology (Borkowska, Molas 2012, Nabel et al. 2017). This perennial plant is successfully grown under agroclimatic conditions in Europe (Franzaring et al. 2015), mainly for energy biomass (Borkowska, Molas 2012). Unlike other energy crops, *Sida* biomass has high protein content, reaching 17-25% DM in the budding stage. Virginia fanpetals is also a honey crop and a rich source of chemical substances for pharmacological use, including alkaloids, flavonoids, phytoestrogens, carotene and tocopherols (Borkowska. Molas 2012, Dinda et al. 2015). *Sida* biomass harvested before the flowering stage has similar chemical composition and a more desirable ratio between water-soluble carbohydrate and buffer capacity than alfalfa herbage in the budding stage (Fijałkowska et al. 2017). Tocopherols and carotenoids are active plant antioxidants supplied by green fodder, which act as biomarkers of a nutritional status in livestock (Nozière et al. 2006). β-carotene is the most important provitamin A carotenoid. In the group of eight natural tocochromanols (α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols), α-tocopherol is the most active form of vitamin E. α-tocopherol predominates in leaves, whereas generative plant parts are abundant sources of α- and γ-tocopherols. The content of α-tocopherol and β-carotene in ruminant diets is determined by a plant species, harvest date, maturity stage (leaf/stem ratio or the ratio between vegetative and generative shoots), degree of wilting and preservation method (Prache et al. 2003, Röhrle et al. 2011*b*). Previous research revealed rapid changes in the chemical composition of Virginia fanpetals during the growing season, with the predominance of cellulose and lignin in the dry weight from the flowering stage (Borkowska, Molas 2012). According to Nozière et al. (2006), lipids present in green forage, including carotenoids, are positively correlated (0.60) with ether extract content and negatively correlated with the content of structural carbohydrates, which affects the concentrations of provitamins and lipophilic vitamins in forage produced from herbage harvested at successive dates. The research hypothesis postulated that Virginia fanpetals (*Sida hermaphorodita* Rusby L.) herbage and silage could be a rich source of carotenoids and tocopherols in ruminant nutrition. The aim of this study was to determine the effect of technological factors on the vitamin content of Virginia fanpetals herbage and silage.

MATERIALS AND METHODS

The experimental material was first-cut Virginia fanpetals herbage harvested in the budding stage, on 11, 18 and 25 June 2017. Herbage was cut at 15, 25, 35 and 45 cm above the ground. Herbage samples from each cutting height were collected in three replicates, at each date from adjacent places, and then chopped with an electric chopper to a theoretical chaff length of 10 mm. Each batch of the plant material (600 g) was ensiled in vacuum bags in triplicate. Fresh herbage samples and silage samples collected after 90 days of ensiling were subjected to chemical analyses. The content of β-carotene (Rodriguez-Bernaldo De Quirós et Costa 2006) and tocopherols α-, β-, γ-, δ- (PN-EN ISO 6867: 2002) was determined in samples of fresh herbage and silage. Hexane extracts obtained under limited access to light were analyzed by high-performance liquid chromatography using the Shimadzu HPLC system, RP, column: Nukleosil C_{18} 250x4.6 mm, 5 µm; β-carotene determination: mobile phase: acetonitrile-dichloromethane (75:25, v/v), flow rate: 1 cm^3 min^{-1}, 20 µl loop, UV-vis detector - 450 nm, external standard: β-carotene type I, synthetic, Sigma-Aldrich; tocopherol determination: mobile phase: methanol: H_2O (95:5 v/v), flow-rate 1 cm^3 min^{-1}, loop 20 µl, RF-20A fluorescence detector, E_x 293 nm and E_m326 nm, external standards: (±)-α-tocopherol (DL-all-rac α-tocopherol), β-tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol, Sigma-Aldrich). The value of vitamin E equivalent (Vit. EEq) was calculated as described by Eittenmiller et al. (1998). The results were analyzed statistically with the use of Statistica ver.13.0 software. The significance of differences between means was verified by the Duncan's test.

RESULTS

Herbage cut at a height of 45 cm and silage made from herbage cut at a height of 25 cm had a higher content of β-carotene, α-tocopherol ($P \leq 0.01$) and total tocopherols ($P \leq 0.01$). Herbage cut at a height of 45 cm had higher β-tocopherol content ($P \leq 0.01$), and silage made from herbage cut at a height of 25 cm had a higher content of the remaining tocopherols ($P \leq 0.01$) – Tables 1, 2. Herbage cut at a height of 45 cm and silage made from herbage cut at a height of 25 cm were characterized by higher values of Vit. EEq ($P \leq 0.01$), which points to the predominance of the most biologically active tocopherol (α-tocopherol) – Tables 1, 2. The results of this study (P-value)

Table 1

Effect of the cutting height and harvest date on the concentrations of β-carotene, α-, β-, γ-, δ-tocopherol (mg kg^{-1} DM) and total tocopherols (mg kg^{-1} DM) and the value of vitamin E equivalent (Vit. EEq, mg kg^{-1} DM) in Virginia fanpetals herbage

Item	β-carotene	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols	Vit. EEq
Cutting height (C)							
15 cm	43.19^{C}	77.50^{B}	11.60^{B}	2.00^{D}	5.20^{B}	96.30^{B}	82.40^{B}
25 cm	69.30^{B}	43.60^{D}	8.60^{C}	19.70^{A}	6.30^{A}	77.60^{C}	48.80^{D}
35 cm	116.40^{A}	72.83^{C}	11.90^{B}	4.30^{B}	6.10^{A}	95.40^{B}	78.60^{C}
45 cm	113.93^{A}	87.20^{A}	15.60^{A}	2.50^{C}	5.10^{B}	110.73^{A}	93.70^{A}
Harvest date (T)							
11 June	82.19	69.53	11.76	7.13	5.13^{B}	94.13	75.32
18 June	86.62	70.40	12.13	6.83	5.61^{B}	94.83	75.61
25 June	88.30	70.91	11.42	7.43	6.29^{AA}	96.07	76.70
Effect **P-value**							
Cutting height (C)	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01
Harvest date (T)	0.893	0.980	0.831	0.982	$\leq$ 0.01	0.926	0.979
Interaction (C×T)	$\leq$ 0.01	0.081	0.020	0.023	0.001	0.529	0.019
SEM	5.276	2.751	0.460	1.237	0.133	1.993	2.808

Values in columns: *A, B, C* – $P \leq 0.01$, *a, b, c* – $P \leq 0.05$

revealed that the concentrations of β-carotene and tocopherols in *Sida* herbage and silage were affected by the cutting height ($P \leq 0.01$) – Tables 1, 2. The average content of β-carotene (85.71 mg kg^{-1} DM), α-tocopherol (70.28 mg kg^{-1} DM) and total tocopherols (95.01 mg kg^{-1} DM), and the value of Vit. EEq (75.82 mg kg^{-1} DM) were higher in *Sida* herbage than in silage by 56, 96, 80 and 95%, respectively (Tables 1, 2). β-carotene loss was over two-fold greater in silage made from herbage cut at 35 and 45 cm above the ground (69%) than in silage produced from herbage cut at a height of 15 and 25 cm (26%). α-tocopherol loss was similar in all silages regardless of the cutting height of *Sida* herbage. Harvest date had no effect on the concentrations of the analyzed compounds in *Sida* herbage and silage. The only exception was δ-tocopherol ($P \leq 0.05$, $P \leq 0.01$) whose content increased at successive harvest dates (11, 18, 25 June), which was confirmed by analysis of variance ($P \leq 0.01$) – Tables 1, 2. A positive interaction between the cutting height and harvest date was noted for the concentrations of β-carotene and δ-tocopherol ($P \leq 0.01$), and β-tocopherol, γ-tocopherol and Vit. EEq ($P \leq 0.05$) in *Sida* herbage as well as for the content of α-tocopherol ($P \leq 0.01$), γ- tocopherol and total tocopherols ($P \leq 0.05$) in *Sida* silage.

Table 2

Effect of the cutting height and harvest date on the concentrations of β-carotene, α-, β-, γ-, δ-tocopherol (mg kg^{-1} DM) and total tocopherols (mg kg^{-1} DM) and the value of vitamin E equivalent (Vit. EEq, mg kg^{-1} DM) in Virginia fanpetals silage

Item	β-carotene	α-tocopherol	β- tocopherol	γ- tocopherol	δ- tocopherol	Total tocopherols	Vit. EEq
Cutting height (C)							
15 cm	35.81^{C}	3.10^{AB}	0.40^{B}	12.50^{B}	5.20^{B}	21.20^{B}	4.60^{B}
25 cm	44.90^{A}	3.50^{AA}	0.48^{A}	15.50^{A}	6.00^{A}	25.51^{A}	5.30^{A}
35 cm	27.60^{D}	1.50^{C}	0.32^{C}	8.20^{D}	2.80^{D}	12.80^{D}	2.50^{D}
45 cm	42.60^{B}	2.10^{B}	0.31^{C}	10.70^{C}	4.20^{C}	17.30^{C}	3.30^{C}
Harvest date (T)							
11 June	38.27	2.82	0.40	11.33	4.64	18.69	4.25
18 June	37.19	2.48	0.35	11.77	4.31	19.04	3.45
25 June	37.73	2.35	0.78	12.07	4.70	19.89	4.08
Effect **P-value**							
Cutting height (C)	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01	$\leq$ 0.01
Harvest date (T)	0.932	0.408	0.230	0.808	0.725	0.830	0.224
Interaction (C×T)	0.801	0.001	0.995	0.054	0.098	0.023	$\leq$ 0.01
SEM	1.144	0.143	0.013	0.455	0.207	0.802	0.198

Values in columns: A, B, C – $P \leq 0.01$, a, b, c – $P \leq 0.05$

DISCUSSION

There is general scarcity of published research into the vitamin content of *Sida* herbage and silage. According to Nozière et al. (2006), Dunne et al. (2009) and Larsen et al. (2012), grass and legume herbage contains 33-700 mg kg^{-1} DM of β-carotene and 6-169 mg kg^{-1} DM of α-tocopherol, and their loss during ensilage can reach 20-80%. In a review article, Müller et al. (2007) summarized the results of many studies and concluded that the β-carotene content of grass fodder ranges from 127 to 529 mg kg^{-1} DM. In the current study, the β-carotene content of *Sida* herbage (85.71 mg kg^{-1} DM on average) remained within the range of 33-700 mg kg^{-1} DM reported by Dunne et al. (2009) for grass and legume forage. Herbage cut at a height of 35 and 45 cm had higher ($P \leq 0.01$) β-carotene content (116.40 and 113.93 mg kg^{-1} DM, respectively) which could be due to a higher share of leaves rich in carotene and tocopherols than stems in the analyzed samples (Table 1). According to Reynoso et al. (2004) and Nozière et al. (2006), leaves contain 5 to 10 times more carotenoids than stems, depending on plant species. In the evaluated *Sida* silage (Table 2), β-carotene content was approximately 56% lower than in herbage (Table 1). Our results corroborate the findings of Chauveau-Duriot et al. (2005), who reported that β-carotene was 52, 60 and 69% lower in grass silage (perennial ryegrass, orchard grass) and red clover silage than in herbage. Carotenoid loss during ensilage varies widely from 10 to 90%. According to Dunne et al. (2009), β-carotene content can be over 10-fold lower in legume silage than in herbage (33-700 mg kg^{-1} DM vs. 11-44 mg kg^{-1} DM). α-tocopherol predominates in the green parts of plants (leaves), whereas photosynthetic tissues contain 10 to 50 μg g^{-1} of α-tocopherol – vitamin E (Dellapenna 2005). γ-tocopherol predominates in stems, roots, fruit, seeds, tubers, cotyledons, germinating seedlings and flowers (Horvath et al. 2006). The α-tocopherol content (70.28 mg kg^{-1} DM on average) of *Sida* herbage (Table 1) remained within the range of 13 - 275 mg kg^{-1} DM reported for grasses and legumes by Schingoethe et al. (1978), Lynch et al. (2001), Müller et al. (2007) and Röhrle et al. (2011a). In the present experiment (Table 1), α-tocopherol loss during the ensiling of *Sida* biomass was somewhat higher (96%) than that determined in grass and legume silage (83%) by Schingoethe et al. (1978).

The content of β-carotene and α-tocopherol in *Sida* herbage increased at successive harvest dates (11, 18 and 25 June) – Table 1, although the noted differences were not statistically significant. Our results are consistent with the findings of Nozière et al. (2006) who demonstrated that the concentrations of β-carotene and total tocopherols increased during the growth and development of plants, and decreased towards the end of the growing season. An analysis of Sida herbage harvested during the 15-day experimental period did not contribute to described changes in the carotene and tocopherol content. However, the results of our study revealed an increase in δ-tocopherol content ($P \leq 0.05$, $P \leq 0.01$) and a statistically non-significant increase in the concentrations of total tocopherols and the value of Vit. EEq at successive harvest dates (11, 18, 25 June) – Table 1. In silage made after successive harvests, a statistically non-significant decrease in the content of β-carotene and α-tocopherol was accompanied by a statistically non-significant increase in the levels of γ-tocopherol and total tocopherols (Table 2). According to Lynch et al. (2001) and Dunne et al. (2009), silage produced after successive cuts of grasses and legumes was characterized by decreasing concentrations of β-carotene and α-tocopherol. Fredriksson Eriksson, Pickova (2007) demonstrated that the ensiling process reduced the content of total tocopherols, mostly α-tocopherol, in the ensiled material.

CONCLUSIONS

Virginia fanpetals herbage cut at a height of 35 and 45 cm had higher concentrations of β-carotene and total tocopherols ($P \leq 0.01$). Herbage harvested at successive dates (from 11 to 25 June) and the corresponding silage did not differ in their content of β-carotene and tocopherols with the highest biological activity. An analysis of β-carotene and δ- tocopherol concentrations, and the value of Vit. EEq in *Sida* herbage as well as α-tocopherol content and the value of Vit. EEq in silage revealed a positive interaction between the cutting height and harvest date. The β-carotene and tocopherol content of *Sida* herbage and silage was comparable with their concentrations in fresh and ensiled roughage fed to livestock.

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