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Effects of different wilting regimes on fatty acid profile of fresh forage

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Introduction

Long chain n-3 polyunsaturated fatty acids (LCFA) have been shown to have beneficial effect on human health (Simopoulos, 2000). A higher proportion of these fatty acids have been found in products from grazing animals than in products from animals fed a diet with conserved forage or high proportion of concentrates (Elgersma et al., 2004a; Fredriksson and Pickova, 2007). The majority of the forage used in beef and milk production is consumed as preserved forage, mainly as silage, due to a limited grazing season. During the ensiling process, it is mainly the wilting procedure that has been shown to have considerable impact on composition and content of LCFA in the forage. In an experiment by Dewhurst and King (1998), extended wilting reduced the content of total LCFA in a crop by 30%, with a reduction of up to 40% of C18:3. The negative effect of wilting on the LCFA profile in the crop was confirmed by Van Ranst et al. (2009). It is assumed that losses of LCFA during the wilting are associated with the activity of lipases, widely present in living plants, during processes of lipolysis (Elgersma et al., 2003). A shortening of the wilting process can thus result in reduction of LCFA losses. Therefore, the aim of the study was to investigate the effect of different wilting regimes on changes in LCFA composition in forage crops.

Materials and Methods

Red clover (*Trifolium pretense)* at early flowering maturity and timothy (*Phleum pretense)* at fully developed heads were used in the study. Forages were harvested manually using a scythe on June 6 2008, nearby Uppsala. Samples were collected in triplicate from each crop and immediately frozen at -18 °C until analysis. The fresh crops were wilted widespread outdoors to four different dry matter (DM) contents. Wilting time varied from 0 and to 48 h, according to the design described in Table 1. The treatments with DM content of 20% were not wilted in the sun but instead placed in the shade and sprayed with water during day time every third hour to simulate unfavorable weather conditions. This combination of wilting times and wilting to different DM contents enabled separation of effect of wilting time and effect of DM content on LCFA content and composition.

Table 1 Experimental design of the study

DM contents	Time of wilting (h)			
(%)	0	12	24	48
20	x	x	x	x
40		x	x	x
60			x	x
80				x

The concentration of DM was analyzed in two steps. First, fresh samples weighing approximately 150 g were dried for 18 h in a ventilated oven at 65°C and milled to pass a 1.0-mm sieve. Final DM concentration was achieved by drying at 103°C for 5 h. Lipids were extracted using the method described by Lourenco et al. (2007). The methylation of samples was performed according to the method described by Appelqvist (1968). Approximately 2 mg of lipids in 0.5 mL of hexane were mixed with 2 mL of 0.01 M NaOH in dry methanol. Samples were incubated at 60°C for 10 min and then 3 mL of BF_3 was added and samples were incubated at 60°C for another 10 min. Afterwards 2 mL of 20% NaCl and 2 mL of hexane were added and lipid phase was separated. The methylated fatty acids in this phase were then chromatographically separated on a BPX-70 fused-silica capillary column (50 m x 0.22 mm x 0.25 μm) in a CP-3800 gas chromatograph.

Results and Discussion

A higher initial DM content and better wilting rate of the timothy crop resulted in higher DM contents during shorter wilting times than planned (Table 2). The opposite was true for the red clover crop, where a lower initial DM content and less good wilting ability of the crop resulted in that planned DM contents were not reached within planned wilting times.

Table 2 Fatty acid (FA) composition (g/100g FAME (fatty acid methyl esters)), FA content (mg/g DM), and crude fat content (g/kg DM) in timothy forage wilted to four DM content levels (%) in four wilting periods (h)

DM contents	Wilting time	C16:0	C18:0	C18:1	C18:2	C18:3	Total FA	Crude fat
32	0	18.2	1.8	3.5	20.1	51.7	17.0	25.8
32	5	18.3	1.9	3.3	19.6	52.1	14.3	26.0
32	21	18.1	1.8	3.4	19.6	52.0	15.3	29.7
32	45	18.4	1.9	3.4	20.6	50.4	12.7	28.1
50	5	17.8	1.8	3.8	20.8	51.1	15.6	26.4
50	21	18.5	1.8	3.3	20.5	51.0	15.5	27.3
50	45	18.2	1.9	2.8	20.0	51.6	11.3	27.2
62	21	16.6	1.9	2.7	19.1	55.5	18.1	34.7
62	45	17.6	1.8	2.8	21.0	51.9	11.5	26.9
77	45	16.2	1.7	2.6	19.7	55.2	15.1	28.5
$LSD_{0.05}$		0.81	0.12	0.68	1.56	2.76	4.93	2.29
Probability	DM	***	*	*	NS	***	NS	***
	Time	NS	NS	NS	NS	NS	**	***
	Int.	NS	NS	NS	NS	NS	NS	***

*, ** and *** at P<0.05, P<0.01 and P<0.001, respectively; NS – not significant; DM – dry matter.

A faster wilting process was probably the reason for limited changes in LCFA composition in timothy forage (Table 2). The increase in forage DM content from 32% to 50% within five wilting hours was not accompanied by any changes in LCFA profile. However, at a DM content of 62% the content of C16:0 and C18:1 was lower in comparison with initial contents.

When timothy had reached 77% DM content, the proportion of C18:0 was reduced (P<0.05). On the other hand, the proportion of C18:3 increased as the crop reached higher DM contents (P<0.001). This result contrasts previous observations (Dewhurst and King, 1998; Van Ranst et al., 2009), where wilting reduced proportion of C18:3 in forage. A possible explanation can be a higher initial DM content of timothy in this study which limited the activity of lipases. In addition, the crop was not mechanically treated, which could contribute to reduce the activity of lipases (Elgersma et al., 2003). There were no differences in FA content in timothy among wilting times within 32 and within 50% DM content, which indicates limited degradation of LCFA due to longer wilting times. A lower initial DM content, and with that an associated higher activity of plant lipases, were probably a reason for large changes in LCFA composition in red clover during wilting compared to timothy (Table 3). Unexpectedly, there were no variation in total FA content in the red clover, but proportions of C18:3 were influenced by an interaction of DM content and wilting time (P<0.01). A reduced proportion of C18:3 was observed due to extended wilting (51 h) or increasing DM content up to 46%. The proportion of C18:3 in the red clover crop containing 62 % DM was however similar to the initial proportion. In the crop containing 16 and 30% DM content, the proportion of C18:1 decreased (P<0.001) during the entire wilting time. Proportion of C18:1 in the red clover crop containing 46 and 62% DM was lower (P<0.03) than the initial proportion. In contrast to timothy, the trend of increased proportions of C16:0 and C18:0 with increasing DM content was observed in the red clover crop, except for C16:0 at 62% DM content. Moreover, extended wilting time (51 h) increased the proportions of C16:0 (P<0.05) and C18:0 (P<0.001), compared to initial proportions. On the other hand, these differences were numerically small, therefore it is uncertain to which extent can these differences can influence the FA composition in animal products.

Table 3 Fatty acid (FA) composition (g/100g FAME (fatty acid methyl esters)), FA content (mg/g DM), and crude fat content (g/kg DM) in red clover forage wilted to four DM content levels (%) in four wilting periods (h)

DM contents	Wilting time	C16:0	C18:0	C18:1	C18:2	C18:3	Total FA	Crude fat
16	0	18.3	2.6	2.1	15.4	57.4	17.9	44.1
16	9	18.4	2.5	1.8	15.2	57.7	20.1	40.7
16	29	18.0	2.5	1.5	14.7	58.9	20.7	43.4
16	51	19.1	2.7	1.6	15.2	56.4	15.9	41.0
30	9	19.3	2.8	2.0	16.3	55.0	21.1	39.8
30	29	18.9	2.7	1.6	15.7	56.4	18.6	41.5
30	51	19.4	2.7	1.8	16.5	54.7	18.1	39.5
46	29	19.4	2.8	1.7	16.3	55.2	17.6	41.0
46	51	19.2	2.7	1.7	16.0	55.8	17.6	41.0
62	51	18.7	2.7	1.6	15.5	56.7	17.0	41.5
$LSD_{0.05}$		0.67	0.12	0.16	0.87	1.42	5.20	3.79
Probability	DM	***	***	***	*	***	NS	NS
	Time	NS	NS	**	NS	*	NS	NS
	Int.	*	***	NS	NS	**	NS	NS

*, ** and *** at P<0.05, P<0.01 and P<0.001, respectively; NS – not significant; DM – dry matter.

Conclusions

Effect of wilting time and DM content influenced the LCFA composition differently in timothy and red clover crops. In timothy forage, extended wilting reduced FA content and a higher DM content caused increase in C18:3 proportion. In red clover, extended wilting reduced the proportion of C18:3 up till 30% DM content. No effect of wilting time or DM content on FA content in the red clover crop was observed.

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