Influence of clover species in mixtures with grasses on fatty acid composition of mixtures

Steinshamn H.[1], Bleken M. A.[2], Langeland Å.[2] and Thuen E.[2]

[1]*Bioforsk-Norwegian Institute for Agricultural Environmental Research, Organic Food and Farming Division, N-6630 Tingvoll, Norway.*
[2]*Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway.*

Abstract

White clover (WC, *Trifolium repens* L.) or red clover (RC, *Trifolium pratense* L.) was grown in mixture with grasses. Samples of the mixtures and pure clovers were taken during three cuts, and the fatty acids (FA) compositions were determined. The clover species had no significant effect on the individual FA contents of the leys (g kg^{-1} dry matter (DM)), nor on the total FA content. Pure clovers had lower content of all individual FA (8.2 vs. 12.4 g kg^{-1} DM) than mixtures; the contents of all FA decreased with increasing percentage of clover in the mixture, but the decrease was weaker for C18:3n-3 than for the other FA. Although pure WC and RC had similar FA contents, the relative proportions (percentage of total FA content) of C16:0 and C18:3n-3 differed; RC had a higher proportion of C18:3n-3 and a lower proportion of C16:0. These results are partly in contradiction with previously reported.

Keywords: fatty acids, temperate clovers, grass-legume mixtures.

Introduction

Recent studies indicate that milk fat from cows fed clover-based silages has higher levels of beneficial n-3 FA than milk from cows fed pure grass silages (Dewhurst *et al.,* 2003). This could partly be ascribed to a higher content of C18:3n-3 in clover than in grass (Dewhurst *et al.,* 2001). As the C18:3 concentration of milk fat is influenced by the C18:3n-3 concentration of forages (Hebeisen *et al.,* 1993), it is interesting to examine agronomic practices that can affect the FA composition of forages. Few studies have compared clover species with respect to FA content. Boufaïed *et al.* (2003) found higher content of total FA (TFA) and C18:3n-3 in white clover (*Trifolium repens* L.) than in red clover (*Trifolium pratense* L.), and higher content of TFA but lower content of C18:3n-3 in legumes than in grasses. However, the study of Boufaïed *et al.* (2003) was carried out in pure stands of legumes and grasses. The objective of the present study was to investigate how the clover species affect the FA content of the herbage when grown in a mixture with grasses.

Materials and methods

Red clover (RC) or white clover (WC) was grown in mixture with timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.) and perennial ryegrass (*Lolium perenne* L.) in three replicates (field size ± 2.3 ha) at the University of Life Sciences, Norway (59°N,10°E) and cut for silage three times in 2004. The leys were established in 2003. For each of three plots, distributed evenly on a line diagonally through each field (replica), a sample was taken by harvesting 0.5 m^2 at a stubble height of 6 cm. The harvested material was split into two sub samples, one for the botanical analysis and one for the FA analysis (Mix). In addition, pure clover samples (Pure) were also taken by harvesting the mixture adjacent to each harvested plot at a stubble height of 6 cm and immediately hand separating the clovers from the other species in field. The samples for the FA analysis were kept in airtight plastic bags, frozen within an hour after harvest, stored at -20°C, and subsequently freeze-dried and prepared for gas chromatography of FA methyl esters according to Sukhija and Palmquist (1988), with slight modifications. The results were analysed by the analysis of variance between treatment differences using the mixed procedure of SAS (SAS, 1998).

Results and discussion

In this paper, results are presented as averages over three harvest dates (cuts). There was a significant effect of the harvest date on FA contents and composition. However, the effect of clover species was consistent across harvest dates.

Table 1. Fatty acid (FA) content (g kg^{-1} DM) and relative proportion (% of total FA) in grassland herbage containing either white clover (WC) or red clover (RC) in mixture with grasses (Mix) and in pure clover (Pure) hand-separated from the same stand; averaged over three cuts and replicates (n=9).

Fatty acid	WC		RC		s.e.d.	Significance		
	Mix	Pure	Mix	Pure		Species	Mix vs. Pure	Interaction
FA content (g kg^{-1} DM)								
Total	11.7	8.03	13.2	8.34	0.92	NS	***	NS
C16:0	2.28	1.80	2.29	1.57	0.17	NS	***	NS
C18:1	0.21	0.14	0.20	0.10	0.03	NS	**	NS
C18:2n-6	1.77	1.46	1.96	1.69	0.16	NS	*	NS
C18:3n-3	5.62	4.05	6.40	4.55	0.51	NS	**	NS
% of total FA								
C16:0	20.6	24.1	18.2	18.9	0.54	***	**	**
C18:1	1.9	1.5	1.5	0.9	0.22	*	*	NS
C18:2n-6	16.0	20.2	16.0	20.8	0.80	NS	***	NS
C18:3n-3	49.0	48.9	51.2	55.7	1.37	*	*	*

s.e.d., standard error of the difference for interaction effect.
Significant differences between means at * P < 0.05; ** P < 0.01; *** P < 0.001; NS, not significant.

There were no significant differences in the dry matter (DM) yield between the two leys. The percentage of clover in the mixture was significantly ($P < 0.001$) higher in the RC ley (53%) than in the WC ley (35%). There were no significant differences between the clover species in the content of total and individual FA (Table 1). These results are in contrast to Boufaïed *et al.* (2003), who found higher content of TFA in WC than in RC. However, Boufaïed *et al.* (2003) samples' were taken from the spring growth of pure stands harvested at blooming that were 3 weeks later for RC than for WC, while our samples were taken from mixed stands harvested at the same date. Pure clovers had lower content of total (8.2 g kg^{-1} DM) and individual FAs ($P < 0.001$) than the mixtures (12.4 g kg^{-1} DM) (Table 1). This indicates that the FA concentrations were higher in the grasses than in the legumes. A linear regression between percentage of clover in the mixture and the content of FA in the mixture supported this result, as the content of total and individual FAs decreased with increasing clover content in the mixture (TFA = 23.8 – 0.20 * C, $R^2 = 0.56$, $P < 0.001$ for RC and TFA = 17.3 – 0.16 * C, $R^2 = 0.18$, $P < 0.05$ for WC, where C is the clover percentage of the DM yield). This is also in contrast with the results of Boufaïed *et al*. (2003), who found a higher content of most FA, except C18:3n-3, in legumes than in grasses.
C18:3n-3, C18:2n-6 and C16:0 were the most abundant FA, averaging 51, 18 and 20% of TFA, respectively. These results are consistent with that reported previously for forage plants (e.g. Boufaïed *et al.,* 2003). Although clover species did not affect the herbage FA content, the FA proportion expressed as percentage of TFA was affected by clover species. The proportion of C16:0 was higher ($P < 0.001$) in WC than in RC (24.1 and 18.9 %, respectively), while that of C18:3n-3 was higher ($P < 0.05$) in RC (55.7 and 48.9 %, respectively) (Table 1).

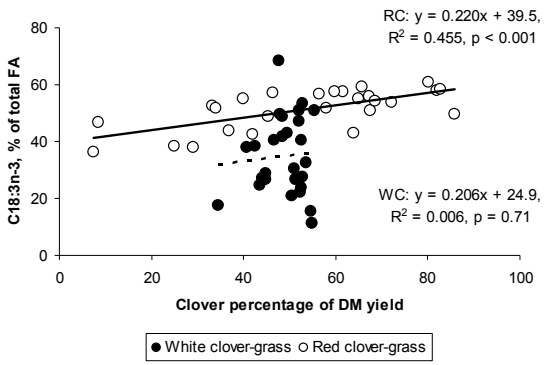

Figure 1. The relationship between clover percentage of DM yield and the proportion of C18:3n-3 (% of total FA) in white clover-grass herbage (WC) and red clover-grass (RC) herbage; samples from 3 cuts, 3 replicates and 3 plots within replicate.

The proportion of C16:0 was higher in pure clovers than in mixtures, particularly for WC (Table 1). Pure RC had a higher proportion of C18:3n-3 than the mixture, what was not found in WC (Table 1; indicated also by the significant interaction between species and mixtures vs. pure). The latter result was also reflected in a positive relationship between the clover percentage in the mixture and the proportion of C18:3n-3 found in RC mixture but not in WC mixture (Figure 1). Therefore, even though the content of all FA decreased with increasing clover percentage in the mixture, the reduction was less pronounced for C18:3n-3 in RC than for the other FA.

Conclusions

Clover species did not affect herbage FA content (g FA kg^{-1} DM). However, the proportion of C18:3n-3 was higher and that of C16:0 lower in RC than in WC leys. The content of FA decreased generally with increasing clover content in the mixture, but the proportion of C18:3n-3 increased with increasing clover proportion in the RC mixture. More research is needed to clarify the cause of discrepant results between studies.

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