Effect of species, cultivar and phenological stage of different forage legumes on herbage fatty acid composition

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ABSTRACT - An experiment was conducted to evaluate the effects of species, cultivar, and phenological stage, on the concentration of fatty acid composition in different forages legumes. Four species and eight cultivars *Vicia sativa* L. (VS cv. Jose JO, and Nikian NI), *Vicia Villosa* Roth (VV cv.Haiymaker HA, and Hungivillosa HU) *Trifolium incarnatum* L. (TI cv. Viterbo VI, and Contea CO), and *Trifolium alexandrinum* L. (TAX cv Marmilla MA and Sacromonte SA) were compared. Overall the main factors which influence fatty acids (FA) profile appear to be forage species and phenological stage but we need to consider the numerous interaction with these factors; besides the second important FA (C16:0) did not change between different phenological stages whereas linoleic acid increases (about 50% $P<0.01$) and linolenic acid decreases (about 10% $P<0.01$) from vegetative to reproductive stage. We observe also a worsening effect ($P<0.05$) on unsaturated/saturated (UNSAT/SAT) ratio from vegetative to reproductive stage. In conclusion these studies demonstrate a significant genetic component to the level and pattern of fatty acid concentration as well as a key role of the association between phenological stage and cultivars which modulated the amplitude and the trend of fatty acid pattern.

Key words: Fatty acid, PUFA, Forage species phenological stage.

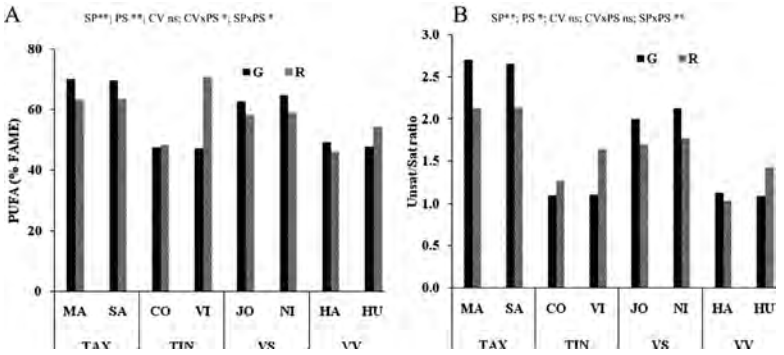
Introduction – Managing the fatty acid composition of grazing ruminant could lead to increase the levels of ω-3 fatty acid and conjugated linoleic acid (CLA) in meat and dairy products. Since the dynamic of forage fatty acid pattern is very poorly studied the results from grazing animals are frequently misunderstand because a lot of variable which influence fatty acid composition of herbage are unknown. For these reasons we need to improve the knowledge about the influence of different forage species, cultivars and phenological stages on forage fatty acid composition, in order to increase the transfer ratio efficiency of these FA from forage to ruminant products. In general the lipid content in forage plants is about 3-5 %, but its variation is considerable. Ruminant products are the only significant source of conjugated linoleic acid (CLA) and they are an alternative as a source of ω-3 fatty acid, which may be beneficial components in the human diet. Fresh forage are a reach source of linoleic and linolenic acids which are the precursors of ω-3 fatty acid in milk and meat. Although forages usually contains a high proportion of ether extract (EE) as linolenic and linoleic acid and this high proportion can have substantial effects on the fatty acid profiles of dairy products. Some authors showed that fatty acid profiles were distinctive to species confirming a strong genetic basis, whereas in others studies within perennial ryegrass varieties no evidence was found about the effect of ploidy in relation to level and pattern of fatty acids. Elgersma *et al.*, 2003 showed in some case that, cultivar can affect the fatty acid composition in perennial ryegrass (*Lolium perenne*) in particular for linoleic and linolenic acid likewise. Boufaied *et al.,* 2003 found a very strong effect of cultivar on linoleic and linolenic acid in both grasses and legumes forage. Since legumes are interesting for their role in sustainable

feeding systems, when the level of these forage species in pasture is linked to the higher concentration of CLA and ω-3 fatty acid in milk (Cabiddu *et al.*, 2003). The objective of the work is to determine how the FA composition within legumes species change during different phenological stages for to increase delivery of fatty acids from plants to ruminant products.

Material and methods – The esperiment was carried out at Bonassai farm (NW Sardinia, Italy) on a flat calcareous soil. Four species and eight cultivars *Vicia sativa* L. (VS cv. Jose JO, and Nikian NI), *Vicia Villosa* Roth (VV cv. Haiymaker HA, and Hungivillosa HU) *Trifolium incarnatum* L. (TI cv. Viterbo VI, and Contea CO), and *Trifolium alexandrinum* L. (TAX cv Marmilla MA and Sacromonte SA) were compared in a randomized experimental design. Samples of herbage were taken at vegetative and reproductive stage and stored at -20°C prior to chemical analysis. These samples were freeze dried and their fatty acid composition analysed. Lipid extraction was carried out as described by Cabiddu *et al.*, (2009), using a mixture of solvents (chloroform:methanol (2/1; v/v)) and lipid were converted to methyl esters. FA were determined according to the method reported by Cabiddu *et al.*, 2009. The results were submitted to GLM analysis to test the effect of forage species (SP) cultivar within species (CV) phenological stage (PS) and their interaction

Results and conclusions – All fatty acid profile (expressed by fatty acid methyl esters, FAME), was influenced by forage species, and even if at lesser extent by cultivars (within species) and phenological stage. Apparently the main factors which influence FA profile appear to be forage species and phenological stage but we need to consider the numerous interaction between these factors (Table 1). Another interesting result is that the second important FA (C16:0) did not change between different phenological stages whereas linoleic acid increases (about 50% P<0.01) and linolenic acid decreases (about 10% P<0.01) passing from vegetative to reproductive stage. Overall we observe also a worsening effect (P<0.05) on unsat/sat ratio from vegetative to reproductive stage (Figure 1). Likewise Boufaied *et al.*, 2003 the main representative FA are linolenic acid, palmitic acid and linoleic acid. About the content of linolenic acid, the Beersem clover Egyptian clover (T alexandrinum) showed the best results (52% vs 37, 45 and 35% FAME; P<0.01 for TAX, TIN VS and VV) whereas the cultivar effect on this FA was detected only during the reproductive stage in TIN (43 vs 32; P<0.01 for VI e CO respectively) and VV (38 vs 30; P<0.05 for HU and HA respectively). Forage species did not influence linoleic acid content whereas a significant effect of cultivar within species was observed, with biggest differences for Crimson clover (*T. incarnatum*) during reproductive stage (16.36 vs 27.97 % FAME; P<0.01 for CO e VI respectively).

Figure 1. Levels of PUFA (A) and unsat/sat ratio (B) in experimental forages during vegetative (G) and reproductive stages (R).



In conclusion these results confirm that also in the Mediterranean region forage species and, even if at lower level, cultivars play a key role for to modulate the fatty acid composition. The trend and the amplitude of fatty acid pattern in the plant could be modulated by the interaction between cultivar and phenological stage, as observed in Crimson clover where passing from vegetative to reproductive stage linolenic acid decreased in CO

and increased in VI. In this species, also during vegetative stage, the pattern and the amplitude of linoleic acid showed differences between cultivars.

Table 1. Fatty acid composition (%FAME) of forage species (SP) and cultivars (CV).

SP	CV	C12:0	C14:0	C14:1 c-9	C15:0	C16:0[a]	C16:0	C17:0	C18:0	C18:1[b]	C18:1[c]	C18:2[d]	C20:0	C18:3[e]	C22:0
Growth stage															
TAX	MA	0.58	0.35	0.15	1.49	5.69	15.04	2.02	2.39	0.78	0.22	11.85	0.39	58.28	0.77
TAX	SA	0.63	0.36	0.17	1.62	5.78	15.09	2.04	2.44	0.76	0.18	12.20	0.38	57.59	0.75
TIN	CO	1.02	0.70	0.43	2.71	10.32	25.30	3.25	4.89	1.48	0.42	11.10	0.84	36.43	1.12
TIN	VI	1.08	0.65	0.41	2.96	10.81	24.54	3.37	5.22	1.59	0.45	10.59	0.80	36.58	0.95
VS	JO	0.72	0.44	0.11	1.96	7.44	16.68	2.83	2.83	1.62	0.25	15.28	1.39	47.51	0.94
VS	NI	0.74	0.46	0.11	1.98	7.21	15.82	2.72	2.56	1.18	0.20	15.05	1.26	49.81	0.88
VV	HA	1.04	0.74	0.22	2.80	10.56	23.92	3.45	3.20	1.30	0.37	13.84	1.38	35.51	1.68
VV	HU	0.92	0.78	0.20	2.54	9.59	25.89	2.85	3.51	1.40	0.33	14.07	2.14	33.83	1.95
Repreductive stage															
TAX	MA	0.71	0.58	0.25	1.90	6.85	16.82	1.13	3.88	1.59	0.36	17.80	1.61	45.50	1.01
TAX	SA	0.71	0.58	0.26	1.85	7.19	16.96	1.24	3.67	1.42	0.30	17.62	1.17	45.97	1.05
TIN	CO	0.72	0.55	0.45	1.99	7.64	26.77	2.06	3.78	4.83	0.53	16.36	1.23	31.75	1.33
TIN	VI	0.78	0.81	0.53	2.09	7.87	25.67	1.47	7.11	8.54	0.73	27.97	7.60	42.83	2.05
VS	JO	0.74	0.55	0.16	2.08	8.77	18.82	2.37	3.07	2.34	0.37	16.51	1.55	41.77	0.90
VS	NI	0.71	0.53	0.16	1.93	7.87	19.19	2.41	3.03	2.45	0.35	16.80	1.40	42.25	0.91
VV	HA	1.01	0.76	0.31	2.71	10.48	26.39	2.13	3.37	2.18	0.49	15.37	1.80	30.81	2.19
VV	HU	0.78	0.56	0.20	2.19	8.19	22.85	1.85	2.98	1.97	0.45	16.47	1.91	38.01	1.60
SEM		0.004	0.002	0.001	0.011	0.039	0.80	0.021	0.011	0.020	0.001	0.090	0.019	0.500	0.007
Source of variation															
SP		**	**	**	**	**	**	**	**	**	**	ns	**	**	**
CV		ns	ns	ns	ns	ns	ns	*	0.05	ns	ns	*	**	ns	ns
PS		0.06	ns	**	*	ns	ns	**	ns	**	**	**	**	**	**
SP*PS		**	*	ns	**	**	ns	**	ns	**	ns	**	**	*	*
CV*PS		*	0.05	ns	*	**	ns	**	ns	**	ns	**	**	0.08	**

*[1]Effects of forage (SP) cultivar within species (CV), phenological stage (PS) and their interactions; *; P<0.05; **, P<0.01; ns, not significant.[a]=C16:0 iso; [b]=C18:1 c-9; [c]=C18:1 c-11; [d]=C18:2 c-9,c-12; [e]=C18:3 c-9, c-12, c-15.*

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