An *in vitro* investigation of forage factors which affect the production of conjugated linoleic acid and *trans* vaccenic acid in the rumen. II. Wilting & cell damage

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Introduction Previous studies have shown that *cis* 9 *trans* 11 conjugated linoleic acid (CLA) concentrations are higher for summer milk produced from cows grazing fresh pastures than for winter milk when conserved forages are fed (Jahreis *et al.,* 1997). Furthermore, Offer (2003) showed that a similar depression in milk fat CLA occurred if grass was simply cut and fed after a short wilt. This experiment investigated the effect of wilting on the production of CLA and *trans* vaccenic acid (TVA) in an *in vitro* rumen simulation, and whether any differences could be related to changes in plant structure. It was hypothesised that fresh turgid grass may have a greater propensity for cell damage during mastication than the much more flaccid wilted grass, which in turn may increase microbial metabolism of the grass lipid.

Materials and methods Fresh grass, eight different grass species, were cut from experimental plots on day 0 and day 1 of the trial (*circa.* 200grams FW) 3cm above soil level. The grass cut on day 0 was left to wilt for 24 hr on a laboratory bench. Half of the fresh and wilted tissue was then crushed and cut (C&C) into 5mm strips and 2 ½ g loaded into incubation bottles under CO_2. The remaining grass was loaded into the incubation bottles and homogenised (H) also under CO_2. Two bottles contained 10ml of strained rumen liquor and 10ml of Van Soest medium were used for each treatment. After gassing the head space and sealing, the bottles were incubated for 6 h in the dark at 39^{o}C. At the end of the incubation the bottles were removed and the lipid extracted and analysed as described by Lee *et al*. (2005). Biohydrogenation of C18:2 and C18:3 were calculated as proportional loss of these fatty acids during the incubation. The effect of wilting and cell damage was assessed using an unbalanced ANOVA model with wilt × cell damage as the treatment and blocking according to grass species. The effect of grass species has previously been reported (Lee *et al.* 2005).

Results The concentration of CLA and TVA in the incubation vessels and the extent of C18:2 and C18:3 biohydrogenation are shown in Table 1. Wilting had no significant effect on the concentration of CLA in the vessels but significantly reduced the concentration of TVA and the biohydrogenation of C18:2 and C18:3. The extent of cell damage appeared to have no effect on the concentration of either CLA or TVA but significantly increased the concentration of C18:0 (data not shown) and the biohydrogenation of C18:2 and C18:3.

Table 1 CLA and TVA concentration (g/kg dry matter input) and C18:2 and C18:3 biohydrogenation

	Unwilted		Wilted			Significance		
	C&C	H	C&C	H	S.e.d	Cell damage	Wilt	Interaction
CLA	0.011	0.011	0.010	0.011	0.0007	NS	NS	NS
TVA	3.79	3.36	2.62	2.81	0.162	NS	***	**
Biohydrogenation								
C18:2	0.37	0.52	0.33	0.43	0.037	***	*	NS
C18:3	0.48	0.65	0.46	0.58	0.027	***	*	NS

Conclusions The low level of CLA production in the batch cultures highlights the important role of TVA as a precursor of milk CLA. Increasing the extent of cell damage significantly increased biohydrogenation, but surprisingly did not increase the concentration of the intermediates CLA and TVA. It, did, however increase the concentration of C18:0, which is the end point of biohydrogenation, which suggests that the reaction went further to completion. The fact that H wilted grass was not similar to H unwilted grass in terms of TVA concentration may suggest that structural differences alone between wilted and unwilted grass are unlikely to explain the large differences in milk CLA produced from fresh pasture and cut/wilted grass (Offer, 2003).

References

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