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Effect of freeze-thaw treatment of herbage on the biohydrogenation of α-linolenic acid

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Introduction

Grazed grass is an important source of the n-3 α-linolenic acid (C18:3) in ruminant diets and products. However, exploitation of C18:3 has been limited because rumen biohydrogenation (BH) is often most extensive for high-forage diets. Consequently, we have investigated plant-based mechanisms that might decrease BH. Disruption to plant cells causes rapid release of volatile plant defence compounds such as methanol and C6 oxygenates including hexenal ('green odour'). Lee *et al*. (2007) identified individual effects of some components or analogues of 'green odour' on BH. This study adopted a complementary approach, using the rapid release of compounds when herbage is frozen and then thawed. One limitation of this model is that the composition of green odour released after freeze-thaw differs from that due to physical damage (Fall *et al*., 2001). Since herbage fatty acid levels increase in autumn, particularly when temperatures are low (Witkowska *et al*., 2008), a further objective of this work was to investigate effects of autumn management of pasture on BH.

Material and Methods

A batch incubation study was conducted to investigate the effects of freeze-thawing of herbage on BH. Measurements of gas production (GP) provided a parallel assessment of effects on overall fermentation activity. Ryegrass/white clover herbage that had regrown for 4 weeks was harvested on May 23 (late autumn). Adjacent plots had either a grazing rotation omitted (RO) or not (C) so that the preceding rotation had either 6- or 3-weeks regrowth respectively. Herbage was carefully harvested and immediately frozen. Half of the herbage was then freeze-dried and ground (<1 mm) and then weighed into fermentation vessels (1 g DM), whilst the remaining herbage was held in the freezer. On the day of the experiment, the frozen herbage was quickly weighed (8 g fresh weight) into fermentation vessels, sealed with parafilm and placed in an incubator at 39^{o}C. There were 3 replicates of a 2×2 arrangement of previous field management (RO vs. C) and sample processing (freeze/thaw (FT) vs. freeze-dry and grind (FD)). Rumen incubations were conducted in 260 ml fermentation vessels of an automated GP system (Ankom, Macedon, NY, USA). When the FT herbage reached 39°C (90 minutes), 80 ml of a 50/50 (v/v) mixture of buffer solution (Lee *et al*., 2007) and rumen fluid was added and the incubations started. The buffer was pre-warmed and gassed with carbon dioxide, whilst the rumen fluid was collected from two grazing cows, blended and strained through 4 layers of muslin. Incubations of the FD herbage were conducted in parallel. GP was recorded at 5 minutes intervals via radio frequency transmitter. At the end of the 6-hour incubation, the fermentations were stopped and the bottle contents were frozen until analysis. The chemical composition of the herbage was analysed using NIR and fatty acid analysis used base methylation (Lee *et al*., 2007). BH of C18:3 was calculated as the proportional loss of

the fatty acid over 6 hours (Lee *et al*., 2007). Gas pressures were converted to volumes and expressed in relation to the amount of OM incubated. Two-factor analysis of variance was performed with the SPSS 17.0 statistical package.

Results

The concentrations (g/kg DM) of crude protein, water-soluble carbohydrates, NDF, total fatty acids and C18:3 fatty acid were 236 and 262, 171 and 176, 303 and 277, 65 and 84, and 47 and 63 for RO and C pasture respectively, demonstrating the exceptionally high quality of this late autumn herbage. Effects of treatments on GP and BH of C18:3 are shown in Table 1.

Table 1. Effects of previous pasture management and processing method on gas production (GP) and biohydrogenation (BH) of C18:3 fatty acid.

	Rotation omitted (RO)		Control pasture (C)		S.E.M.	*P*-value	
	FD[1]	FT[2]	FD	FT		Management	Processing
GP, ml/g OM	36.5	45.6	35.4	43.9	0.64	n.s.	<0.001
BH of C18:3, g/g	0.75	0.27	0.77	0.26	0.06	n.s.	<0.001

[1]FD = freeze-dry and ground (<1 mm); [2]FT = freeze-thaw

Conclusion

It is possible that the reduced BH of FT herbage resulted from its different physical form. However, Kim *et al*. (2005) found extensive BH when crushed herbage, which is physically similar to FT herbage, was incubated *in sacco*. The freeze-thaw treatment increased GP relative to freeze-drying and grinding, confirming the extensive release of cell contents. The results are consistent with the hypothesis that green odour selectively inhibits bacteria involved in BH. Herbage fatty acid levels were exceptionally high at this time, probably as a result of the low mean daily temperature (6.5^{o}C; Witkowska *et al*. 2008). Previous pasture management affected fatty acid level, with exceptionally high levels for autumn pasture that had been managed intensively, but there was no effect on BH.

Acknowledgements

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