Metabolic profiling of heathland plants in the diet of sheep

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Introduction Little is known about how plant biochemistry influences the grazing behaviour of animals grazing heterogeneous vegetation communities. Furthermore, most biochemical profiles of grassland species are restricted to major nutritional characteristics. Recent developments in analytical techniques have made possible the detailed analysis of minor components, which can potentially affect animal feeding preferences, performance and health. Gas chromatography/time of flight mass spectroscopy (GC/TOF-MS) coupled with automated library annotation is ideally suited to the acquisition of detailed metabolite profiles of plant extracts (Wagner *et al*., 2003) and can be applied to other matrices such as blood and faeces. In this study GC/TOF-MS was used to identify metabolites within heathland plants, and to investigate which of these metabolites were present and absent within plasma and faeces from sheep consuming mixtures of these plants.

Material and methods Twelve mature Welsh Mountain ewes were zero-grazed on defined diets of heathland plant species for 29 days. The average diet composition was (as a percentage of total dry matter): *Molinia caerulea*, 41.4%; *Calluna vulgaris*, 18.2%; *Vaccinium myrtillus*, 0.21%; *Erica tetralix*, 0.17%; *Juncus effuses*, 6.96%; *Festuca* spp., 1.72%; *Carex* spp., 3.95%; dead grass, 21.1%; and moss, 6.4%. Methanolic extracts were prepared from freeze-dried and milled samples of each diet mixture, each major plant group within the diet mixtures and faecal output. These extracts, together with blood plasma samples were analysed by GC/TOF-MS (Wagner *et al*., 2003). Individual compounds that were predominantly found in one of the nine plant groups (i.e. more than 75% of the quantity of a compound summed across all plant groups was attributable to a single plant group) were investigated further to determine their presence in plasma and faeces of the sheep fed the diets.

Results Metabolite profiling by GC/TOF-MS resolved several hundred compounds in the plant, faeces and plasma samples. Library searching allowed over 100 of these to be tentatively identified. All of the plant groups, with the exception of dead grass, contained several metabolites that were largely specific to that group. These group specific metabolites represent many metabolic processes and include plant sugars and phosphorylated sugars, e.g. mannitol (*Molinia*) and inositol-1-phosphate (*Erica*); amino compounds, e.g. allantoin and spermidine (*Vaccinium*); and one phenolic compound, 1-caffeoylquinic acid (*Erica*). Some of these plant group-specific metabolites were also detected in the sheep plasma and faeces (Table 1). Although many of the metabolites found in the samples remain unidentified, it may become possible to identify some of these unknown metabolites as new additions are made to the mass spectral library or by use of alternative analytical approaches.

Table 1 Numbers of identified (ID) and unidentified (UID) metabolites that predominated in each plant group, together with the numbers of these also found in plasma and faeces (all categories were live growth except dead grass)

	Plant group		Faeces		Plasma	
Plant group category	ID	UID	ID	UID	ID	UID
Molinia caerulea	2	3	1	0	1	1
Calluna vulgaris	0	11	0	4	0	3
Vaccinium myrtillus	2	17	1	10	2	9
Erica tetralix	2	9	0	6	0	4
Juncus effusus	1	5	1	3	1	2
Festuca spp.	0	6	0	4	0	1
Carex spp.	0	2	0	0	0	2
Dead grass	0	0	0	0	0	0
Moss	0	1	0	0	0	0

Conclusions Using GC/TOF-MS it is possible to quickly characterise plant species beyond basic nutritional composition. Further work is required to determine the nutritional significance of the presence or absence of indicator plant compounds in plasma or faeces, in order to interpret their role in influencing diet selection.

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References

Wagner, C., M. Sefkow, J. Kopka (2003). Construction and application of a mass spectral and retention time index database generated from plant GC/EI-TOF-MS metabolite profiles. *Phytochemistry,* 62, 887-900.