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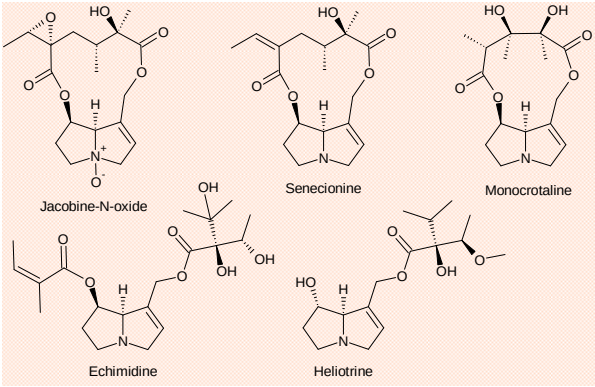
Pyrrolizidine alkaloids in animal feed
A survey conducted in the Netherlands

Patrick P.J. Mulder*, Mirjam Klijnstra, Jacob de Jong

Introduction

Pyrrolizidine alkaloids (PAs) are secondary plant metabolites present in many plants. They act as chemical defence compounds against herbivore attack. Unfortunately, they are also toxic for mammals and humans, causing hepatic veno-occlusive disease (HVOD), liver cirrhosis and ultimately death. Particularly notorious are PAs present in ragwort and groundsel species (*Senecio*), as well as those present in various *Boraginaceae, Heliotropium* and *Crotalaria* species, which are held responsible for hepatic disease in horses and cows and causing livestock losses worldwide. PAs can be found in nature in a wide array of structures (over 600 PAs are known), presenting a considerable analytical challenge.

Following an EFSA opinion and call for data (EFSA Journal, 2007, 447:1-51) a monitoring program was started in the Netherlands directed at the presence of PAs in animal feed. In total 400 samples have been analysed for 70 different PAs. The results are presented in the Table below. The samples have been classified according to the new EU catalogue of feed materials (CR 575/2011).



Representative PAs that can occur in animal feed

Methodology

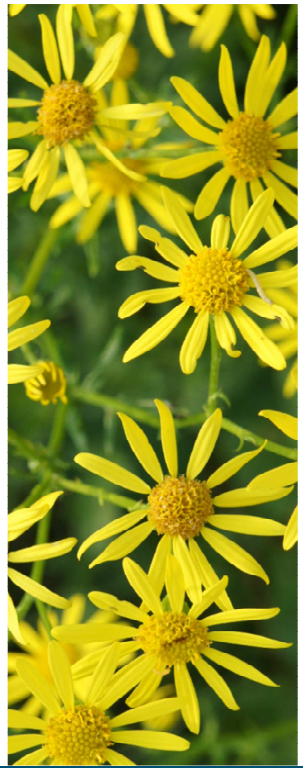
Samples were collected in the framework of the Dutch National Monitoring Plan on animal feeding stuffs 2006-2011. Samples were dried, ground and homogenised. Extraction was performed with 2% formic acid, followed by SPE (Strata X, 60 mg) clean-up.

LC-MS/MS analysis: Waters Acquity UPLC coupled to a Waters Quattro Premier XE tandem MS. Chromatography: UPLC BEH C18, 150x2.1 mm, 1.7 µm column, run with a H_2O/MeCN/NH_4OH (0.01%) gradient (15 min). Detection: Sample extracts were run with two methods covering 39 *Senecio,* 20 *Boraginaceae,* 7 *Heliotropium* and 4 *Crotalaria* PAs. Quantification was performed by standard addition (30 standards available) or by area response comparison.

Survey results

- In grains, seeds and fruits (entries 1-5) a low incidence and relatively low PA concentrations are found (max 343 µg/kg).
- In herbal supplements (entry 7) a high incidence and a high mean PA content is found (max 3209 µg/kg). The majority of PAs is of the heliotrine type, which suggests a non-EU origin.
- In the animal forage category (6), green silage contains few PAs. Dried grass and hay may contain PAs of the *Senecio* and *Boraginaceae* (e.g. echimidine) type, but generally in modest amounts (max 813 µg/kg). One hay sample was however highly contaminated with PAs (22.7 mg/kg). In this sample ragwort (*Jacobaea vulgaris*) was visually detected.
- Lucerne (alfalfa) showed the highest incidence of PAs, as well as the highest average PA concentration (max 6216 µg/kg). Based on the PA profile, common groundsel (*Senecio vulgaris*) was identified as the most likely source of contamination.
- Lucerne is a fodder of increasing popularity, in particular for horses. However, long term consumption of contaminated lucerne could potentially lead to adverse health effects.

		No of		Average concentration main PA groups (µg/kg)						%
	EU classification	samples	% Pos	*Senecio*	*Borago*	*Heliotrope*	*Crotalaria*	Total	Max	Free base	N-oxide
1	Cereal grains	9	11.1	0.0	3.3	0.0	0.0	3.3	30	100	0
2	Oil seeds and fruits (soya)	59	23.7	4.1	8.1	0.0	0.3	12.5	343	36.2	63.8
3	Legume seeds	2	0	0.0	0.0	0.0	0.0	0.0	0		
4	Tubers and roots	1	0	0.0	0.0	0.0	0.0	0.0	0		
5	Other seeds and fruits	4	50.0	0.0	9.1	10.8	0.0	19.9	43	36.1	63.9
6	Forages and roughage	293	54.6	231.2	38.7	0.0	0.0	269.9	22753	56.1	43.9
	Green silage	61	13.1	2.2	0.0	0.0	0.0	2.2	29	25.4	74.6
	Dried grass/hay	92	29.3	269.3	17.2	0.0	0.0	286.5	22753	38.6	61.4
	(highest sample removed)	(91)	(28.6)	(22.3)	(17.2)	(0.0)	(0.0)	(39.5)	(813)	(60.9)	(39.1)
	Lucerne	140	88.6	305.9	69.7	0.0	0.0	375.6	6216	64.9	35.1
7	Other plants (herbal supplements)	32	62.5	26.7	69.4	219.8	0.0	315.9	3209	8.2	91.8
	All samples	400	49.2	169.5	36.6	20.0	0.1	226.2	22753	50.6	49.4



1 **RIKILT – Institute of Food Safety, Wageningen UR**
P.O. Box 230, NL-6700 AE
Wageningen, the Netherlands
Phone +31 317 48 02 56
Internet www.rikilt.wur.nl
* Corresponding author: @wur.nl

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