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Exposure assessment of cattle via roughages to plants producing compounds of concern

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ABSTRACT

Food producing animals are exposed to biologically active plant compounds through feed and roughages, presenting a potential risk to the animal but also consumers of food of animal origin. To evaluate to which plant compounds of concern dairy cows in the Netherlands are exposed, a ranking filter model was developed, combining information on abundance of plant species in vegetation plots in the Netherlands (183,905 plots of three different vegetation types) with plant-compound combinations (700), and with consumption data of fresh grass, grass silage and corn silage by cattle.

The most abundant plant genera are those producing cyanogenic glycosides, coumarins and benzofuranocoumarins, being predominantly fodder plants (alfalfa, clover and some grasses) considered to be safe. Highest exposures were estimated for plant genera producing piperidine alkaloids (horsetail), furanocoumarins (parsley and relatives), pyrrolizidine alkaloids (*Symphytum*, *Senecio*, *Leucanthemum*, *Eupatorium*) and essential oils. The current results allow to prioritise future scientific research on these compounds.

1. Introduction

Food producing animals are exposed to various herbs or botanicals through feed and roughages. Virtually each angiosperm (flowering plant species) produces a wide range of biologically active compounds. These compounds can be indicated as secondary metabolites with "secondary" defined as all metabolites outside the primary metabolic pathways (i.e. glycolysis, citric acid cycle, DNA/RNA metabolism). The variation in molecular structures of secondary metabolites is immense and is summarised in overviews (e.g. Frohne & Pfänder, 2005; Hegnauer, 1962–2001). The compounds indicated as "plant toxins" can be considered as subset of these secondary metabolites. Examples are pyrrolizidine, tropane and piperidine alkaloids, saponins, protoanemonin, oxalic acids and furanocoumarins. The study described in this paper was initiated from a feed safety perspective and, therefore, secondary plant metabolites are further referred to as "compounds of concern".

The naturally evolved feeding strategies of farmed animals offer a principal safety level for animals themselves and for the products thereof. Examples are the avoidance of ragworts (*Senecio* and *Jacobaea*), species of buttercups (*Ranunculus*) and docks (*Rumex*) under natural grazing conditions (Haeggström, 1990; Frohne & Pfänder, 2005; Cortinovis & Caloni, 2013; personal observations). However, several circumstances and recent developments might jeopardise this natural occurring protection, e.g. ragworts remain undetected by animals when consuming roughage (Molyneux & Ralphs, 1992). Practises such as feeding cattle year round in stables may thus lead to increased exposure.

Only a limited range of plants or plant-related compounds is currently regulated in the European Union (EU) for their presence in feed (Decision 2002/32/EC; European Union, 2002). A study of van Raamsdonk, VanCutsem, and Jørgenson (2009) showed that effective monitoring for the botanic components in the EU was limited. However, a large range of risk assessment opinions developed by or under the auspices of the European Food Safety Authority (EFSA), documentation for risk assessors on "naturally occurring substances of possible concern" (EFSA Guidance document) and a Compendium prepared by the Scientific Cooperation

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Working Group (ESCO) have been published (EFSA, 2009a, 2012). A broad chemical survey of plant compounds occurring in feed would provide further knowledge for prioritizing the most abundant compounds of concern. However, the full list of over 550 entries on combinations of these compounds and their producing plant species resulting from EFSA (2009a) extends the reasonable limits for such a survey. Any exposure of farmed animals to such compounds is directly related to the animals' exposure to the producing plants. Therefore, as an alternative, a survey to prioritise the most relevant plant species in roughage producing those compounds, can provide the link between the theoretically possible choices for research and the optimal monitoring for assuring feed safety. In addition to monitoring, the results of this survey would facilitate HACCP systems for animal production.

The current paper focuses on exposure of cattle, as major roughage consuming farmed animal, to plants producing compounds of concern. Ruminants are fed compound feeds, roughage, silage but also herbs for appetite and for enhancing animal health and welfare. The origin and way of production is different for the various sources. Compound feeds are composed for an important part of a limited group of well-defined by-products often resulting from human food processing. Herbs and roughage, however, are based on ingredients harvested under local and relatively uncontrolled circumstances. Fortunately, in the Netherlands extensive data are available from decades of vegetation analysis on the occurrence of plant species in all existing plant communities (Schaminée, Hennekens, & Ozinga, 2007, 2012). When focusing on pastures, meadows and corn fields as major sources of roughage in North-western Europe, short lists of most frequently occurring plants can be composed. The list of occurring plant species can subsequently be filtered by data available on plant compounds of concern. This paper describes the use of a ranking filter (RF) model to assist in determining the clusters of plants producing compounds relevant to cattle via free grazing and roughage. The most relevant compound groups, resulting from the modelled levels of plant consumption, are further discussed. Recommendations for future use of databases for the identification of plant compound hazards are presented.

2. Materials and methods

2.1. Model approach

The principle of the RF model is to rank the plant species or genera producing compounds of concern pooled and filtered for the compound groups. The model is based on the consumption of fresh grass, grass silage harvested from pastures (eutrophic production fields) or from meadows, and corn silage harvested from corn fields, since these are the areas (vegetation types) providing roughage for cattle in the Netherlands. To calculate the exposure to plant groups of concern, a two-tiered approach was followed. The first tier included the following two steps:

1. Analyse of the database with vegetation plots, expressed as lists of plants according to their cover-abundance (relevés) of the three vegetation types included in the study. List of plant species present in the included relevés in decreasing order of abundance (frequency × coverage).
2. Correlation between plant genera and compounds of concern, clustered according to compound group (e.g. pyrrolizidine alkaloids) as filter criterion.

The combination of 1 and 2 resulted in a shortlist per vegetation type of clusters of plants producing a specific group of compounds of possible concern. Adjustment for selective grazing by cattle is made by using weighting factors.

The second tier included:

3. Share of the various harvested products in the diet of cattle. Pastures are used for both free grazing and roughage production.

Combination of 1/2 and 3 resulted in a weighted shortlist pooled over all sources of clusters of plants producing a specific group of compounds of possible concern.

An overview of the RF model is given in Fig. 1.

2.2. Vegetation relevés

Since 1900, as part of vegetation analysis, large numbers of small plots (ranging from 1×1 to 10×10 m) in all types of vegetation across the Netherlands have been inventoried for the plant species present and for their 'cover-abundance' (the percentage of the plot-area covered by their biomass). For the Dutch situation these plot descriptions (so called "relevés") have been stored in The Dutch National Vegetation Database (Schaminée et al., 2007, 2012). The information from plots of three different vegetation types collected between 1970 and 2010 according to a standardised protocol was abstracted from this database (see Table 1 for details on the plot numbers and species numbers per vegetation type). To detect potential time trends, the abstracted relevés were divided in a group collected during 1970–1989 and during 1990–2010. For each plant species occurring in a vegetation type, the median coverage and the frequency of occurrence in that group (i.e. the percentage of relevés in which the species occurs) was calculated.

2.3. Basic data for plant secondary metabolites

The Compendia on Botanicals of the ESCO working group (EFSA, 2009a, 2012) were converted to a table with 559 entries with genus and compound group combinations. This table was verified with data from Frohne and Pfänder (2005), and complemented with an additional 141 entries of plant genera producing substances of concern extracted from other sources (Frohne & Pfänder, 2005; Regulation (EC) 1334/2008; Regulation (EC) 574/2011). The total list of 700 entries is accessible through internet (http://www.wageningenur.nl/en/Expertise-Services/Research-Institutes/rikilt/Research/Natural-toxins/Plant-toxins.htm; to be referred to as RIKILT., 2011). The terminology used by EFSA in the compendium was followed in the analysis. As a consequence, a general group of "essential oils" was included in the list, together with separate compound groups of monoterpenoids, furanocoumarins and others, although these compounds can be part of essential oils. The conclusive lists of all plant species in order of abundance for the three vegetation types resulting from the Dutch National Vegetation Database (Table 1) were filtered in order to focus exclusively on the lists of plant species producing compounds of concern, using the database with 700 entries of plant genera containing such compounds as filter (RIKILT, 2011).

2.4. Weighting factors for selective grazing

In free grazing circumstances, cattle usually avoid the consumption of certain plant species. However, it can be assumed that not all toxic plant species can or will be avoided completely. Therefore, in some well documented situations for free grazing, the exposure was given a reduced weighting factor: ragworts (genera *Senecio* and *Jacobaea*; Frohne & Pfänder, 2005; Cortinovis & Caloni, 2013) and docks (*Rumex*; Haeggström, 1990) were given the factor 0.1 instead of 1.0. Species of buttercups (*Ranunculus*) were given the factor 0.5, because some *Ranunculus* species are