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Pyrrolizidine alkaloids in natural and experimental grass silages and implications for feed safety

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ABSTRACT

Hepatotoxic 1,2-dehydro-pyrrolizidine alkaloids (PA) and their *N*-oxides (PANO) in feed are a potential threat for animal and human health. However, their risk assessment in preserved animal forage is difficult due to data gaps regarding their occurrence in field samples and contradictory results regarding their behavior during the ensilage process. In this study, 115 samples of grass silage originating from different districts in Bavaria (Germany) were analyzed for their PA and PANO contents. A sensitive LC–ESI-MS/MS method for the detection of 10 PA and 4 PANO was developed including a clean-up of the aqueous acidic extract by cation-exchange cartridges. The mean recoveries were between 70% for seneciphylline-*N*-oxide and 104% for senecionine-*N*-oxide. The limits of detection ranged between 0.14 μg senkirkine/kg dry matter (DM) and 1.3 μg retrorsine-*N*-oxide or monocrotaline-*N*-oxide/kg DM. Eighteen percent of the samples contained one or more of the analyzed compounds. The highest observed value, calculated as the sum of seneciphylline and senecionine, was 30 μg/kg DM. Other samples contained senecionine-*N*-oxide, seneciphylline-*N*-oxide, lycopsamine, intermedine, echimidine, and heliotrine in lower amounts. An accompanying laboratory scale ensilage trial showed a compound-dependent transformation of PANO during the fermentation process while PA-amounts remained stable or even increased. Impacts on safety of ensiled animal feed are discussed with regard to animal health.

1. Introduction

The increasing spread of plants containing toxic 1,2-dehydro-pyrrolizidine alkaloids (PA) (LfU, 2012; German Federal Agency for Nature Conservation, 2015) and their potential to cause chronic effects at very low levels of exposure raised

Abbreviations: DM, dry matter; Em, echimidine; Hn, heliotrine; Im, intermedine; La, lycopsamine; Mc, monocrotaline; McNO, monocrotaline-*N*-oxide; PA, pyrrolizidine alkaloid free base (tertiary amine); PANO, PA-*N*-oxide; PA/PANO, PA tertiary amines and *N*-oxides; Re, retrorsine; ReNO, retrorsine-*N*-oxide; Sc, senecionine; ScNO, senecionine-*N*-oxide; Sk, senkirkine; Sp, seneciphylline; SpNO, seneciphylline-*N*-oxide; Td, trichodesmine.

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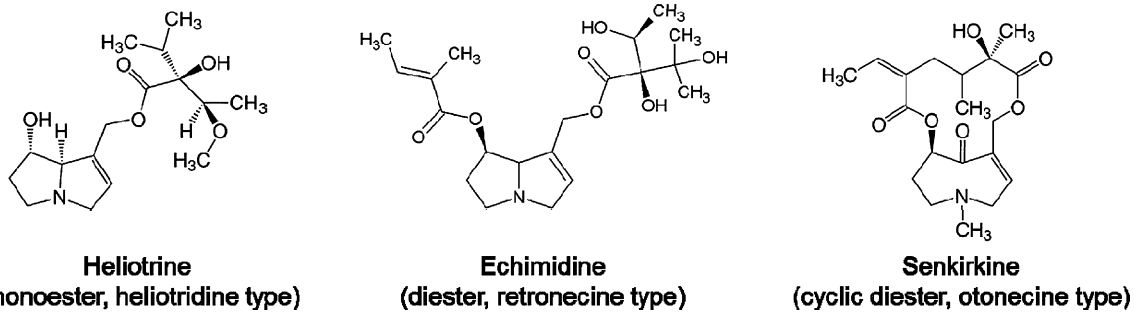

Fig. 1. Examples for 1,2-dehydro-PA (retronecine, heliotridine, otonecine-type) with different kinds of esterification (monoester, diester, and cyclic diester).

the special attention of food and feed safety authorities in Germany and many countries worldwide in the past few years (documented by various scientific opinions, discussion papers, and guidance documents, e.g., BfR, 2007, 2011, 2013; DEFRA, 2007; EFSA, 2011; JOINT FAO/WHO, 2011, 2012). Altogether, more than 6000 plant species are estimated to contain PA or their *N*-oxides (PANO) (Smith and Culvenor, 1981). A majority of the more than 500 different compounds of these secondary plant metabolites is synthesized by species belonging to the *Boraginaceae*, *Asteraceae* (*Compositae*), or *Fabaceae* (*Leguminosae*) families. In Germany, various indigenous *Senecio* (S.) species such as *Senecio jacobaea* (tansy ragwort), *S. vulgaris* (common groundsel), *S. aquaticus* (marsh ragwort), but also the invasive neophyte *S. inaequidens* (narrow leafed ragwort), *Echium vulgare* (blueweed), *Symphytum* spp. as well as *Borago* spp. (German Federal Agency for Nature Conservation, 2015) can represent a risk as contaminants of food and feed due to their content of PA/PANO.

PA are mono- or diesters of 7-hydroxy-1-hydroxymethyl-pyrrolizidine (necine base) and aliphatic mono- or dicarboxylic acids (necic acids). Depending on the structure of the necine base mainly four groups of PA exist: the retronecine-type, the heliotridine-type, the otonecine-type, and the platynecine-type. According to the type of esterification, they can be differentiated into monoesters, diesters as well as macrocyclic diesters (Hartmann and Witte, 1995). 1,2-dehydro PA (i.e. PA of the retronecine-, heliotridine-, and otonecine-type) with at least one esterified hydroxyl group (Fig. 1) are suspected to cause acute and progressive health effects on the liver, liver cirrhosis, pulmonary arterial hypertension, and cancer (Mattocks, 1986; Edgar et al., 2011). The *N*-oxidized forms of retronecine- and heliotridine-type PA possess the same toxic effects like the corresponding free bases. PANO are reduced to the PA free bases during passage through the human or animal gut (Wang et al., 2005). Therefore, the German Federal Institute for Risk Assessment (BfR) recommended that a daily intake of 1,2-dehydro PA/PANO of 0.007 μg per kg body weight (sum of all compounds) preferably should not be exceeded in the human diet, where especially honey and herbal teas currently are considered the main source of exposure (BfR, 2011, 2013; Bodi et al., 2014). This health based guidance value is in accordance with the recent scientific opinion of the European Food Safety Authority (EFSA, 2011). Other approaches on this topic were intensely reviewed in a discussion paper compiled by the Codex Committee on Contaminants in the Food Chain (JOINT FAO/WHO, 2011). Recently, this document was supplemented by a discussion paper on management practices for the prevention and reduction of PA/PANO-contamination of food and feed (JOINT FAO/WHO, 2012).

So far, there are only a few studies on the natural occurrence of PA/PANO in grass silages (Mulder et al., 2009). Reports on PA/PANO-decomposition or metabolism during the ensilage process or by composting are contradictory. While some authors reported a complete degradation (Crews et al., 2009; Hough et al., 2010), others only observed a partial degradation (Candrian et al., 1984; Berendonk et al., 2010; Berendonk and Hünting, 2011; Wiedenfeld, 2011), or even a structure-related behavior (Becerra-Jimenez et al., 2013; Ronczka et al., 2012). However, it is known that food of animal origin such as milk and cheese can contain these phytotoxins after a carry-over from PA/PANO-contaminated forage (EFSA, 2011; JOINT FAO/WHO, 2011). Generally, for cattle grazing on pasture *Senecio* spp. are unpalatable and livestock usually discriminates toxic weeds from grasses and clovers as long as pastures are not overgrazed. However, they suffer intoxications after inadvertent consumption or when fed more palatable hay or silage contaminated with toxic weeds (DEFRA, 2007; Stegelmeier, 2004).

With respect to both food safety (carry-over) and animal health occurrence data in feed are of high priority for risk assessment purposes. In this study, 115 samples of grass silages originating from different districts of Bavaria (Germany) were analyzed for 14 PA and PANO by LC–ESI-MS/MS. Furthermore, a small laboratory-scale model ensilage trial was conducted to examine the influence of the ensilage process on the total PA-content and on single PA and PANO compounds.

2. Materials and methods

2.1. Samples

In this study, random field samples ($n = 115$) were taken of common grass silages from 10 different farms in 8 districts of Bavaria, Germany (Weiden, Schwandorf, Roth, Passau, Ebersberg, Rosenheim, Kempten, Landsberg am Lech). Sampling was performed according to official procedures under control of the responsible authority (LfL – Bavarian State Research Center