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Toxic pyrrolizidinalkaloids as undesired contaminants in food and feed: degradation of the PAs from *Senecio jacobaea* in silage

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Dedicated to Prof. Dr. Th. Dingermann on the occasion of his 65th birthday.

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Pyrrolizidine alkaloids (PAs) can show a hazardous potential for men and animals. They can act as cancerogenic, mutagenic, teratogenic and fetotoxic agents. One pathway of a human intoxication is its occurence as contaminants in food and feed. Here, the contamination of cereals already led to severe and fatal intoxication episodes. Besides this, milk is of special concern as it is the main food for children which show a very high susceptibility for a PA intoxication. Milk can contain PAs in case the milk producing animals have access to contaminated feed. In this context it is of special interest whether the PA content of contaminated silage remains stable during the ensiling procedure or show a more or less high level of decomposition. We could show that ensiling will not lead to PA-free silage.

1. Introduction

Pyrrolizidine alkaloids (PAs) are wide-spread in the plant kingdom; they are present in about 3% of all flowering plants. If PAs show a 1,2 double-bond in their base moiety (necine) and are additionally esterified in both OH groups of the necine they possess hepatotoxic, carcinogenic, genotoxic, teratogenic and sometimes pneumotoxic properties and are therefore hazardous to livestock (Gilruth 1903, 1904; Bull and Dick 1959).
For many years there have been reports describing these toxicity problems (see overview: Wiedenfeld 2011; Wiedenfeld and Edgar 2011).
Besides medicinal plants (whose use and the use of preparations from them is controlled and restricted in several countries), the main sources of PA uptake are food and feed. It is proven that PAs may occur in honey (Deinzer et al. 1977; Culvenor et al. 1981; Roeder 1995, 2000; Edgar et al. 2002; Beales et al. 2004; Boppré et al. 2005; Betteridge et al. 2005), milk (Schoental 1959; Dickinson et al. 1976; Dickinson 1980; Johnson et al. 1978; Goeger et al. 1982; Lüthy et al. 1983; Candrian et al. 1984a; Molyneux and James 1990; Roulet et al. 1988, Hoogenboom et al. 2011), eggs (Edgar and Smith 1999) and salads (BfR 2007). Milk is of special concern as it is the main diet for infants who show a very high sensitivity to a PA intoxication. PAs are transferred into milk by milk-producing animals consuming PA plants.
Recently, *Senecio jacobaea* L. (tansy ragwort) has extensively spread on pastures and meadows and it can therefore easily contaminate hay and silage. We could show that the PA content in hay deriving from contaminated tansy ragwort remains stable and undergoes no reduction or degradation.
From silage, it was reported that the PAs (from *Senecio alpinus*) showed different degradation levels depending on the amount of contaminated plant material: Increased levels of PA contamination led to a higher degradation: 100% contamination showed a reduction of 4.5%, whereas 3.5% contamination showed 45.7% from the initial PA value (Candrian et al. 1984b). Recently, a complete degradation of the PAs from ragwort during composting was reported (Hough et al. 2010).
To verify the conditions of a possible reduction of the PA content during ensiling, 24 silage samples with different levels of contamination by tansy ragwort were analyzed. We could show that the reduction of the initial PA level depends not only on the percentage of ragwort contamination but also on the different structures of the PAs: reduced amounts of PAs only occurred in the case of senecionine, seneciphylline, integerrimine and erucifoline whereas the other PAs from ragwort (jacobine, jacoline, jaconine, jacozine) showed lower degradation levels.

2. Investigations and results

Due to the fact that the reduction of the PA content in silage is caused by an enzymatic chemical decomposition, it becomes obvious that the amount of the remaining PAs is dependent on several factors like temperature, storage, influence of air and amount of ensiling material. To obtain valid data in our study, standardized conditions were set. Silage samples were produced with 8 different levels of tansy ragwort (TR) contamination (1, 5, 10, 25, 50, 75, 100%; each in triplicate).
Figure 1 shows the total amount of toxic PAs in 1 g of silage (mean values; st.d.: 2–20%). Values between 495 (100% TR contamination) down to 5 μg (1% TR contamination) could be measured. The exact reduction values of the PAs are shown in Figs. 2 and 3. Figure 2 (mean values; st.d.: 2–20%) shows the total percentage of PAs in 1 g of dried silage in relation to the percentage of ragwort contamination (100% RT contamination = 100% PA-value), whereas Fig. 3 shows the total PA values relative to an equal percentage of contamination (100%).
In Fig. 4 the single PA distribution corresponding to one gram of tansy ragwort is shown. It becomes obvious that the decay of each PA is not identical: whereas senecionine, seneciphylline,

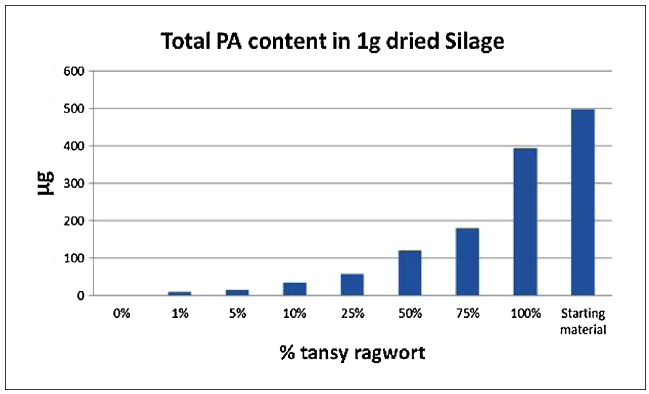

Fig. 1: Total PA content in 1 g silage

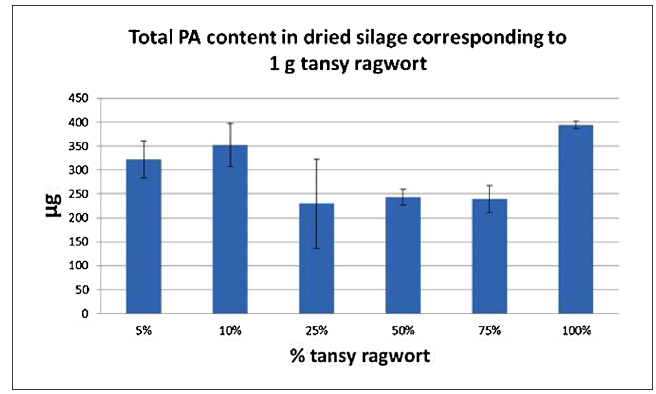

Fig. 3: PA content corresponding to 1 g tansy ragwort

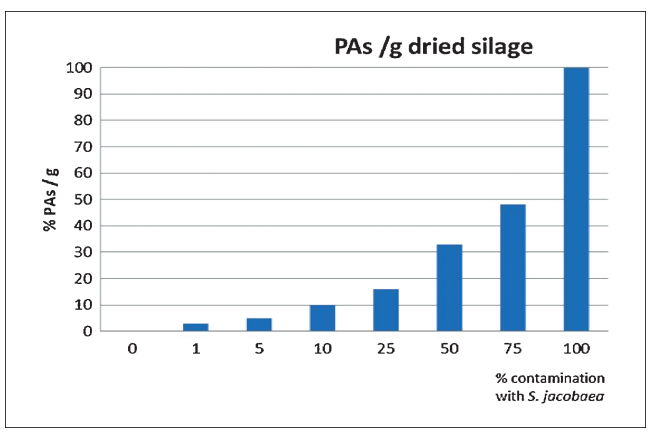

Fig. 2: PA content (%) in 1 g silage in relation to the percentage of ragwort contamination

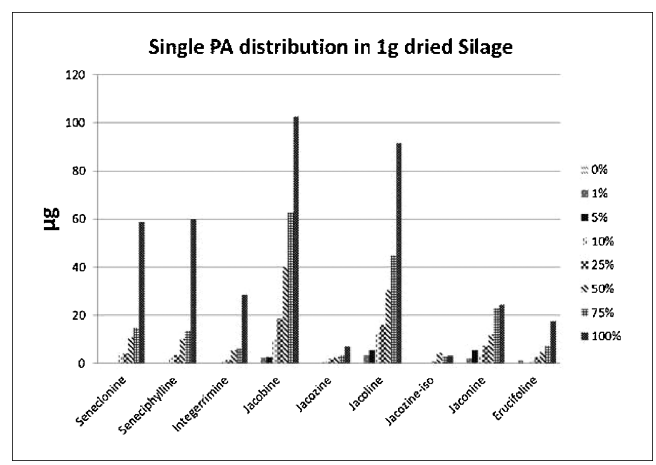

Fig. 4: Single PA distribution in 1 g silage

integerrimine and erucifoline show a clear decrease, the other PAs (jacobine, jacoline, jaconine, jacozine) remain less effected.

3. Discussion

An important aspect during ensiling is the pH value and exclusion of air: it is known that under anaerobic conditions optimal silage shows pH values ~ 4 leading to a stable ensiling process during 100 days (Thaysen 2011). In all our samples an equal pH value of ~ 3.9 was found, which proved that the ensiling process was running under optimal conditions and this ensured that enzymatic reactions can take place during the whole ensiling process; stronger acidic environment stops the enzymatic activity and a possible decomposition of the PAs whereas under higher pH values the anaerobic conditions and the ensiling procedure are not stable.

Under these standardized conditions it could be found that, comparing dried 100% ragwort silage with pure dried ragwort, the total PA amount is about 20% higher in pure ragwort (498 µg/1 g) than in the corresponding ragwort silage (394 µg/1 g) (Fig. 1); other reports found that there was a difference of 4.5% (Candrian et al. 1984b). Furthermore, a reduction of the initial PA levels between 56 to 69% could be observed in the samples with 75, 50 and 25% ragwort contamination (Fig. 2): 75% contamination = 48% PAs / 50% contamination = 33% PAs / 25% contamination = 16% PAs); in contrast, in silages with a lower ragwort percentage the PA degradation is reduced leading to the initial PA level in silage with 5–10% ragwort contamination (Fig. 3). These data confirm that, in general, ensiling leads to a PA degradation. But, the level of degradation is depending on the level of contamination; and that in a low percentage of contamination the degree of decomposition is negligible. Additionally, studying the single PA distribution (Fig. 4) it could be shown that the main PAs in silage are jacobine, senecionine, seneciphylline and jacoline.

It was shown (Mattocks 1986) that in macrocyclic diester PAs the ester function at C-7 is the most reactive one, which leads to the conclusion that an enzymatic degradation may start at this position. It could be shown by X-ray analysis (Wiedenfeld et al. 2008) that PAs which possess a double-bond adjacent to this ester function form highly conjugated systems which facilitate a cleavage of the ester bonds. This may be the reason for the observed enzymatic decomposition of the PAs senecionine, integerrimine, seneciphylline and erucifoline while the other PAs do not show such a double-bond resulting in stronger ester bonds and in a higher stability for decomposing attacks, respectively.

Summing up, it could be shown that in all silage samples good ensiling conditions were found (= pH: ~ 3.9) and that under this environment a degradation of the PAs can take place. On the other hand PA-free silage cannot be found (PAs could be detected even in those samples with a low percentage of tansy ragwort contamination: 1% contamination = 5 µg PA/1 g) for which reason ensiling is finally not a solution to obtain PA-free feed when using materials with ragwort contaminations.

4. Experimental

4.1. PA-Determination

Dried silage samples (300 g, each in triplicate) were extracted with MeOH for 3 days (soxhlet). After evaporation to dryness of the alcoholic solution under reduced pressure, the residue was resolved in 2.5% HCl, Zn-dust was added and stirred for 1 h to reduce N-oxides to free bases. The aqueous phase was alkalized with ammonia (25%), saturated with NaCl and extracted several times with CH_2Cl_2. The organic phase was evaporated to

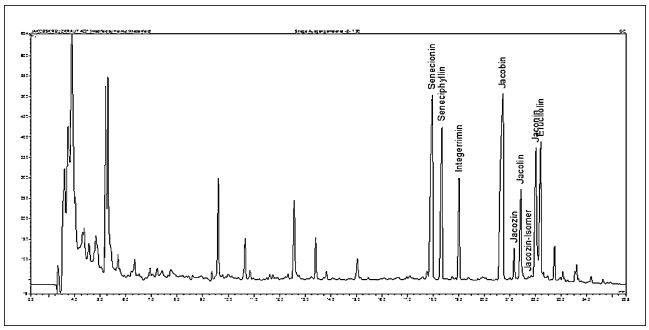

Fig. 5: Gas chromatogram of the PAs from *Senecio jacobaea.*

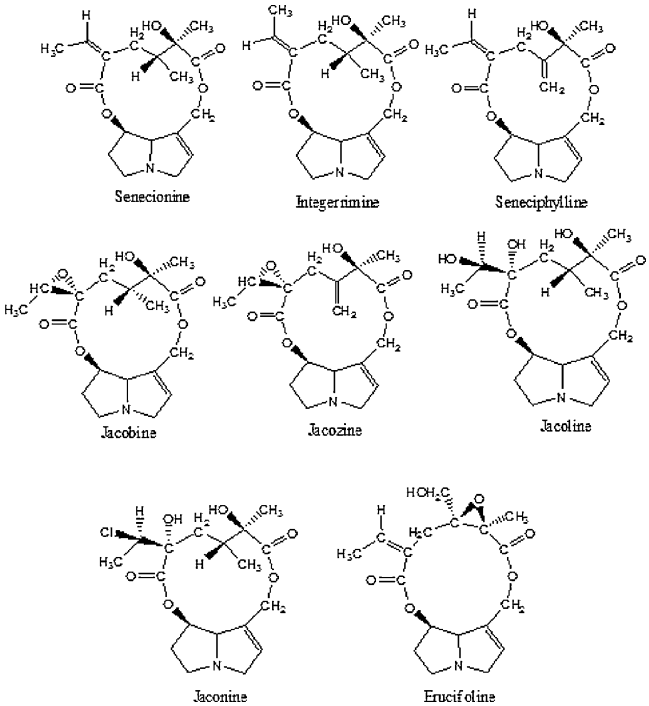

Fig. 6: Structures of isolated PAs from *Senecio jacobaea.*

dryness, resolved in 1 ml CH_2Cl_2 and applied on a diol solid-phase column. After washing steps with CH_2Cl_2, the PAs were eluted with AcCN/MeOH (1:1). After evaporation to dryness, the alkaloid residue was resolved in 1.0 ml CH_2Cl_2/MeOH (1:1) and analyzed by GC and GC-MS (analytical data for extraction and quantification: see: Wiedenfeld et al. 1996; Wiedenfeld 1997). The GC measurement was done using reference samples (isolated from *S. jacobaea, S. erucifolius, S. inaequidens*. The structures were determined by MS and 2D-NMR). Fig. 5: GC from tansy ragwort plant material used for the contamination in ensiling. Fig. 6: Structures of isolated PAs.

4.2. Plant material

Lolium perenne, collected in 2010, leave state; *Senecio jacobaea,* collected in 2010, flowering state; both plants were pre-dried to 40% weight of starting material.

4.3. Ensiling

The ensiling procedure was performed by C. Berendonk and K. Hünting at the Landwirtschaftskammer Nordrhein-Westfalen; LWZ Haus Riswick, Fachbereich Grünland und Futterbau, Elsenpaß 5, D-47533 Kleve, Germany. According to the DLG guidelines (DLG 2000) sealed 500 ml glass jars were used; constant temperature; ensiling time 90 days.

4.4. Reference samples

The PAs from tansy ragwort were isolated, their structures elucidated and their contents in the plants analyzed during the vegetation period (Berendonk et al. 2010).

4.5. GC-, GC-MS conditions

Hewlett Packard 5890, Series II, NPD-Detector, Autosampler 7673A. PC-system Chromeleon V. 6.20. Fused-Silica-Gel capillary column, Optima-5, DF-60m, 01.25 µm X ID. He; splitless, 1.5 PSI. Inj./Det.-Temp.: 300 °C; Temperature program (180–280 °C; 5 °C/min.; Endt.: 15 min.). Injection: 3 µl.

Hewlett Packard 5890, Series II, Quadrupol MS, autosampler 7673. Injection 3 µl. column CP-Sil m8, 50 m, 0,25 µm x 0,25 mm. MS: Source: 180 °C, Interface: 290 °C; Injector: 290 °C; Full-scan mode.

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