

## MICROSCOPIC FEED EXAMINATION – A USEFUL METHOD FOR ESTABLISHING THE CONSTITUENTS OF ANIMAL ORIGIN AND BOTANICAL IMPURITIES

## MIKROSKOPSKA PRETRAGA STOČNE HRANE – USPJEŠNA METODA ZA UTVRĐIVANJE TKIVA ŽIVOTINJSKOG IZVORA I BOTANIČKIH NEČISTOĆA

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### SUMMARY

Microscopic methods are often used to check for fake feeds, made of substances of lower nutritive and digestible value, which can significantly decrease their quality or can even be toxic to animals and subsequently to people. In accordance with the EU laws, the official EU method (Commission Directive 2003/126/EC) for the determination of constituents of animal origin in feeds is used in Slovenia. On the basis of the results we can confirm that the feed for domestic animals in Slovenia is safe regarding the presence of the constituents of animal origin. There is a Directive in the EU (Directive of the European Parliament and of the Council 2002/32/EC) on undesirable substances in animal feed, containing a list of undesirable chemical and botanical impurities. Also in this case microscopic feed examination is adequate and applicable. Among the botanical impurities, mentioned in the Directive, is the plant named Thorn apple (*Datura stramonium*). Seeds of this plant contain alkaloids like hyoscyamine, atropine and scopolamine, which are toxic for animals, therefore the maximum Thorn apple seeds content is regulated for all feedstuffs. In the past two years microscopic examination showed that 10 of 40 samples of different feeds for wild birds contained seeds of *Datura stramonium* (1 to 124 seeds). The same samples were also used to examine the presence of seeds of the named Common ragweed (*Ambrosia artemisiifolia*) that is not toxic for animals but for humans, because its pollen is a strong allergen. 21 of 40 examined samples contained 1 to 235 seeds of Common ragweed. With regard to the fact that these different feeds for wild birds are available in, there is a permanent possibility that people involuntarily spread this plant in their surroundings.

Key words: microscopic examination, constituents of animal origin, botanical impurities, *Datura stramonium*, *Ambrosia artemisiifolia*

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## INTRODUCTION

It is generally known that animal feed products, used properly, should not be dangerous to animal health and consequently to humans. These products should not present risks to the environment and the production of livestock. To determine whether animal feed is of high quality and safe it is essential to constantly examine it. In many cases an organoleptic examination is sufficient, but to determine the quality and wholesomeness microbiological, microscopic, chemical and other analyses are to be carried out.

Compared to the most recent methods, mainly chemical methods, the microscopic examination of feed is a well-known method for feed evaluation that determines the quality of ingredients and feed composition in a relatively short time and a simple way. This is helpful especially when there is a suspicion that the feed could be the reason for health problems and decreased production in animals (Kamphues et al., 1998).

The microscopic examination of feed is frequently used to identify the substances or individual ingredients, contaminants and other supplements that can be found in feeds. None of the raw materials for feed production is absolutely clean, so in most cases a small amount of different contaminants (straw, seeds and fruits of other types of food crops, husk, sand, soil, etc.) is allowed. It is different with poisonous seeds or other parts of poisonous plants harmful to animal health and therefore among prohibited substances in raw materials and feeds (Vöhringer, 1997). It is impossible to completely eliminate undesirable substances but it is also of vital importance to decrease the presence of substances, which are regarded as being acutely poisonous and to avoid biological accumulation and decomposition in animal feed products (European Community, 2002).

To stop the BSE from spreading it is very important that official bodies control presence of constituents of animal origin in feeds. For this purpose the European Commission has first presented the basic directives (European Community, 1998) to determine constituents of animal origin in feeds using a microscopic method. In some situations the modified microscopic method has been approved (van Raamsdonk et al., 2004; van Raamsdonk et al., 2005; von Holst et al., 2006). In

2003 the European Commission presented the official microscopic method (European Community, 2003) and determined that every single member of the community must appoint an official or National Reference Laboratory (NRL) to carry out examinations. The demand that such a laboratory uses a reliable and accredited method in accordance with the European and International standards is of great importance (European Community, 2004).

For determining the presence of animal tissues in feeds for the needs of official control the official microscopic method (European Community, 2003) is appropriate and useful. Its effectiveness has been proved in concentrations of animal tissues in feeds lower than 0.1 % (Gizzi et al., 2003; Ujčić and Vengušt, 2006). The Community Reference Laboratory for the detection of animal proteins in feed (CRA-W) also carried out, as part of its annual work programme, a proficiency test to evaluate laboratories, which demonstrated a good laboratory performance in the detection of small amounts of constituents of animal origin in feeding stuffs using the analytical method as described in the Directive 2003/126/EC (European Community, 2009).

From 1999 to 2008 in Slovenia 2748 samples of different feeds were examined to determine the presence of constituents of animal origin. Only 95 samples (3.46 %) contained constituents of animal origin. Most of these samples (73.68 %) contained fish meal was in some cases even mentioned in the declaration or the feed was intended for animals that were allowed to be fed on fish meal. The analyzed situation in the field of feed safety in Slovenia, regarding the presence of constituents of animal origin in the nutrition of domestic animals, shows that feed in Slovenia is from this point of view safe, which can also be attributed to the microscopic examination of feed within the Veterinary Administration of the Republic of Slovenia (VARs) yearly monitoring.

There is a Directive in the EU (European Community, 2002) concerning undesirable substances in animal feed, containing a list of undesirable chemical and botanical impurities, where a microscopic feed examination is also adequate and applicable. Among the botanical impurities, mentioned in the Directive, also is the plant named Thorn apple (*Datura stramonium*). Seeds of this plant contain alkaloids, which are toxic for animals.

For the identification of seeds of different plants experiences and comparison with samples in archive databases or illustrations in various books can be of great help. In most cases size, shape, colour, surface marks, texture and shape and the position of the seed scar (NIAB, 2004) help to identify the seeds. Other characteristics such as husk, thorn, starch grain and the presence of whiskers are of great help as well. These structures, both in field and herb seeds, can be destroyed during harvesting or later during the process of cleaning or other working processes, which makes them even more difficult to identify. In some cases plant seeds do not develop all or any typical characteristic, which can be a consequence of different growth conditions, degree of development etc. It is necessary, for the examination to consider a large number of characteristics of seeds, although it is not always possible to determine the exact species (NIAB, 2004).

Our wild bird feed examination verified the presence of another weed named Common ragweed (*Ambrosia artemisiifolia*), which is not mentioned in the Directive (European Community, 2002) and is not toxic for animals but for humans, because its pollen is a strong allergen. The aim of this work is to present the microscopic feed examination as a useful method for the detection of prohibited and undesirable substances in feed.

**Table 1. Number of seeds of *Ambrosia artemisiifolia* and *Datura stramonium* in wild birds feed (declared content as sunflower seeds)**

**Tablica 1. Broj sjemenki *Ambrosia artemisiifolia* i *Datura stramonium* u krmi za ptice u prirodi (deklarirano kao sjeme suncokreta)**

	Winter 2007/08	Winter 2008/09
Number of examined samples	10	10
Number of samples contaminated with <i>Ambrosia artemisiifolia</i> seeds	6	3
Number of samples contaminated with <i>Datura stramonium</i> seeds	1	1
Number of samples contaminated with both types of seeds	1	0
Average (min – max) number of <i>Ambrosia artemisiifolia</i> seeds per sample	122.3 (1 - 235)	11.0 (2 - 28)
Average (min – max) number of <i>Datura stramonium</i> seeds per sample	124*	5*

\*the value of one positive sample

## MATERIAL AND METHODS

Forty packs of wild bird feed, each of 1000g, offered on the Slovenian market, for feeding wild birds in winter 2007-08 and 2008-09, were examined: Twenty packs with a seed mix and 20 packs with sunflower seeds (as set out in the declaration). The contents of the 20 packs with a seed mix varied according to the producer declaration. In the divider, the bird feeds were divided into two halves, 500g each. One half of the feed was analyzed while the other half was stored in bags.

For the detection of *Datura stramonium* and *Ambrosia artemisiifolia* seeds a macroscopic examination method (with and without a magnifying glass) and a stereomicroscope were used.

## RESULTS AND DISCUSSION

Microscopic examination performed in the past two years showed that 10 of all 40 samples of different feeds for wild birds contained seeds of *Datura stramonium* (1 to 124 seeds). The same samples were also used to examine the presence of seeds of Common ragweed (*Ambrosia artemisiifolia*). 21 of 40 examined samples contained 1 to 235 seeds of Common ragweed. The results are summarized in table 1 and 2.

**Table 2. Number of seeds of *Ambrosia artemisiifolia* and *Datura stramonium* in wild birds feed (declared content as seed mix)****Tablica 2. Broj sjemenki *Ambrosia artemisiifolia* i *Datura stramonium* u krmi za ptice u prirodi (deklarirano kao mešanina sjemenki)**

	Winter 2007/08	Winter 2008/09
Number of examined samples	10	10
Number of samples contaminated with <i>Ambrosia artemisiifolia</i> seeds	7	5
Number of samples contaminated with <i>Datura stramonium</i> seeds	3	5
Number of samples contaminated with both types of seeds	1	4
Average (min – max) number of <i>Ambrosia artemisiifolia</i> seeds per sample	49.3 (1 – 146)	10.8 (2 – 21)
Average (min – max) number of <i>Datura stramonium</i> seeds per sample	1.3 (1 – 2)	3.0 (1 – 5)

The genus *Datura* contains more than a dozen species. Among the most common species are *D. stramonium*, *D. metal*, *D. metaloides* and *D. suaveolens* (Radeleff, 1970). *Datura* poisoning has been reported throughout the world in humans and all classes of livestock (Radeleff, 1970; Friedman and Levin, 1989; Tostes, 2002; Hansen and Clerc, 2002; Binev et al., 2006; Soler-Rodriguez et al., 2006). The species of *Datura* mentioned above contain solanaceous alkaloids of the tropane configuration (atropine, hyoscyamine and hyoscine – scopolamine) (Radeleff, 1970). *Datura stramonium*, also mentioned in the Directive (European Community, 2002), has different names: Thorn apple, Jimson weed, Jamestown weed (Radeleff, 1970). It is commonly found in barnyards, cultivated fields of sunflowers, maize and lucerne, roadsides, wasteland and other disturbed habitats (Oladosu and Case, 1979). *D. stramonium* is an annual weed that grows 75 to 90 cm tall, with an erect, stout stem and spreading branches near the top, unpleasant-smelling, alternating leaves that are unevenly or sharply toothed and glabrous, a funnel-shaped corolla, and hard, prickly fruit whitish or purplish in colour. The fruits ripen in autumn and split open to reveal numerous wrinkled, black seeds (Cooper and Johnson, 1998). All parts of the plant are poisonous, especially the seeds that contain higher amounts of toxic alkaloids (Soler-Rodriguez et al., 2006), which

are toxic for animals, therefore a maximum content of 1000 mg/kg of Thorn apple seeds is proposed for all feed stuffs (European Community, 2002).

Regarding our investigations the wild bird feeds declared as seed mix had a higher contamination with seeds of Thorn apple than those declared as sunflower seeds only. If we take an average weight of a seed (6 mg per seed) there was just one sample that did not comply with the legislation. Anyhow, it is of great importance to take into consideration that the seeds can spread with the wild bird feed and affect animals and humans.

The Common ragweed (*Ambrosia artemisiifolia*) is an annual, monoecious (male and female flowers on the same plant) weed from the Asteraceae family, usually sized 20 to 120 cm. It grows along roads, cultivated fields, in fallow lands, meadows and gardens (Tamarcaz et al., 2005). The ragweed has its origin in North America. Its seeds are 2-4 mm large. A plant can carry between 30 and 3000 nutlets (Schulz-Schroeder and Russ, 2007). The pollen of the *Ambrosia artemisiifolia* is produced in enormous amounts and one single plant alone can produce millions of pollen grains. Since the pollen grains are small (18-22 µm) they are often involved in episodes of long distance transport (D'Amato et al., 2007). In Europe, the presence of ragweed and the ragweed allergy phenomenon is rapidly increasing, particularly in certain areas of France, Italy, Austria,

Hungary, Croatia and Bulgaria (Tamarcaz et al., 2005; Wopfner et al., 2005).

On the basis of our research, we share the opinion that wild bird feeds play an important role in spreading *Ambrosia artemisiifolia* seeds. In winter 2007/08 and 2008/09, 40 packs of wild bird feed offered on the Slovenian market were examined to detect *Ambrosia artemisiifolia* seeds. A macroscopic and microscopic examination showed that in feeds with seed mix 12 of 20 packs contained seeds of *Ambrosia artemisiifolia* and in feeds with sunflower seeds 9 of 20 packs contained seeds of *Ambrosia artemisiifolia*. The number of *Ambrosia artemisiifolia* seeds varied from 1 to 146 (winter 2007/08) and 2 to 21 (winter 2008/09) in feeds with seed mix and in feeds with sunflower seeds from 1 to 235 (winter 2007/08) and from 2 to 28 (winter 2008/09). In our case we determined that the wild bird feed with seed mix was very often contaminated, but the feed with sunflower seeds could, on average contain a remarkably higher number of *Ambrosia artemisiifolia* seeds.

There is presently no Community legislation setting maximum levels of *Ambrosia* seed in feeding stuffs. Switzerland has introduced an intervention value at 50 mg/kg feed, which equals around 10 seeds/kg feed (Jørgensen, 2008). Despite the fact that several countries have implemented various eradication methods, ragweed continues to spread on. The effectiveness of individual eradication methods has not been known yet (Tamarcaz et al., 2005). Knowing that the pollen of *Ambrosia artemisiifolia* has a high allergenic potential (Tamarcaz et al., 2005; Wopfner et al., 2005; Schulz-Schroeder and Russ, 2007), its spreading is not only a high health risk but it also endangers agriculture and natural protected areas (Schulz-Schroeder and Russ, 2007). Regarding to the fact that these different feeds for wild birds are accessible in the ordinary sale, there is a permanent possibility that people involuntarily spread this plant in their surroundings.

## CONCLUSION

Microscopic feed examination is very suitable to control specific demands of feed regulations and can also give useful indications for further investigations.

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## SAŽETAK

Mikroskopskom pretragom može se otkrivati loša krma odnosno dodavanje tvari slabije kakvoće ili lošije iskoristivosti, što može bitno smanjiti njezinu kakvoću ili čak biti toksično za životinje i posredno za ljude. U Sloveniji, u skladu s Europskim zakonodavstvom upotrebljava se EU mikroskopska metoda (Direktiva Komisije 2003/126/EC) za utvrđivanje tkiva životinjskog porijekla. Na osnovi dosadašnjih istraživanja možemo tvrditi, da je krma u Sloveniji, u pogledu prisutnosti sastojaka životinjskog izvora, sigurna. U EU važeći pravilnik o nepovoljnim tvarima u krmu za životinje (Direktiva Europskog parlamenta i Savjeta 2002/32/EC) sadrži i spisak zabranjenih kemijskih i botaničkih nečistoća. I za tu vrstu analiza mikroskopska pretraga je podesna i upotrebljiva metoda. Među botaničkim nečistoćama, spomenutim u Direktivi, nalazi se i bijeli kužnjak (*Datura stramonium*). Sjeme te biljke sadrži alkaloide hiosciamin, atropin i skopolamin, koji su toksični za životinje, pa se zato u svim vrstama krme propisuje njihova najveća dozvoljena količina. U posljednje dvije godine, mikroskopskom metodom pretraženo je 40 uzoraka različitih krmnih smjesa za ptice u prirodi, a u 10 uzoraka pronađene su sjemenke *Datura stramonium* (1 do 124 sjemenki). Isti uzorci pretraženi su i na sjeme biljke ambrozije (*Ambrosia artemisiifolia*), koja inače nije opasna po zdravlje životinja ali je štetna za ljude, zbog cvjetnog praha, koji je jak alergen. U čak 21 od 40 pregledanih uzoraka pronađeno je od jednog do 235 sjemenaka ambrozije. Budući da su različite krmne smjese za ptice u prirodi dostupne ljudima u redovitoj prodaji, biljka se može nehotice širiti u okolinu.

Ključne riječi: mikroskopska pretraga, tkiva životinjskog izvora, botaničke nečistoće, *Datura stramonium*, *Ambrosia artemisiifolia*