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THE DETERMINATION OF DIFFERENT FEED CONSTITUENTS USING THE MICROSCOPIC METHOD

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Determining the proper quality and safety of animal feed requires constant surveillance and examination. Because of that, along with an organoleptic examination, microbiological, microscopic, chemical and other analyses need to be carried out. To perform the microscopic examination, which is the only official method to determine constituents of animal origin in feed (European Community, 2003), instruments such as mills, mixers, and strainers as well as a stereomicroscope and a light microscope with polarization, fluorescent light and phase contrast are needed. The aim of the microscopic examination is to identify constituents on the basis of their morphological and histological characteristics, to estimate the quantity and the proportion of the constituents in the feed mixture and to detect pollution of the feed. A microscopic feed examination is also adequate and applicable for detection of botanical impurities, like poison plants and their toxic seeds. There is an EU Directive (European Community, 2002) concerning undesirable substances in animal feed, containing a list of undesirable chemical and botanical impurities. Among the botanical impurities mentioned in the Directive, there are also: *Datura stramonium*, *Ricinus communis*, *Claviceps purpurea*, *Crotalaria spp.*, *Prunus armeniaca,* containing alkaloids and glycosides. Undesirable substances of plant origin that are not mentioned in the legislation, but are spread with raw material for animal feed could also be detected with this method. One example are seeds of the plant *Ambrosia artemisiifolia,* whose pollen is a strong allergen for humans.

Key words: feed composition; feed analysis; microscopy

ОДРЕДУВАЊЕ НА РАЗЛИЧНИТЕ КОНСТИТУЕНТИ ВО СТОЧНАТА ХРАНА СО УПОТРЕБА НА МИКРОСКОПСКИОТ МЕТОД

Одредувањето на соодветен квалитет и безбедност на сточната храна бара постојано надгледување и испитување. Заради тоа, заедно со органолептичкото испитување, мора да бидат извршени и микробиолошки, микроскопски, хемиски и други анализи. За да се изведе микроскопското испитување, кое е единствениот официјален метод за одредување на конституенти од животинско потекло во сточната храна (Европска Зедница, 2003), потребни се инструменти како што се мелници, миксери и филтри, како и стереомикроскоп и мал микроскоп со поларизација, флуоресцентно светло и фазен контраст. Целта на микроскопското испитување е да се идентификуваат конституентите врз база на нивните морфолошки и хистолошки карактеристики, да се проценат квантитетот и соодносот на конституентите во комбинацијата со сточната храна и да се одреди нивото на загадување на сточната храна. Микроскопското испитување на сточната храна исто така е адекватно и применливо за откривање на загадувања од растително потекло, како што се отровни растенија и нивно отровно семе. Постои Директива на ЕУ (Европска Заедница, 2002) која се однесува на непосакувани супстанции во сточната храна, содржејќи листа на непосакувани хемиски и растителни загадувачи. Покрај растителните загадувачи спомнати во Директивата, тука се и *Datura stramonium*, *Ricinus communis*, *Claviceps purpurea*, *Crotalaria spp.*, *Prunus armeniaca,* кои содржат алкалоиди и гликозиди. Непосакувани супстанции од растително потекло кои не се споменати во законот, но се шират со суровините за сточна храна, можат исто така да се откријат со овој метод. Пример се семињата од растението *Ambrosia artemisiifolia,* чиј полен е силен алерген за луѓето.

Клучни зборови: состав на сточна храна; анализа на сточна храна; микроскопија

1. INTRODUCTION

Stockbreeding or livestock production occupies a very important place in farming. Satisfactory results in terms of public and animal health, animal welfare, the environment and the livestock producers' finances depend to a large extent on the use of appropriate good quality feeding stuffs (European Community, 2002). The production of faultless feeding stuff depends on different factors. The most important factors are: the quality of the raw material (stability, purity), the quality of the bought additives like premixes and pure substances, the quality of the made feed mixture (the harmonization with the recipe, homogeneity, stability), and techniques (transport of the raw material, grinding, mixing, extrudation, packing, storing) (Vöhringer, 1997).

We cannot maintain the requested high quality of the feed without constant examination of factors stated above (Vöhringer, 1997). In many cases an organoleptic examination is sufficient, still to determine the quality and wholesomeness microbiological, microscopic, chemical and other analyses are needed 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 simple way. This is helpful especially when there is suspicion that the feed could be the reason of health problems and decreased production in animals (Kamphues et al., 1998).

2. MICROSCOPIC FEED EXAMINATION

The aim of the microscopic feed examination is the identification of the ingredients on the basis of their morphological and histological characteristics, estimation of the quantity and of the ration of the ingredients in the feed mixture, and the detection of the feed pollution (Kamphues et al., 1998). None of the raw materials for feed production is absolutely clean, this is why 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 when there are poisonous seeds or other parts of poisonous plants harmful to the 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).

It is also important that microscopic methods are often used to check for fake feeds made of less nutritive and worse digestible substances, which can significantly decrease the quality of feeds or can even be toxic to animals and subsequently to people. The information about the ratio of this substances in feed is very useful. With the microscopy it could be possible to find out the procedure of making and the influence of it on the quality of the feed, the contamination with bacteria, moulds, insects and other pests.

The first step in microscopic feed examination is the optical examination of the feed with the use of the stereomicroscope. In this way we get some raw data about the feed composition and contents of e.g. moulds, undeclared substances etc. If the answers with this examination are not satisfied, an accurate microscopic examination is necessary, that could be performed just by an expert (Kamphues et al., 1998). There are some parallel procedures that help us in our work, for example sieving and separating particles of different sizes, flotation, sedimentation and concentration of the investigating structures, and use of specific reagents, that colour the individual structure in the way that we can see them.

If we want to find out the feed composition, it is necessary to check several fractions of the sample with the microscope. The particles of the sample have to be determined, sorted and weighted. The percentage of the examined component is calculated on the whole sample. This work demands huge knowledge about the components and their by products that are used for the feed mixtures. The literature for this field is important for the acquirement of the knowledge, but above all an archive collection of single component from the feed and feed mixture is needed (Roetschi et al., 2003).

Examination on the presence of constituents of animal origin

The very important part of the microscopic feed examination occupies the microscopic feed

examination on the presence of constituent of animal origin. In the period before BSE (Bovine Spongiform Encephalopathy) the use of the different feeds of animal origin was widespread. It was in force that these feeds were the cheapest and even the least problematic source of proteins. It was used for preparation for feed mixtures for all kinds of domestic animals and also other production animals. Most of all meat, meat and bone, bone meal, cracklings, poultry, feather and blood meal, animal fat, fish meal, condense fish juice and fish oil, and also milk powder, whey and other secondary products in the milk production were used (Pravilnik o kakovosti krme, 1989; Hahn, 1999; Pravilnik o kakovosti, označevanju in pakiranju krme v prometu, 2003).

After the appearance of BSE it was found that its appearance in ruminants was probably caused by feeding animals with not enough heat treated meat and bone meal, they limited or prohibited the use of majority of feeds of animal origin (Mondini et al., 2008). With this the need of an efficacious control of constituents of animal origin in the feeds came. Experts stared to develop methods, and they hoped, that the methods would give enough precise and quick results and they would be at the same time more economical methods. But the only official method for detection of constituents of animal origin in feed is still the microscopic method described in the Commission Directive 2003/126/EC (European Community, 2003).

The constituents of animal origin are identified on the basis of typical, microscopically identifiable characteristics (i.e. muscle fibres and other meat particles, cartilage, bones, horn, hair, bristles, blood, feathers filaments, eggshells, fish bones, scales) (European Community, 2003). The main fragments of animal origin that could be present in the feed are bones and muscle fibres. Also other fragments of animal origin such as cartilage, hairs, feathers filaments, eggshells, fish scale and ligaments could be found. Fragments that derived from the organs, skin and other soft tissues normally are not present or are not recognisable. To the prescribed condition of sterilization of the material (133º C, 3 bare, 20 min.) the bone fragments are the most resistant. Also in the processed animal products some base characteristics of the fragments of animal origin could be still recognised. In the long bones of the vertebrate animals the pattern of lacunae (contain bone cells – osteocytes) is visible, that is usually organized around the central canal (Havers canal) in series of circular lamella. Between the lacunas and also between the central canal there is a net of tiny tubes (canaliculae). In the cartilage the cartilage cells (chondrocytes) form holes that are more around shaped. Here there are no connective tubes, because the cells are fed by diffusion. There are some general descriptions of tissues of animal origin in the important classes of vertebrate (mammals, poultry and fish), but also in these we could find some differences (Gizzi et al., 2003; van Raamsdonk et al., 2005).

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 fishmeal that was in some cases even mentioned in the declaration or the feed that was intended for animals that are allowed to be fed on fishmeal. 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, that can also be attributed to the microscopic examination of feed within the Veterinary Administration of the Republic of Slovenia (VARS) yearly monitoring.

Examination on the presence of impurity and toxic substances of botanic origin

With the microscopic examination poisoning plants or their toxic seeds could also be found out. The identification of seeds is usually by comparison with a mental image, a reference collection or with illustrations. If seeds are regularly handled and examined it is possible to build up and maintain an extensive knowledge. In most cases the useful clues for the identification of seeds comes from shape, size, colour, surface markings, texture and the shape and position of the attachment scar. Wings, scales, spines, awns or presence of hairs may also be useful. Unfortunately in agricultural seeds, and the weed seed impurities, these structures are often damaged or last during harvesting, cleaning and treatment processes. Seeds, like living organisms, are inherently variable and an individual seed may vary from the »normal« in one or more characters. Not all the usually typical features may be developed; at times a particular feature may not appear at all or may be present in an

atypical form. Care should therefore be taken to examine all the features of a seed.

Lighting is also important, the light used should not throw a colour cast and the direction may be critical for revealing features. It is often helpful to move a seed around to view it from different angles so that all features may be seen clearly. A reliable reference collection is an essential tool in the identification of seeds. Personal reference collections become a prized possession and life's work for many seed analysts who take great care to prevent loss, damage or contamination of samples. It is not always possible to identify a seed to the species level. In cases of doubt, determination of identity may have to be left at genus or even family level (NIAB, 2004).

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, e.g. *Datura stramonium*, *Ricinus communis*, *Claviceps purpurea*, *Crotalaria spp.*, *Prunus armeniaca*, different *Brassicae*, seeds of weeds and fruits, that contains alkaloids, glycosides etc., where a microscopic feed examination is also adequate and applicable. To Member states of European Union it is permitted, when a danger to animal or human health or to the environment exists, to reduce temporary the existing maximum level of the mentioned undesirable substances. It is also permitted to define the maximum level of other substances or prohibit the presence of those undesirable substances in products intended for animal feed (European Community, 2002). Some laboratories made a proposal to place additionally, on the official list of botanical undesirable substances, some other plants and their seeds, e.g. common ragweed (*Ambrosia artemisiifolia*) and ragwort (*Senecio jacobaea*) (van Raamsdonk, 2007).

Among the botanical impurities, mentioned in the Directive (European Community, 2002), is also the plant named Thorn apple (*Datura stramonium*). Seeds of this plant contain alkaloids, which are toxic for animals. The genus *Datura* contains more than a dozen species (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* 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).

Common ragweed (*Ambrosia artemisiifolia*) is not known to be toxic for animals but cause severe allergy in humans. The seeds of this plant are relatively often spread via bird feed and other connected products (Schulz Schroeder in Russ, 2007). With this kind of feeds, people unknowingly, spread seeds of high allergenic plants in their close surroundings. In Europe, the presence of the ragweed and the ragweed allergy phenomenon is rapidly increasing, particularly in certain areas of France, Italy, Austria, Hungary, Croatia and Bulgaria (Taramarcaz in sod., 2005; Wopfner in sod., 2005; Schulz Schroeder in Russ, 2007). Despite the fact, that several countries have implemented various eradication methods, the ragweed continues to spread on. The effectiveness of individual eradication methods has not been known yet (Taramarcaz el al., 2005).

Because of the present problems with spreading the ragweed and allergic problems with the ragweed pollen, we examined, in Slovenia at the end of 2007 and beginning of 2009, 40 samples of different feed mixtures for wild birds, which were available to people in the ordinary sale. The microscopic examination of 40 samples 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 plant 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 accessible in the ordinary sale, there is a permanent possibility that people involuntarily spread this plant in their surroundings.

3. CONCLUSION

Microscopic feed examination is a universally useful method for hygiene and quality assessment of the feed. On the one hand it is the only official method for determination of constituents

of animal origin in feed, on the other hand in a quite easy and fast way we could have a look in the quality and hygiene quality of the ingredients and composition of the feed. From the point of view of the composition of the feed it is very important to use the possibility of detecting the presence of undesirable substances and toxic compounds of botanical origin, e.g. thus mentioned in the European and Slovene legislation or thus that are not mentioned in the legislation, but for the reason of the globalisation of the market, more and more frequent in the feed for domestic and wild animal. We must not forget that some of these substances of the feed, e.g. ambrosia, could be harmful for humans.

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