Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the Commission related to ergot as undesirable substance in animal feed

Question N° EFSA-Q-2003-38

Adopted on 19 April 2005

SUMMARY

The term ergot refers to fungal structures from *Claviceps* species replacing kernels on grain ears or seeds on grass heads, being visible as large discoloured sclerotia. These sclerotia contain different classes of alkaloids, the most prominent being ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and their related -inines. The amount and pattern of alkaloids vary between fungal strains, depending on the host plant and the geographical region. Ergot alkaloids (ergolines) exert toxic effects in all animal species, and the most prominent toxic signs can be attributed to the interaction of ergot alkaloids with adrenergic, serotinergic and dopaminergic receptors. Typical clinical symptoms are vasoconstriction that may progress into vaso-occlusion and gangrenous changes, but also into abortions. The neurotoxic signs comprise feed refusal and dizziness but also convulsions. Typical dopaminergic effects are agalactia accompanied with insufficient nursing of suckling animals such as piglets and foals. Available data indicate that adverse effects may occur in agricultural animals particularly in pigs after intake of feed contaminated with ergot at levels close to the current EU limit.

At present, the rate of contamination of feed materials is expressed as percentage of sclerotia present in feed material. This physical determination of the contamination rate is often inaccurate, as size, weight and composition of the sclerotia may vary considerably. Moreover, sorting is impossible in processed feed materials. Hence, it has been suggested to replace the physical methods by chemical analysis. At present, the data on the toxicological properties of individual ergot alkaloids are too limited to select individual marker toxins for monitoring the extent of contamination. Data on the toxicity of individual ergot alkaloids are scarce, as under field conditions animals are exposed to the complex mixtures with a varying composition of ergot alkaloids depending on the fungal strain, the host plant and on environmental factors. This implies that at present neither the total alkaloid content, nor a single alkaloid can be recommended as reliable indicator of the potential adverse effects to livestock associated with the ingestion of ergot contaminated feeds. Systematic analyses of common grains and forage grasses would be necessary to establish a correlation between exposure to ergot alkaloids and adverse effects in individual animal species. The few data available, however, do not provide any evidence that ergot alkaloids accumulate in edible tissues, including milk and eggs and thus food from animal origin is unlikely to be an important source of human exposure.

KEY WORDS:

Claviceps, ergot alkaloids, ergolines.

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BACKGROUND

1. General background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed[1] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[2].

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination ("early warning system") and to take measures to reduce or eliminate the contamination ("pro-active approach").

In particular the introduction of the principle of non-dilution is an important and far- reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful mean to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)[3].

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[1] OJ L140, 30.5.2002, p. 10
[2] OJ L 115, 4.5.1999, p. 32
[3] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)

It is worthwhile to note that Council Directive 1999/29/EC is a legal consolidation of Council Directive 74/63/EEC of 17 December 1973 on the undesirable substances in animal nutrition[4], which has been frequently and substantially amended. Consequently, several of the provisions of the Annex to Directive 2002/32/EC date back from 1973.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[5] provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

On the basis of this opinion, some provisional amendments are proposed to the Annex of Directive 2002/32/EC in order to guarantee the supply of some essential, valuable feed materials as the level of an undesirable substance in some feed materials, due to normal background contamination, is in the range of or exceeds the maximum level laid down in the Annex I of Directive 2002/32/EC. Also some inconsistencies in the provisions of the Annex have been observed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health that additional detailed risks assessments are necessary to enable a complete review of the provisions in the Annex.

2. Specific Background

The term ergot refers to the dark sclerotia formed by several species of the genus *Claviceps. Claviceps purpurea* is the most important in terms of frequency of occurrence. It is mainly found on rye, triticale and wheat, but also on other cereals and grasses. A number of alkaloids are formed in the sclerotia and the total alkaloid content of the sclerotia is quite variable and may differ by a factor of ten.

SCAN concluded[6] that current EU legislation limits the occurrence of ergot on the basis of weight of sclerotia present but that this is not sufficient to protect animal health for following reasons:

- separation of contaminated grain and non-contaminated grain on the basis of size can be inaccurate;
- the toxic potential of ergot and consequently its impact on animal health is dependent on its alkaloid content and composition.

[4] OJ L 38, 11.2.1974, p. 31

[5] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)

[6] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 7.6. Conclusions and recommendations.

Therefore current legislation on ergot should be revised and individual ergot alkaloids should be considered rather than ergot sclerotia.

TERMS OF REFERENCE

The Commission requests the EFSA to provide a detailed scientific opinion on the presence of ergot, in particular of ergot alkaloids in animal feed.

This detailed scientific opinion should comprise the

- identification of the ergot alkaloids, relevant for their impact on animal health or their impact on public health through a possible carry-over into food of animal origin
- determination of the toxic exposure levels (daily exposure) of relevant ergot alkaloids for the different animal species of relevance (difference in sensitivity between animal species) above which
  - signs of toxicity can be observed (animal health / impact on animal health) or
  - the level of transfer/carry over of relevant ergot alkaloids from the feed to the products of animal origin results in unacceptable levels of these ergot alkaloids or of their metabolites in the products of animal origin in view of providing a high level of public health protection.
- identification of feed materials which could be considered as sources of contamination by these relevant ergot alkaloids and the characterisation, insofar as possible, of the distribution of levels of contamination
- assessment of the contribution of the different identified feed materials as sources of contamination by the relevant ergot alkaloids
  - to the overall exposure of the different relevant animal species to relevant ergot alkaloids,
  - to the impact on animal health
  - to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry over rates.
- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

ASSESSMENT

1. Introduction

The term ergot alkaloids (also denoted ergolines) refers to a diverse group of up to forty toxins, comprising clavines, lysergic acids, lysergic acid amides and ergopeptines (Betina, 1994). These toxins are produced by members of the fungal family of *Clavicipitaceae* represented among others by *Claviceps purpurea, C. paspaspali,* and *C. fusiformis*, occurring predominantly on rye, wheat and barley, but also on rice, maize, sorghum, oats and millet. Other members of the *Clavicipitaceae*, such as *Neotyphodium* and *Epichloe* species, infect exclusively forage and turf grasses, including perennial rye grass (*Lolium perenne*) and tall fescue (Festuca grass, *Festuca arundinacea*). *Neotyphodium* species produce ergovaline (an ergopeptide) as most prevalent ergot alkaloid, but also structurally less related toxins such as indolediterpenes (lolitrems), 1-aminopyrolizidines (lolines) and a pyrrolopyrazine (peramine). In the United States over 90 % of the tall fescue pastures harbour plants infected with *Neotyphodium coenophialum* and the economic losses due to livestock intoxications approach one billion dollars annually (Panuccione and Annis, 2001). In Europe, intoxications of livestock associated with *Neotyphodium* toxins are rare and confined to certain forage grasses. Hence, these toxins will not be addressed in this opinion.

Claviceps purpurea infests living plants at the time of flowering, and while in principle all species in the Poaceae family are potential hosts for *Claviceps purpurea,* infections are most prevalent in rye and triticale that have open florets. In addition, various grasses can be infected, forming a natural reservoir for the fungus. Sclerotia are resistant and remain intact during the winter and during storage of grains. Under favourable conditions they develop into *perithetia* that produce and shade ascospores, which reach flowering plants and infect these. Following infection, a yellow-white mycelium is formed that produces honeydew, containing infectious conidia. Honeydew attracts insects that disseminate the conidia between plants resulting in secondary infections. The primary ergot mycelium progresses, and darkens, finally visible as hardened sclerotium (Smith and Henderson, 1991).

Mature sclerotia vary in number and size from a few millimetres to more than 4 centimetres according to the host plant (Meyer, 1999; Kamphues and Drochner, 1991), and differ in mass from a few grams to 25 grams for 100 sclerotia. Ergot sclerotia also vary in colour from white (*C. tripsaci*), to brown (*C. glabra*) to yellow (*C. hirtella*) and purplish-brown (*C. purpurea*). In addition, sclerotia show significant differences in their total alkaloid content that varies between 0.01 % and 0.21 % (Lorenz, 1979; Wolff, 1989) and show differences in the pattern of produced alkaloids that is determined by the individual fungal strain in a geographical region and the host plant.

Investigations in Germany indicated an increase in the occurrence of *Claviceps purpurea* infections in the last 10 years. This increase seems to be associated with the more extensive use of hybrid varieties of rye and perannial rye breeds (Amelung, 1999; Engelkes, 2002). Hence, infectivity may reach (in Germany) 40 to 50 % of all investigated rye samples and may comprise also sclerotia from grass contaminating harvested cereals.

Intoxications induced by *Claviceps purpurea* have been known in Europe for many centuries. In humans these intoxications are described in the medieval literature as St. Anthony's Fire or Holy Fire, with reference to the intense pain resulting from vasoconstriction and subsequent gangrene, as well as the neurotoxic symptoms associated with the ingestion of ergot alkaloids. The last recorded outbreak of gangrenous ergotism occurred in Ethiopia in 1977 - 1978 when 140 persons showed signs of intoxication with high mortality (34 %). A milder convulsive form of ergotism with no fatalities occurred in India in 1975. Recently, a severe outbreak of gangrenous ergotism was again reported from Ethiopia (Urga, 2002).

Based on these biological effects ergot alkaloids have also been used for medical purposes since the beginning of the 19th century. For example, ergotamine and dihydro-ergotamine are acting as partial agonist and antagonist at α-adrenoceptors; bromocriptine is an agonist on dopamine receptors, particularly in the central nervous system and methysergide acts as an antagonist of 5-HT_2-receptors.

Ergotamine causes a sustained rise in blood pressure due to α-adrenoceptors mediated vasoconstriction and is considered to be responsible for the peripheral gangrene of St. Anthony's Fire. Dihydro-ergotamine has a less pronounced effect. Methysergide, a potent 5-HT_2-receptor agonist is used in the treatment of migraine attacks and for the alleviation of the symptoms of carcinoid tumours. Ergometrine is a moderate vasoconstrictor but initiates strong contractions of the gravid uterus and is used in the management of the third stage of labour and to control bleeding in the peri-partal phase. However, unless their established clinical use, the exact mechanism of action of ergot alkaloids remains to be elucidated.

2. Chemical and physical properties of ergot alkaloids

Ergot alkaloids share as common structure the tetracyclic ergoline ring systeme of lysergic acid. The clavines consist only of this basis structure, whereas the ergopeptides have an additional peptide moiety linked to the basic tetracyclic ergoline (see figure 1 and 2). The main ergot alkaloids produced by *Claviceps* species are ergometrine, ergosine, ergotamine, ergocornine, ergocristine, ergocryptine, and the group of agroclavines. Ergot alkaloids appear as colourless crystals that are readily soluble in various organic solvents, but insoluble or only slightly soluble in water. As indicated, most alkaloids can form stereoisomeric *–inines* that are biologically less active, and easily convert back into the genuine *–ine* form (Buchta and Cvak, 1999). A distinct group are the ergoclavines formed not only by *Claviceps* species but also by various other fungi. They are considered to be less toxic.

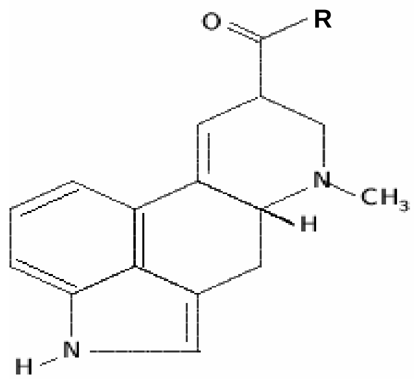

Figure 1. Structure of lysergic acid amines, representing the basic structure of ergot alkaloids.

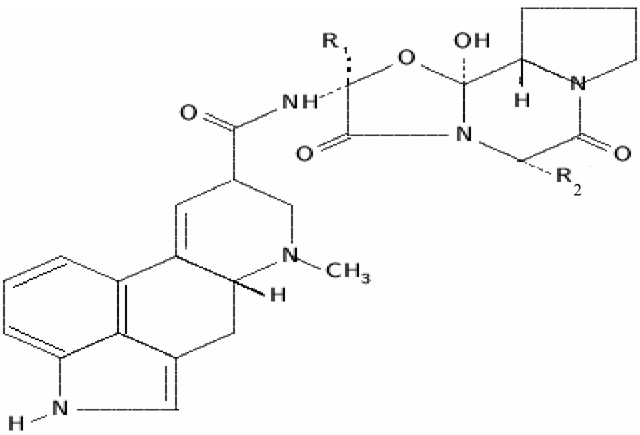

Figure 2. Structure of peptide alkaloids: in ergotamine, for example, R_1 is a methyl group and R_2 is methylbenzene. These peptide alkaloids have chiral centres that allow the presentation as –ine (here ergotamine) and –inine (for details see annex).

3. Methods of analysis and statutory limits

3.1. Analysing the presence of sclerotia

Kernels infected with *Claviceps spp*. are malformed, discoloured and enlarged and can be eliminated by simple sorting (centrifugation) during cleaning and milling. This method has been used for many years, but is rather inaccurate. For example, grass ergots contaminating grains will remain undetected as they differ in size from normal grass seeds, but not from grain seeds.

At present, the maximum permissible level for example in the US and Canada is 300 mg ergot per kg grain. Feed materials exceeding this limit are labelled ergoty rye or ergoty wheat and are discharged or mixed with non-contaminated batches to dilute the burden of total alkoloids (Weipert, 1996). Australia has a limit for ergot of 0.05 % in (food) cereal grains. In Canada various tolerance limits exist for ergot alkaloids in food commodities (wheat, oats, barley, rye, solin, flaxseed, canola, buckwheat, soybeans, mustard seed, peas, Canadian pea beans, Canadian triticale, and Canadian lentils).

In the EU the maximum limit is set to 0.1 % for rye ergot in all feedingstuffs containing non-ground cereals[7]. No limits have been set for individual alkaloids as yet.

3.2. Chemical analysis of ergot alkaloids

For the direct monitoring on the occurrence of ergot alkaloids in food and feed commodities, mainly chromatographic methods of analysis are used. All the common analytical techniques such as thin layer chromatography (TLC), liquid chromatography (LC), and gas chromatography (GC) have been used for the determination of ergot alkaloids. Most methods have been reported for cereals and cereal products. A review of methodology for ergot alkaloids was published by Scott (1995) and is recently updated by the same author (Scott, 2005). The analytical state-of-the-art is yearly reviewed in the AOAC General Referee Reports on Mycotoxins of AOAC International (Trucksess, 2003; Trucksess, 2004). In the 1960's and 1970's TLC was the major technique, which, in principle, allowed the separation of most on the ergot alkaloids. The use of TLC was largely superseded by revered phase LC with fluorescence detection in the 1980's, which is now the most frequently used analytical technique. This technique allows the separation of major ergot alkaloids such as ergometrine, ergosine, ergotamine, ergocornine, alpha-ergocryptine and ergocristine. For example, Wolff *et al.* (1988) describe a HPLC method with fluorescence detection allowing the quantification of ergometrine, ergotamine, ergocornine, L-ergocryptine and ergocristine at levels as low as 2 - 5 ng/g. These individual ergot alkaloids can be related to the total concentration of ergot alkaloids, defined as sum of all substances disappearing after UV treatment, including the –inine isomers of the mentioned 5 ergot alkaloids (Hofmann, 1964; Wolff *et al.*, 1988).

An AOAC collaborative study of this technique carried out in the early 1990s was rejected by AOACs Methods Committee on Natural Toxins, because an insufficient number of collaborators submitted results and recoveries were too low. Capillary electrophoresis, enzyme-linked immunosorbent assay and near infrared spectroscopy were also explored for the determination of ergot alkaloids. Apart from the LC methodology no other technique to determine ergot alkaloids ever reached the stage of formal inter-laboratory validation, so that the performance characteristics are not fully known. Certified matrix reference materials are not available for ergot alkaloids. Pure standards of several ergot alkaloids are commercially available. Currently no analytical proficiency studies on ergot alkaloids have been published.

[7] Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition. OJ L 115, 04.05.1999, p. 32-46.

Sclerotia of *Claviceps* species contain high amounts of ricinoleic acid (Bharucha and Gunstone, 1957; Lorenz, 1979; Coenen *et al.,* 2000), and the analysis of recinoleic acid has considered as surrogate marker to assess the presence of *C. purperea* sclerotia (Buchta and Cvak, 1999). If the free fatty acids released are methylated with diazomethane, these methyl esters can be analysed by gas liquid chromatography. This method allows the detection of 0.3 % ergot in samples as small as 1 - 2 g feed or food (Whittemore *et al*., 1976), and thus does not meet the requirements of the current EU regulations.

3.3 Statutory limits for ergot alkaloids

Currently, no country could be identified that regulates ergot alkaloids in food, and only in a few countries ergot alkaloids in feeds are regulated (FAO, 2004). These are Canada and Uruguay. In Canada guideline limits exist for ergots in feed for swine (6000 µg/kg), in feed for dairy cattle, sheep and horses (3000 µg/kg) and chicks (9000 µg/kg). In Uruguay a limit exists for ergot alkaloids in animal feed (450 µg/kg), whereas ergot alkaloids in animal feed for pigs and female rabbits may not be detectable. Details on the prescribed methods of analysis for the control of compliance to these limits are not presented.

4. Occurrence of ergot in animals feeds

Ergot sclerotia are often larger in size than cereal grains and thus can be removed by mechanical means with conventional grain cleaning equipment such as sieves and separators used during the harvesting process, as mentioned above. Up to 82 % of ergot removal is generally possible by this process with the ergot sclerotia ending up in the 'dockage'. Ergot sclerotia remain intact when cereal grains are in storage but during transport the ergot tends to break into smaller fragments, making cleaning procedures less reliable. The data available on occurrence of ergot in cereals and specifically feedingstuffs is scant. In most instances the occurrence is reported on a weight (%) basis as this relates more directly to controls, which range from 0.1 to 0.3 % in grains. Previous surveys had suggested that in central Europe the total alkaloid concentrations in sclerotia of cereals vary between 0.09 and 0.21 % (Wolff, 1989), and sclerotia originating from grass seeds may contain 0.16 to 0.23 % of alkaloids (Wolff and Richter, 1989). Other data on ergot alkaloids in cereals relate to processed finished products for human consumption rather than to animal feed.

Quantitative analysis for total and individual ergot alkaloids in 14 samples of rye and wheat flour (Scott and Lawrence, 1980) indicated contamination with 6 ergot alkaloids (ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine) at concentrations ranging from 0.3 to 62 µg/kg for individual ergot alkaloids. Ergocristine was the major ergot alkaloid present in the flours with 62 µg/kg being the maximal concentration found. In a survey of ergot alkaloids in ergot-contaminated cereals, in North America, the average total ergot alkaloid content was 0.24 % (Young, 1981a,b; Young and Chen, 1982). On average, the ergot alkaloids in sclerotia from rye, wheat, and triticale comprised ergocristine (31 %), ergocristinine (13 %), ergotamine (17 %), ergotaminine (8 %), ergocryptine (5 %), ergocryptinine (3 %), ergometrine (5 %), ergometrinine (2 %), ergosine (4 %), ergosinine (2 %), ergocornine (4 %), and ergocorninine (2 %). The individual ergot alkaloid composition was uniform throughout a single sclerotium or in different sclerotia from the same head, somewhat less uniform from head-to-head in a given field and highly variable between different fields throughout a region. Moreover, the total alkaloid content was highly variable within-sclerotium, within-head, head-to-head, and on a field-to-field basis. In a comparative study of the total alkaloid concentration in ergots from rye and wheat (caused by infection with *C. purpurea)* and in ergots from pearl millet (caused by infection with *C. fusiformis)* in South-East Asia, it was found that the total ergoline content in the sclerotia of pearl millet was much lower (320 mg/kg) than that in the sclerotia of rye (700 mg/kg) and wheat (920 mg/kg) (Krishnamachari and Bhat, 1976). The ergot alkaloids in the sclerotia of pearl millet were reported to comprise agroclavine, elymoclavine, chanoclavine, penniclavine, and setoclavine.

Monitoring of flours in Swiss mills (Baumann *et al.,* 1985) found that the total alkaloid content of white, semi-white and brown wheat flours varied between 4.2, 30.7 and 103.4 µg/kg, respectively, and in rye flours between 14.5 to 397.4 µg/kg. Data from Sweden (Moller *et al.,* 1993) is comparable, with the highest concentration in wheat and rye products being 24 µg/kg of total alkaloids, and with ergotamine being the most frequently detected toxin.

In contrast, in survey work from Canada ergocristine was the major alkaloid detected in commercial samples of wheat and rye flour at levels up to 62 µg/kg (Scott and Lawrence, 1980). More recent work, again from Canada (Lombaert *et al.,* 2003), focussing on cereals used in infant foods, found the highest incidence of occurrence (31 out of 55 samples) and the highest level (108 µg ergots/kg) in barley-based cereals in comparison with multi-grain cereals (6 out of 75 samples) with the highest level of 47 µg ergot alkaloids per kg. In relation to risks of ergot alkaloids in animal feeds, the levels found in human food are insignificant, but they do provide insights into the prevalence of individual ergot alkaloids in feedingstuffs in the absence of direct survey data. If ergot contaminated grain is milled, between 80 and 90 % of the ergot can be directed to the bran and shorts feed streams. These fractions are regularly destined for animal feed but there is no available data on contamination levels. Care also needs to be taken that grain 'dockage' does not ultimately get routed into animal feed.

5. Exposure

As mentioned before, the alkaloid concentration and -composition (patterns of individual alkaloids) are highly variable (Bush *et al.,* 1997).

Assuming that the susceptibility of cereals to *Claviceps spp* under European conditions can be ranked as follows: Hybrid rye > population rye > triticale > durum wheat, maize > wheat, barley > oats, exposure can be estimated according to common feeding practices. For example, the use of rye (which is the major source of ergot) is restricted in young growing monogastric animals due to the presence of anti-nutritive substances, others than ergot. The rye percentage in the daily ration of fattening pigs may reach 50 %, whereas rye should be completely avoided in feeds for piglets with a live-weight of less than 15 kg. A rye percentage of 30 % and 20 %, respectively, should not be exceeded in feeds for gravid or lactating sows.

The composition of feeds for cattle depends largely on the production state. For examples, high yielding dairy cows and fattening beef can obtain up to 70 % of their diet in the form of concentrates. These concentrates may contain more than 50 % cereals and in turn the exposure level may thus reach the same magnitude as for monogastric animals. Hence, the rate of exposure may become critical in ruminants, particularly if the animals are also exposed to ergot alkaloids from roughages containing grass infected with various *Clavicipitaceae*.

In complete diets for horses and rabbits, the proportion of rye, as the major ergot plant, should not exceed 5 % and 10 %, respectively. The diets of these species are completed with roughage such as hay or grass, contributing also to total exposure as mentioned above for ruminant species.

In summary, herbivorous animals consuming small grains and pasture grasses as prominent part of their diets are likely to be exposed in practice to a complex mixture of ergot alkaloids. Exposure to these mixtures may result in synergistic, but also in antagonistic effects exerted by the individual alkaloids. Moreover, animals can be exposed at the same time to other fungal toxins present in their diet. Hence, the case reports as presented in the literature and the various studies describing only the percentage of ergots in the feeds (but not the absolute amount and composition of the ergot alkaloids) are difficult to interpret as long as the toxicological potency and the biological effects of individual alkaloids and mixtures thereof remain unknown (Gareis and Wolff, 2000; Swamy *et al.,* 2002).

6. Toxic effects in livestock

6.1. Acute toxicity

Ergot alkaloids vary in their acute toxicity as demonstrated in the comparison of LD_{50} values for individual compounds in rabbits, rats and mice. For example, following intravenous injection, the LD_{50} for ergometrine was 3.2 mg/kg b.w. in rabbits, 120 mg/kg in rats and 160 mg/kg in mice. The LD_{50} for ergotamine varied between 3.0 mg/kg in rabbits, 38 mg/kg in rats and 265 mg/kg b.w. in mice, respectively (Griffith *et al.,* 1978). The same investigations also demonstrated the differences in oral bioavailability, as for example the oral LD_{50} for ergotamine in rabbits was 550 mg/kg b.w. in contrast to 3.0 mg/kg b.w. after IV injection, whereas the oral LD_{50} for ergometrine increases by a factor of 2.8 to 8.7 in mice and rats, respectively, as compared to the intravenous LD_{50} (Griffith *et al.,* 1978). Symptoms associated with these acute intoxications include vomiting, diarrhoea, cardio-vascular collapse, convulsions, agony and abortions (Guggisberg, 1954; Lewin, 1962).

Based on their use as medicinal products, ergot alkaloids are known to act as partial agonists and antagonists of α-adrenergic, dopaminamergic and serotinergic receptors. The major biological effects such as vasoconstriction, uterus contraction, emesis and reduced prolactin secretion with subsequent agalactia are related to these principal mechanisms. The acute form of ergotism, which is seldom observed, is characterized by lethargy, convulsions and depressions, followed by death due to paralysis of the respiratory centre (Lorgue *et al.,* 1987). Chronic exposure to moderate amounts of ergot alkaloids results in reduced weight gain, low reproductive efficiency and agalactia (Floss *et al.,* 1973; Döcke, 1994; Janssen *et al.,* 2000). Other clinical symptoms of chronic ergotism are gangrene of extremities (ear, tail, hoofs, combs), abortion, convulsions, hypersensitivity and ataxia (Benett and Klich, 2003; Diekman and Green, 1992; Hussein and Brasel, 2001).

Under natural circumstances the total alkaloid as well as the level of individual ergot alkaloids vary (dependent on the fungal strain, the host plant, environmental conditions). Hence, it is virtually impossible to relate specific data as described in the literature to the exposure to individual toxins, as mentioned above (Bailey *et al.,* 1973; Mühle and Breuel, 1977; Klug, 1986). Moreover, it should be mentioned that sclerotia contain also various dyes, representing anthrachinone derivatives and ergochromes as well as secalonic acid (Franck, 1984), which may contribute to the toxicity but which are less stable during storage than other ergot alkaloids (Richter *et al.,* 1990; Wolff, 1992).

Therefore, only a general description of the susceptibility of the major farm animal species can be presented.

6.2. Adverse effects in Pigs

Pigs become exposed to ergot alkaloids following the ingestion of infected rye, wheat and barley (*Claviceps purpurea*) or by sorghum ergot (*Claviceps africana*). The most prominent clinical sign is agalactia in sows, often leading to starvation of piglets. Other symptoms are feed refusal, reduced weight gain and eventually abortion.

In general it can be assumed that 0.1 % ergot in grains will be able to reduce weight gain in fattening pigs; higher levels (3 %) have been implicated in feed wasting and significant growth retardation. For example, in a feeding trial with weaned pigs in Canada ergot sclerotia were added to the basal diet at ratios of 0.05, 0.10, 0.25, 0.50 and 1.0 % respectively. Analysis revealed that these amounts corresponded to a total alkaloid concentration of 1.04, 2.07, 5.21, 10.41 and 20.82 mg/kg feed, respectively (Oresanya *et al.,* 2003). Data show that the average daily weight gain was significantly reduced if sclerotia exceeded 0.25 % and in the highest exposure group the animals gained 37 % less than the controls. Moreover, average daily feed intake was reduced by 10 % and feed efficiency by 29 % over the entire period. In addition, serum prolactin concentrations were reduced by 43 % in the highest dose group as compared to controls. The authors concluded that the maximum tolerable level based on average daily weight gain and average daily feed intake corresponded to 2.07 mg and 1.04 mg (0.05 % sclerotia) alkaloid/kg feed, respectively (Oresanya *et al.,* 2003).

In a report of the Iowa State University (Holden and Zimmerman, 1998) experiments with growing and finishing pigs are described. The basal diet contained 0.52 mg/kg of total ergot alkaloids, the experimental diets 1.04 mg/kg and 1.62 mg/kg, respectively. Data show a reduced feed intake only during the growing phase in the group of pigs exposed to the highest concentration. However, at the end of the trial no significant differences were observed between the three groups, neither in weight gain nor in feed intake or feed conversion.

Feeding experiment with sorghum ergot (*Claviceps africana*, containing predominantly dihydro-ergosine representing 80 % of total alkaloids) showed that diets containing 1.5 % sorghum ergot given 6 - 10 days prior to farrowing, impairs milk production in sows (Kopinsky *et al.,* 1999).

In a subsequent trial with 0.3 % ergot given from 6 weeks prior to farrowing until piglets were weaned at 4 weeks of age, no significant adverse effects were observed in multiparous sows, but in gilts blood prolactin levels were significantly reduced. The authors concluded that the current tolerable level in the USA of 0.3 % ergot (equivalent to 1.5 mg/kg total ergot alkaloids) is safe for feeding sows around farrowing, but might exert undesirable adverse effects in gilts (Kopinsky *et al.,* 1999).

In a review on the effect of ergot alkaloids in pigs and poultry Mainka *et al.* (2003) summarized data from 14 individual reports related to the effect of different ergot levels on feed intake and growth performance in pigs. The data are difficult to compare and hardly representative as in most publications only the percentage of sclerotia in the diet, and the relative concentrations (in %) of individual alkaloids are presented. As mentioned above, feed intake, feed conversion and daily weight gain might be impaired in the presence of higher amounts of ergot alkaloids, but the presented data do not allow conclusions on a NOAEL or minimal toxic doses for typical ergot alkaloids. Even at the lowest concentration tested (0.02 % and 0.05 % sclerotia in the diet) a reduced feed intake could be observed (Dignean *et al.,* 1986; Mainka *et al.,* 2003). At a level of 0.4 % ergot sclerotia, feed intake and weight gain were significantly reduced. Interestingly, the piglets exposed to 0.1 and 0.2 % ergot sclerotia, respectively, showed an optimal feed conversion rate, that was even better than that of the control group, and it has been hypothesized that this improvement is related to an ergoline-induced release of growth hormone.

Experiments with fattening pigs indicated that even a concentration of 0.05 % ergot sclerotia in feed might exert a slight effect on feed intake and weight gain. These adverse effects became significant at levels from 0.1 % (Harols *et al.,* 1974). In contrast, even 0.2 % ergot sclerotia exerted no adverse effects in finishers (Meyer, 1999). Reduced nitrogen retention was observed following exposure to 5.0 % ergot sclerotia (Whittemore, 1976), but also following exposure to 0.1 % ergot sclerotia (Friend and MacIntyre, 1970) which points again to the difficulties in comparing data from different geographical regions with different compositions of ergot alkaloids in the sclerotia.

In adult sows, 0.1 % ergot sclerotia were found to reduce milk production and a level of 0.5 % ergot sclerotia in the diet prior to farrowing resulted in an increased number of weak or stillborn piglets (Nordskog and Clark, 1945). Abortions occurred when sows were fed diets containing 0.53 % ergot sclerotia (Campbell and Burfening, 1972). In an experiment with Canadian cereals a reduced birth weight of piglets was recorded already at levels of 0.2 % ergot sclerotia (Dignean *et al.,* 1986).

6.3. Adverse effect in cattle

Lameness, sometimes leading to gangrene, is a common symptom observed in cattle when the feed contains more than 10 g ergot/kg. The symptoms are more pronounced when the animals are kept outside under cold weather conditions (Mantle, 1977). Four out of 6 animals administered ergotamine tartrate, orally, at 1 mg/kg body weight per day, died within 10 days (Woods *et al*., 1966). The animals became acutely ill within 1 - 2 days, the principal signs being anorexia, hyperventilation, cold extremities, salivation, and, occasionally, tongue necrosis. Post-mortem examination of the most seriously affected animals revealed extensive intestinal inflammation.

In an Australian study (Blaney *et al.,* 2000), cattle (heifers and young steers) were kept on grain sorghum for a period of 28 days. Analysis indicated 0.44% of ergot sclerotia (w/w) with an average alkaloid content of the primary grain heads of 3.5 mg/kg dry matter and of tiller heads of 4.6 mg/kg dry matter (the alkaloid concentration of leaf and stalks was < 1 mg/kg dry matter).

None of the animals showed clinical signs of intoxications, but serum prolactin levels were significantly decreased, particularly in the young steers, which already physiologically have lower levels than heifers. The *major* alkaloid detected was dihydro-ergosine, which is consistent with the known alkaloid production by *C. africana*. The estimated daily intake was about 35 mg (total alkaloids) per animal (the body weight varied between 400 - 405 kg for the heifers and 283 - 295 kg for the young steers) (Blaney *et al.,* 2000).

An outbreak of bovine abortion associated with the ingestion of ergot was reported by Appleyard (1986). Eleven out of 36 suckler cows, all in late pregnancy, aborted in 7 - 11 days following introduction to a rye grass pasture heavily infested with ergot. At least 25 % of the rye seed heads contained sclerotia of *C. purpurea,* with up to 8 sclerotia present on one seed head. The individual sclerotia contained 1.57 mg total ergot alkaloids/g, consisting of 67 % ergotamine and 17 % ergotaminine, and smaller amounts of ergometrine. Ergocryptine and ergosine were detectable in these sclerotia as a mixture of their natural occurring isomers (-ines and -inines).

As mentioned before, cattle might be concomitantly exposed to alkaloids originating from different *Claviceps* species forming sclerotia and small grains and grasses and also to related toxins originating from *Neothyphodium* and *Epicloe* species (Paterson *et al.,* 1995; Porter, 1995; Fink-Gremmels, 2005).

6.4. Adverse effects in sheep

Four lambs were administered aqueous suspensions of milled ergot (from *C. purpurea)* through a stomach tube, over a 2-month period (Loken, 1984). The doses ranged from 0.12 to 0.75 g sclerotia/kg body weight. The sclerotia contained approximately 4 g ergot alkaloids/kg, composed of ergotamine (15 %), ergosine (35 %), and ergocristine (5 % each). One lamb, dosed with 0.12 g sclerotia/kg body weight, was kept indoors at 15 - 17°C and did not develop any symptoms. The other 3 animals, given higher doses and kept outdoors, became ill after 2 - 6 days, with signs that included dullness, inappetence, high pulse rate, diarrhoea, edema of the hind legs and tail, and lameness. Post-mortem findings included inflammation and necrosis of the forestomach and intestinal mucosa.

6.5. Adverse effects in poultry

Poultry has long been considered as sensitive species towards ergot alkaloids. This assumption was based on results of the so-called comb test (parenteral injection of extracts followed by discoloration of the comb due to alkaloid-induced vasoconstriction) that has been used as bioassay to demonstrate the presence of ergot alkaloids in food materials in the past.

Data on the tolerance of poultry to individual ergot alkaloids present in feed materials is inconsistent. O'Neil and Rae (1965) describe increased mortality in broilers exposed to a diet with 0.3 % ergot sclerotia, whereas Bragg *et al.* (1970) found no adverse effects up to a contamination level of 0.8 % ergot sclerotia. In the latter study mortality occurred following exposure to 1.6 % ergot sclerotia. Rotter *et al.* (1985a) exposed male hybrids to increasing concentrations of ergot-contaminated wheat. At a level of 2 - 5 % ergot in the diet a transient decrease in feed intake was observed. However, already at 4 % ergot in the diet, mortality rates increased with prolonged exposure. In contrast, Mainka *et al.* (2003) did not observe adverse effects at a concentration of 0.4 % ergot in rye given to broiler chicks.

Leghorn chickens were fed diets containing ergotamine tartrate at levels up to 800 mg/kg in 7 to 10-day trials as well as in a 51-day trial (Young and Marquardt, 1982). In the short-term trials, only the highest level (800 mg/kg) had an effect on performance, in terms of a slight decrease in growth rate and a slight increase in feed consumption. At the 250 mg/kg level, toe necrosis was observed, as well as cardiomegaly. There were no pathological effects on the brain, liver, or muscle tissues, even at the highest level. In the long-term study (51 days), effects were similar to those observed in the 7 to 10-day trials. No residues of ergotamine were detected in tissues.

Data from O'Neil and Rae (1965) suggest that adult layers are less sensitive as compared to broiler chicks, as even a concentration of 9 % ergot in the feed did neither increase mortality nor affected egg size, eggshell quality and hatchability. Klein and Steinruck (1987) reported, however, that in layers feed intake and egg production were significantly reduced when 2 % ergot sclerotia, originating from rye or triticale were included in the diet of layers.

Taken together, these data show a considerable variability in the response to ergot exposure in poultry broilers, which seem to be more sensitive than adult layers. In an attempt to explain the variability in different feeding trials it has been suggested that long-term exposure to ergot alkaloids results in an adaptive response (reversing the reduced feed intake) that is known also for various other dopaminergic compounds (Burfening, 1973; Rotter, 1985b). It is worthwhile to mention, that based on the nutritional considerations, rearing chicks and laying hens can accept feed containing up to 15 and 20 % of rye, respectively, whereas as balanced diet for chickens and broilers contains as a maximum 5 % rye. These nutritional requirements provide a natural protection of the most sensitive age groups against rye sclerotia.

6.6. Adverse effects in horses

A large number of reports address the adverse effects of ergovaline, originating from the contamination of pasture grasses with *Neotyphodium spp* in horses (Abney *et al.,* 1993; Cheeke, 1995; Cross, *et al*., 1995; Vivrette *et al.,* 2001). Major effects include delayed parturition, agalactia of mares (associated with altered prolactine levels), and incidentally neurotoxic symptoms (Cross et al., 1995). Mares are sensitive to ergovaline levels as low as 50 – 100 ppb, whereas cattle seem to tolerate 1000 - 2000 ppb.

In contrast, data on intoxications in horses related to the different ergot alkaloids that are commonly found in *Claviceps* contaminated feed materials are scarce. Adverse effects are associated with exposure to ergot sclerotia include depression of serum prolactin and progestagens and animals show a prolonged gestation, a thickened oedematous placenta and agalactia. Foals born without the normal increase of maternal progestagenes suffer from hypo-adrenocortical function and are small, weak or even stillborn (Bendemeuhl *et al.,* 1995).

6.7. Adverse effects in rabbits

Experimental data with certain ergot alkaloids that have been tested for their application in human or veterinary medicine suggest that rabbits are among the most sensitive animal species (Griffith *et al.,* 1978). Specific data other than experimental data on the acute toxicity are, however, lacking in the available literature.

7. Toxicokinetics

Kinetic data on individual ergot alkaloids are scarce, even for those used as therapeutic agents. It is generally assumed that the hydrophilic amides (for example ergometrine) are rapidly absorbed whereas the less water-soluble alkaloids of the ergotamine group have a lower oral bioavailability, approaching 62 % in humans (Aellig and Nüesch, 1977). In contrast, Whittemore *et al.* (1976 and 1977) described an oral availability of approximately 90 % of ergotalkaloids (not specified) in pigs.

Following oral ingestion ergot alkaloids undergo extensive first-pass metabolism in the liver. A prominent example is methysergide, which has an oral bioavailability of approximately 13 % and is converted in the liver into the active methylergometrine.

Data on tissue distributions remain incomplete although it is (empirically) known that some compounds cross the blood-brain barrier (see hallucinogenic effect of lysergic acid derivatives and the use of ergot alkaloids in the treatment of Parkinson patients) and the placenta barrier. Ergot alkaloids are biotransformed in the liver, but the structure of many metabolites has not been elucidated yet, although it is likely that these retain biological activity (Lorenz, 1979; Tfelt-Hansen *et al.,* 1995). The excretion is often biphasic depending on the degree of enterohepatic recirculation. Specific kinetic data for individual ergot alkaloids in food producing animals are not available, with the exception of few data for ergovaline in horses.

Comparative investigations in rabbits, with dihydroergocryptine, dihydroergotamine and dihydroergocornine given intravenously at a dosage of 20 mg/kg b.w. showed that the highest concentrations were measurable in liver, kidney, spleen and muscle tissue. None of these dihydro-compounds were able to cross the blood-brain barrier. Total body elimination was calculated to be < 20 hours (Filipov *et al.,* 1999).

8. Carry-over and residue formation

Corresponding to the few available data on the toxicokinetics of ergotalkaloids in target animal species, the information regarding a potential carry-over into edible tissues is scarce. Young and Marquardt (1982) fed poultry chickens various concentrations of ergotamine tartrate. Only at the highest concentrations (810 mg/kg feed) residual amounts of ergotamine could be detected in tissues (liver, muscle), which did not exceed 10 µg/kg. However, in these experiments only the parent compound (ergotamine) was analysed and hence the presence of metabolites cannot be excluded. Following oral exposure via feed to ergot alkaloids, no residues were detectable in the major tissues of pigs (Whittemore *et al.,* 1976, 1977) although in this study quantitative data on total exposure and limit of quantification for individual alkaloids in animal tissues are lacking.

The carry-over following exposure of dairy cows to 50 g ergot sclerotia per animal (approximately 400 kg b.w.) resulted in milk concentrations (total alkaloid content) reaching 0.086 mg/L (Parkheava, 1979). Wolff *et al.* (1995) exposed dairy cows with feed to an alkaloid concentration corresponding to 1835 µg/animal/day (equivalent to approximately 3 µg/kg b.w.) and failed to detect residues of ergot alkaloids in milk. The authors concluded that the carry-over rate into milk is less than 10 % of the applied dose.

CONCLUSIONS

- Ergots, the sclerotia formed by various fungal species belonging to the genus *Clavicipitaceae*, occur in rye and other grains, as well as in sorghum and in cold season grasses. The alkaloid concentrations are very variable and a consistent relationship between the amount of sclerotia and the total ergot alkaloid (ergoline) concentration cannot be established.
- At present, the degree of variability in ergot alkaloid pattern in relation to fungal species, geographical distribution as well as in relation to the host plant is not known.
- Although the exact mechanism of action at the receptor level remains to be elucidated, most of the adverse effects of ergot alkaloids can be associated with interactions with α-adrenergic, dopaminergic and serotonergic receptors.
- Individual ergot alkaloids have different and dose-dependent receptor selectivity (binding affinity). Consequently, the biological effects of the various complex mixtures to which animals are exposed remain unpredictable. Hence, due to the variations in ergot alkaloid pattern, measurements of a single marker alkaloid or the total ergot alkaloid concentration could not be used to predict the nature and/or the intensity of adverse effects in animals.
- Data on the sensitivity of agricultural animal species towards ergot alkaloids are incomplete and do not allow the establishment of tolerance levels for individual ergot alkaloids and mixtures thereof. Available data indicate that adverse effects may occur in agricultural animals particularly in pigs after intake of feed contaminated with ergot at levels close to the current EU limit.
- The available data do not allow identifying marker ergot alkaloids that could be monitored in all feed materials as indicators for ergot contamination.
- The very limited and often incomplete data on tissue distribution and residual concentrations in edible tissues, milk and eggs do not allow an estimate of carry-over rates. Available data, however, provide no evidence that ergot alkaloids accumulate in edible tissues.
- Levels of alkaloids measure as yet in animal tissues indicate that these are unlikely to be an important source of human exposure to ergot alkaloids.

RECOMMENDATIONS

- Data is needed to define the variability of ergot alkaloid patterns in European feed materials.
- A representative standard mixture of frequently occurring natural ergot alkaloids mixtures should be established.
- Validated analytical methods for the quantification of ergot alkaloids in feed materials are needed as a prerequisite for a survey on the occurrence of ergot alkaloids in feed materials in Europe. Analytical techniques should aim to detect the major ergot alkaloids as well as their corresponding biologically active metabolites formed in exposed animals.
- More data should become available on toxic effects (and toxic interactions) in target animal species of the ergot alkaloids prevailing in European grains and grasses.

REFERENCES

Abney, L.K., Oliver, J.W. and Reinemeyer, C.R. 1993. Vasoconstrictive effects of tall fescue alkaloids on equine vasculature. J Equine Vet Sci 13:334-340.

Aellig, W.H. and Nuesch, E. 1977. Comparative pharmacokinetic investigations with tritium-labeled ergot alkaloids after oral and intravenous administration in man. Int J Clin Pharmacol 15:106-112.

Amelung, D. 1999. Mutterkorn im Roggen. Getreide Magazin 5:138-145.

Appleyard, W.T. 1986. Outbreak of bovine abortion attributed to ergot poisoning. Vet Res 118:48-49.

Bailey, J. Wrathall, A.E. and Mantle, P.G. 1973. The effect of feeding ergot to gilts during early pregnancy. Br Vet J 129:127-133.

Baumann, U., Hunziker, H.R. and Zimmerli, B. 1985. Muterkornalkaloide in schweizerischen Getreideprodukten. Mitt Geb Lebensmittelunters Hyg 76:609-630.

Benett, J.W. and Klich, M. 2003. Mycotoxins. Clinical Microbiology Reviews 16:497-516.

Betina, V. 1994. Alkaloids. In: Bioactive secondary metabolite of microorganisms. Chapter 8, p 136-144.

Bharucha, K.E. and Gunstone, F.D. 1957. Vegetable oils 6. The component acid of ergot oil. J Chem Soc 610-614.

Blaney, B.J., McKenzie, R.A., Josey, B.J., Ryley, M.J. and Downing, J.A. 2000. Effect of grazing sorghum (sorghum bicolour) infected with ergot (Claviceps africana) on beef cattle. Austr Vet J 78:124-125.

Bragg, D.B., Salem, H.A. and Devlin, T.J. 1970. Effect of dietary triticale ergot on the performance and survival of broiler chicks. Can J Anim Sci 50:259-264.

Buchta, M. and Cvak, L. 1999. Ergot alkaloids and other metabolites of the genus Claviceps. Ergot: the genus Claviceps 6:173-200.

Burfening, P.J. 1973. Zit. Nach: Rotter, R.G., Marquardt, R.R. and Crow, G.H. 1985b. A comparison of the effect of increasing dietary concentration of wheat ergot on the performance of leghorn and broiler chicks. Can J Anim Sci 65:963-974.

Bush, L.P., Wilkinson, H.H. and Schardl, C.L. 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol 114:1-7

Campbell, C.W. and Burfening, P.J. 1972. Effects of ergot on reproductive performance in mice and gilts. Can J Anim Sci 52:567-569.

Cheeke, P.R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. J Anim Sci 73:909-918.

Coenen, M., Landes, E. and Kamphues, J. 2000. Mutterkorn und Ergotalkaloidmuster in Futtermitteln sowie Reduktion des Ergotalkaloidgehaltes durch Hitze und Alkalisieren. Unveröffentlicht. (Unpublished).

Cross, D.L., Redmond, L.M. and Strickland, J.R. 1995. Equine fescue toxicosis: signs and solutions. J Anim Sci 73:899-908.

Diekman, M.A. and Green, M.L. 1992. Mycotoxins and reproduction in domestic livestock. J Anim Science 70:1615-1627.

Dignean, M.A., Schiefer, H.B. and Blair, R. 1986. Effects of feeding ergot- contaminated grain to pregnant and nursing sows. J Vet Med A 33:757-766.

Döcke, F. 1994. Prolaktin. In: Döcke, F.(ed.). 1994. Veterinämedizinische Endokrinologie 3. Aufl. Gustav Fischer Verlag Jena Stuttgart, 204-207.

Engelke, T. 2002. Ansätze für eine integrierte Bekämpfung des Mutterkorns im Roggen. Dissertation Universität Göttingen, 1. Aufl., Cuvillier Verlag Göttingen.

FAO (Food and Agriculture Organization), 2004. Worldwide Regulations for Mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper 81, Rome, Italy.

Filipov, N.M., Thompson, F.N., Tsunoda, M. and Sharma, R.P. 1999. Region-specific decrease of dopamine and its metabolites in brains of mice given ergotamine. J Toxicol Env Heal 56:47-58.

Fink-Gremmels, J. 2005. Mycotoxins in forages. In: Diaz, D. The Mycotoxins Blue Book, Nottingham University Press. pp. 249-268.

Floss, H.G., Cassady, J.M. and Robbers, J.E. 1973. Influence of ergot alkaloids on pituitary prolactin and prolactin-dependent process. J Pharm Sci 62:699-715.

Friend, D.W. and MacIntyre, T.M. 1970. Effect of rye ergot on growth and N-retention in growing pigs. Can J Comp Med. 34:198-202.

Gareis, M. and Wolff, J. 2000. Mykotoxinbelastung der Nutztiere über Futtermittelaufnahme und der Verbraucher durch Lebensmittel tierischer Herkunft. Mycoses 43:79-83.

Griffith, R.W., Grauwiler, J., Hodel, C., Leist, K.H. and Matter, B. 1978. Toxicologic considerations. Chapter 12, 805-838. In: Berde, B.; Schild, H.O. 1978. Ergot alkaloids and related compounds. Handb Exp Pharm 49, Springer Verlag Berlin, Heidelberg, NY.

Guggisberg, H. 1954. Mutterkorn. Vom Gift zum Heilstoff. Verlag S. Karger Basel, NY.

Harrold, R.L., Dinussen, W.E., Hangse, C.N., Johnsosn, J.N. 1974. Ergot in growing and finishing swine rations. N.Dank.Farm.Res. 32, 13.15.

Hofmann, A. 1964. Die Mutterkornalkaloide. Stuttgart: Enke Verlag.

Holden, P. and Zimmerman, D. 1998. Effect of Ergot-Contaminated Barley on Growing-finishing Pig Performance. Asl-R1482, report of the Iowa State University.

Hussein, S.H. and Brasel, J.M. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167:101-134.

Janssen, G.B., Beems, R.B., Speijers, G.J.A. and Egmond, H.P. 2000. Subacute toxicity of α–ergocryptine in sprague-dawley rat. 1: General toxicological effects. Food Chem Toxicol 38:678-688.

Kamphues, J. and Drochner, W. 1991. Mutterkorn in Futtermitteln-ein Beitrag zur Klärung moglicher mutterkornbedingter Schadensfälle. Tierärztl Praxis 19:1-7.

Klein, F.W. and Steinruck, U. 1987. Untersuchungen mit mutterkorn in der hühnerfütterung. Kraftfutter 9, 336-341.

Klug, C. 1986. Bestimmung von Mutterkornalkaloiden in Lebensmitteln. Max von Pettenkofer-Insitut des Bundesgesundheitsamtes, MvP-Hefte 2.

Kopinsky, J.S., Blaney, B.J., McVeigh, J.F. and Murray, S.A. 1999. Tolerance of sows to the regulatory limit of 0.3 % sorghum ergot feed before and after farrowing. Darling Downs Bacon Pig Science Seminar p 8-9. www.dpi.gld.gov.au/pigs/7506.html.

Krishnamachari, K.A.V.R., and Bhat, R.V. 1 976. Poisoning by Ergoty Bajra (Pearl Millet) in Man. Indian Journal of Medical Research 64:1624 1628.

Lewin, L. 1962. Gifte und Vergiftungen, Lehrbuch der Toxicologie. 5. Aufl., Karl F. Haug Verlag, Ulm/Donau.

Loken, T. 1984. Ergot from meadow grass in Norway: chemical composition and toxicological effects in sheep. Nord vet Med 36:259-265.

Lombaert, G.A., Pellaers, P., Roscoe, V., Mankotia, M., Neil, R. and Scott, P.M. 2003. Mycotoxins in infant foods from the Canadian retail market. Food Additives and Contaminants 20:494-504.

Lorenz, K. 1979. Ergot on cereal grains. CRC Critical Reviews in Food Science and Nutrition 11:311-354.

Lorgue, G., Lechenet, J. and Riviere, A. 1987. Ergot. In: précis de toxicology clinique veterinaire.1st ed, Editions du Point Veterinaire, Maisons-Alfort, France. p97.

Mainka, S., Dänicke, S. and Coenen, M. 2003. Zum Einfluss von Mutterkorn im Futter auf Gesundheit und Leistung von Schwein und Huhn. Übers. Tierernährg 31:121-168.

Mainka, S., Dänicke, S., Wolff, J., Matthes, S. and Böhme, H. 2003. Zum Einfluss von Mutterkorn im Futter auf Absetzferkel und Masthähnchen. Tagungsband 25. Mykotoxin-Workshop Giessen:19-21.

Mantle, P.G. 1977. Ergotism in cattle, sheep, swine. In: Wyllie, T.D. and Morehouse, L.C., ed. Mycotoxic fungi, mycotoxins, mycotoxicoses: an encyclopedia handbook, New York, Marcel Dekker, Vol. 2, pp. 145-151, 207-213, 273-275.

Mantle, P.G. 1996. Detection of ergot in a dairy cattle feed component by GC/MS. Journal of Dairy Science 79:1988-1991

Meyer, A. 1999. Mutterkorn in der Ration. Hannoversche Land- und Forstwirtsch. Zeitung 28:32-33.

Moller, T., Brostedt, S. and Johansson, M., 1993, Ergot alkaloids in cereal products in Sweden. Var Foda 45:308-311.

Nordskog, R and Clark, B.J. 1945. Ergotism in pregnant sows, female rat and guinea pigs. Am. J. Vet. Res. 6, 107-116.

Mühle, E. and Breuel, K. 1977. Das Mutterkorn. 2. Aufl. A.Ziemsen Verlag Wittenberg Lutherstadt.

O'Neill, J.B. and Rae, W.J. 1965. Ergot tolerance in chicks and hens. Poult. Sci. 44, 1404.

Oresanya, T.F., Patience, J.F., Zijlstra, R.T., Beaulieu, A.D., Middleton, D.M., Blakey, B.R. and Gillis, D.A. 2003. Defining the tolerable level of ergot in the diet of weaned pigs. Can. J. Anim. Science 83, 493-500.

Panaccione, D.G., and Annis, S.L. 2001. Significance of fungal peptide secondary metabolites in the agri-food industry. In Applied Biology and Biotechnology: Food and Fungi, vol. I., Khachatourians, G.G., and Arora, D.K. (Eds.) Elsevier, The Netherlands, pp. 115-143.

Parkheava. 1979. Zit. nach: Wolff J, Richter WIF, Spann B. 1995. Mutterkornalkaloide in der Milch? VDLUFA-Schriftenreihe, Kongressband 18:515-524.

Paterson, J., Forcherio, C., Larson, B., Samford, M. and Kerley, M. 1995. The effects of fescue toxicosis on beef cattle productivity. J Anim Sci 73:889-898.

Porter, J.K. 1995. Analysis of endophyte toxins: fescue and other grasses toxic to livestock. J Anim Sci 73:871-880.

Van Rensburg, S.J. and Altenkirk, B. 1974. *Claviceps purpurea:* ergotism. In: Purchase, I.F.H., ed. Mycotoxins, Amsterdam, New York, Oxford, Elsevier Science Publishers, pp. 69-96.

Richter, W.I.F., Röhrmoser, G., Komusinksi, S., Rindle, C, and Wolff, J. 1990. Einfluss der Lagerdauer von mit Mutterkorn kontaminiertem Futtergetreide und dessen Rationsanteil auf Zuwachs und Futterverwertung von Ferkeln. Das wirtschaftseignen Futter 36:236-245.

Rotter, R.G., Marquardt, R.R. and Young, J.C. 1985a. Effect of ergot from different source and of fractionated ergot on the performance of growing chicks. Can J Anim Sci 65:953-961.

Rotter, R.G., Marquardt, R.R. and Crow, G.H. 1985b. A comparison of the effect of increasing dietary concentration of wheat ergot on the performance of leghorn and broiler chicks. Can J Anim Sci 65:963-974.

Scott, P.M. and Lawrence, G.A. 1980. Analysis of ergot alkaloids in flour. J Agric Food Chem 28:1258-1261.

Scott, P.M. 1995. Mycotoxin methodology. Food Additives and Contaminants 12:395-403.

Scott, P.M. 2005. Ergot alkaloids – Analysis. In: Management of Mycotoxins in Food and Feeds for Improving Public Health. International Agency for Research in Cancer, Lyon, France, *in press.*

Smith, J.E. and Henderson, R.S. 1991: Mycotoxins in Animal Food. CRC Press, Inc. Boca Raton.

Sülflohn, K. 2005. Das geltende Futtermittelrecht mit Typenliste für Einzel- und Mischfuttermittel- Stand December 2004. Grüne Broschüre 2005.

Swamy, H.V.L.N., Smith, T.K., Cotter, P.F., Boermans, H.J. and Sefton, A.E. 2002. Effects of feeding blends of grains naturally contaminated with mycotoxins on production and metabolism in broilers. Poult Sci 81:966-975.

Tfelt-Hansen, P., Saxena, P.R. and Ferrari, M.D. 1995. Ergot alkaloids. Handbook of Clinical Neurology 21:61-78.

Trucksess, M.W. 2003. General Referee Reports. Committee on Natural Toxins and Food Allergens. J AOAC International 86:129-138.

Trucksess, M.W. 2004. General Referee Reports. Committee on Natural Toxins and Food Allergens. J AOAC International 87:275-284.

Urga, K., Debella, A., Medihn, Y.W., Agata, N., Bayu, A. and Zewdie, W. 2002. Laboratory studies on the outbreak of gangrenous ergotism associated with consumption of contaminated barley in Arsi, Ethiopia. Ethiopian Journal of Health Development 16:317-323.

Vivrette, S., Stebbins, M.E., Martin, O., Dooley, K. and Cross, D.L. 2001. Cardiorespiratory and thermoregulatory effects of endophyte-infected fescue in exercising horses. Equine Vet Sci 21:65-67.

Weipert, D. 1996. Zit. nach: Engelke T. 2002. Ansätze für eine integrierte Bekämpfung des Mutterkorns im Roggen. Dissertation Universität Göttingen, 1. Aufl., Cuvillier Verlag Göttingen.

Whittemore, C.T., Macer, R.C.F., Miller, J.K. and Mantle, P.G. 1976. Some consequences of the ingestion by young and growing pigs of feed contaminated with ergot. Res Vet Sci 20:61-69.

Whittemore, C.T., Miller, J.K. and Mantle, P.G. 1977. Further studies concerning the toxicity of ingested ergot sclerotia (*Claviceps purpurea*) to young and growing pigs. Res Vet Sci 22**:**146-150.

Wolff, J., Neudecker, C., Klug, C. and Weber, R. 1988. Chemische und toxikologische Untersuchungen über Mutterkorn in Mehl und Brot. Z Ernährungswiss 27:1-22.

Wolff, J. 1989. Mutterkorn in Getreide. Getreidekonservierung und Futterschäden durch Getreide 30. Bericht der Praktiker- Informationstagung; 14.03.1989; Grub. Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten, editors.

Wolff, J. and Richter, W.I.F. 1989. Chemische Untersuchungen an Mutterkorn. Getreide, Mehl und Brot 43:103-108.

Wolff, J. 1992. Mutterkorn in Getreide und Getreideprodukten. In: Ocker, H.D. (ed). Rückstände und Kontaminanten in Getreide und Getreideprodukten. Behrs Verlag, Hamburg, Chapter 7.

Wolff, J., Richter, W.I.F. and Spann, B. 1995. Mutterkornalkaloide in der Milch? VDLUFA-Schriftenreihe, Kongressband 18:515-524.

Woods, A.J., Bradley-Jones, J. and Mantle, P.G. 1966. An outbreak of gangrenous ergotism in cattle. Vet Rec 78:742-749.

Young, J.C. 1981a. Variability in the content and composition of alkaloids found in canadian ergot. 1. rye. J Environ Sci Health 16:83-111.

Young, J.C. 1981b. Variability in the content and composition of alkaloids found in canadian ergot. 2. wheat. J Environ Sci Health 16:831-393.

Young, J.C. and Chen, Z. 1982. Variability in the content and composition of alkaloid found in canadian ergot. 3. triticale and barly. J Environ Sci Health 17:93-107.

Young, J.C. and Marquardt, R.R. 1982. Effects of ergotamine tartrate on growing chickens. Can J Anim Sci 62:1181-1191.

ANNEX

Table 1. Physical and chemical properties of selected ergot alkaloids (ergolines)[a].

Group	Alkaloid	Formula	Melting point[b]
	Ergometrine	$C_{19}H_{22}O_2N_3$	162
Ergometrine[c]	Ergometrinine		196
	Ergotamine	$C_{33}H_{35}O_5N_5$	180
	Ergotaminine		241-243
Ergotamine	Ergosine	$C_{30}H_{37}O_5N_5$	220-230
	Ergosinine		228
	Ergocristine	$C_{35}H_{39}O_5N_5$	160-175
	Ergocristinine		226
	Ergocryptine	$C_{32}H_{41}O_5N_5$	212-214
Ergotoxine	Ergocryptinine		240-243
	Ergocornine	$C_{31}H_{39}O_5N_5$	182-184
	Ergocorninine		228
	Agroclavine	$C_{16}H_{18}N_2$	206
Ergoclavine	Elymoclavine	$C_{16}H_{18}ON_2$	249
	Chanoclavine	$C_{16}H_{20}ON_2$	222
	Penniclavine	$C_{16}H_{18}O_2N_2$	222

[a] From: Van Rensberg and Altenkirk (1974) and Lorenz (1979).

[b] Most of the ergot alkaloids decompose at melting temperatures.

[c] Also denoted ergonovine, ergobasine and ergotoxine.