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FUNGI IN MINK FEED AND ORGANS

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Abstract
The research of feed components of minks (frozen fish and meat offal, dried haemoglobin, dried protein, wheat, barley, wheat and barley meal), and ready-mixed mink feed were investigated by mycological method in Sabouraud's Agar and Czapek Agar. The mycological examination of mink feedstuffs veriffed its contamination with *Acremoniella atra, Alternaria spp., Aspergillus spp., Aureobasidium pullulans, Candida spp., Chaetomium spp., Cladosporium spp., Coremiella cubispora, Crysonilia sitophila, Curvularia spp., Fusarium spp., Gliocladium spp., Moniliella acetoabutans, Mortierella spp., Mucor spp., Penicillium spp., Sporothrix cyanescens, Stemphylium spp., Trichophyton terrestre, Zygosporium masonii,* and *Wangiella spp.* Mycological examination of the mink liver, lungs and kidneys showed contamination with *Acremonium spp., Actinomyces israelli, Arthrographis kalrae, Aspergillus spp., Aureobasidium pullulans, Candida spp., Chaetomium spp., Cladosporium bantianum, Cladosporium sphaerospermum, Conidiobolus coronatus, Curvularia spp., Emmonsia spp., Fonsecaea pedrosoi, Geotrichum candidum, Mucor spp., Penicillium spp., Scedosporium prolificans, Sporothrix cyanescens,* and *Wangiella spp.*
Key words: mink, feed, fungi.

Introduction
At present animal diseases such as mycoses (mycopathies) caused by fungi are considered as one of the most essential urgent issues of veterinary medicine in the world. As the possible reasons for the increase of the number of mycopathies in animals the researchers consider the immunity reducing factors – environmental pollution, the use of plant protection chemicals and fertilizers in a variety of branches of crop production as well as the administration of broad scale antibacterial preparations in the practice of veterinary medicine. Mycoses are caused by the use of feedstuffs infected by fungi (Спесивцева, 1964; Кузнецов, 2001).

A prerequisite of successful development of fur-farming branch is the safety and quality of animal feed that affects directly the herd health. It is determined by the fur-bearing animal keeping and feeding pecularities – great animal density in a small territory, preparation of mixed feed on the spot in the farm kitchen, high protein concentration of animal origin and fat content in the mixed feed, and the use of thermally unprocessed products of animal origin for preparing the mixed feed (Перельдик et al., 1981).

It has been reported (Juokslahti, 1978; 1979) on the bacteriological examinations of the ready-mixed feed and feed components, however, there are no data on the feedstuffs mycological contamination and its effect on the fur-bearing animal body. The data about the effect of farm animal feed mycological contamination on the animal health are still incomplete (Саттон et al., 2001).

Feedstuffs of the plant origin (cereals and rough forage) contain broad spectrum of microorganisms (bacteria, fungi). A reservoir of fungi in nature is the soil. Part of the fungi existing in the soil gradually move to the overground parts of the plants – stalk, leaves, and then to the seeds. Part of the fungi (facultatives) continues to develop also after the plant destruction thus affecting the quality of produce of crop production during its storage (Кузнецов, 2001).

In Latvia, investigations have been carried out on the spread of fungi in the feedstuffs of farm animals and on the negative effect of mycological agents on the semen quality (Емельянов, 1990), but there are no investigations on their effect on the mink body.

This investigation is based on a hypothesis that in the feedstuffs, besides the bacteriological contamination, the presence of fungi is possible that causes disturbances of development in minks, their death and essential economic losses in the branch development due to diseases.

The objective of this research was to establish the distribution of fungi genera in the parenchymal organs of minks and their possible relation with feedstuffs.

Materials and Methods
The mink feed specimens and clinical material were collected on four fur-bearing animal farms in various regions of the Republic of Latvia. Mycological inoculation of feed and parenchymal organ specimens was carried out in the Laboratory of Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra', LLU in 2004 and 2005 from July to December.

To detect the fungal effect on the mink body, 34 dark brown minks at the age of seven months without clinical signs of any disease were selected by random on the Latvian fur-bearing animal farms. After parenteral euthanasia of the selected animals (Jepsen et al., 1981) with 1 ml of ditilin solution (10 g per litre of water), pathoanatomical necropsy

was performed and inner organs (liver, lungs, kidneys) were sampled.

Sabouraud's agar was used as a primary isolation medium for the fungal cultures from the mink internal organs specimens. A small surface of the changed tissue was burned on a flame, and small pieces of tissue from the middle were cut out by sterile scissors. The tissue cuts were used for a stripe-like inoculation onto media (Спесивцева, 1964) or they were placed on the agar surface (4-5 small tissue pieces in size of 0.5 cm X 0.5 cm (Quinn et al., 1994).

Feedstuffs for mycological examination were sampled by a random selection: 11 specimens were collected from ready-mixed feed, six – from frozen pig and cattle offal (lungs, larynx, trachea, fat, cattle forestomachs), three – from frozen fish offal, two – from dried haemoglobin, two – from bone meal, two – from dried protein, three – from cereal bran, six – from barley/wheat meal, and eight specimens from barley/wheat cereal feed.

Mycological inoculates from 43 specimens of the mink feed and their components were cultured on Sabouraud's and Czapek media. The preparation of specimens and dilution series was done in compliance with the standart ISO 7954:1987 „Determination of the number of yeasts and moulds in animal feed".

All mycological inoculates on Petri plates were incubated in thermostat for 4 weeks at the temperature of +26 °C, but some cultures, when transfered onto blood agar medium, were incubated 7-10 days at +37 °C (Quinn et al., 1994; Кузнецов, 2001).

The microscopic identification of the isolated fungi was carried out at the Mycology Department of the Laboratory of the Plant Quarantine Organisms according to the conventional methods (Саркисов et al., 1953; Kwon-Chung et al., 1992; Bridson, 1993; Larone, 1995; Кириленко, 1997; Ulloa et al., 2000; Саттон et al., 2001).

Results and Discussion

Many species of fungi causing illnesses (mycoses) are moulds. In immunosuppressive animals, microscopic fungi can disseminate in the organism (Кузнецов, 2001). A summary of the identified fungi from the mink tissue (liver, kidneys, lungs) in this investigation is given in Table 1.

Disseminated infection agents *Aspergillus spp.* and *Candida spp.* (Саттон et al., 2001) were isolated from the animal liver, lungs, and kidneys. Kuznecov (Кузнецов, 2001) also confirms that *Aspergillus spp.* cause dystrophic changes in the liver as well as inflammation of the urogenital and respiratory system. Macromorphology of the two above mentioned genera, however, was different. Colonies of *Candida spp.* were small, creamy, smooth, while *Aspergillus spp.* colonies were comparatively fast growing, wide, with white, blueish or greenish mycelium.

As to the lungs, *Conidiobolus coronatus* was isolated, the colony of which was flat, yellowish brown with a white reverse side. Sporangiophores with large round spores were seen. Other authors (Kwon-Chung et al., 1992) also have reported to have isolated this fungi from dogs and horses where it had caused nasal mucous membrane infections.

Table 1

Microscopic fungi in the mink organs

Microscopic fungi	Liver	Lungs	Kidneys
Acremonium spp.	-	-	+
Actinomyces israelli	+	-	-
Arthrographis kalrae	+	-	-
Aspergillus spp.	+	+	+
Aureobasidium pullulans	+	-	-
Candida spp.	+	+	+
Chaetomium spp.	+	-	-
Cladosporium bantianum	-	-	+
Cladosporium sphaerospermum	+	-	-
Conidiobolus coronatus	-	+	-
Curvularia spp.	-	-	+
Emmonsia spp.	+	-	-
Fonsecaea pedrosoi	-	+	-
Geotrichum candidum	-	-	+
Mucor spp.	-	+	-
Penicillium spp.	+	+	-
Scedosporium prolificans	+	-	-
Sporothrix cyanescens	+	-	-
Wangiella spp.	+	-	-

The second representative in the lungs was *Fonsecaea pedrosoi* that had black colonies with a velvety mycelium. At the ends of conidiophores, the primary conidia were separating from which the secondary ones were formed additionally. Conidia were of dark shade.

Acremonium spp., Cladosporium bantianum, Curvularia spp., and *Geotrichum candidum* were isolated from the kidneys. *Curvularia spp.* colonies averse and reverse were black, but conidiophores were septate and brown. The conidia had 3 crosswalls with a tannish shade. Although scientists (Cattoн et al., 2001) consider *Curvularia spp.* as widely distributed phytopathogens they can cause a disseminated infection. Carter and Chengappa (1993) have also reported on diseases in horses, cattle, dogs, and cats caused by this agent.

The microscopic fungus *Geotrichum candidum* causes an uncommon disease (geotrichosis) in cattle, dogs, poultry and other species, and most often involve bronchi, lungs, and kidneys. Although the infection is disseminated, usually one or several organs are affected (Carter et al., 1993). Within the framework of this investigation, *G. candidum* was isolated only from the kidneys, and it had a glabrous or yeastlike form without conidiophores, but the vegetative hyphae were breaking up into fragments (artrospores). Carter and Wise (2004) also confirmed that the *G. candidum* glabrous form is the only one most often associated with geotrichosis.

The liver is the most important organ of metabolism in the animal body of which most of the fungi types were isolated. *Arthrographis kalrae* colonies were slowly growing with a light brown shade. Micromorphologically colourless arthroconidia were observed. Tewari and Macpherson have also found that *A. kalrae* possess dimorphism, and they are pathogenic for mice (Kwon-Chung et al., 1992).

Chaetomium spp. colonies were cottony, white or tannish. The hyphae were septate with large, oval, dark brown ascospores. Sutton et al. (Cattoн et al., 2001) have also indicated that *Chaetomium spp.* can be the cause of systemic mycoses.

Table 2

Microscopic fungi in the ready-mixed mink feed and raw materials

Microscopic fungi	Ready-mixed feed	Cereal meal	Cereals	Bran	Pig meat offal	Fish offal	Dried protein
Acremoniella atra	-	-	+	-	-	-	-
Alternaria spp.	-	+	+	-	-	-	-
Aspergillus spp.	+	+	+	+	+	+	+
Aureobasidium pullulans	+	-	+	-	-	-	-
Candida spp.	+	+	+	+	+	-	-
Chaetomium spp.	+	-	+	-	-	-	-
Cladosporium bantianum	+	-	-	-	+	-	-
Cladosporium herbarum	+	+	+	-	-	-	-
Cladosporium sphaerospermum	+	+	+	-	-	-	-
Coremiella cubispora	-	-	+	-	-	-	-
Crysonilia sitophila	-	+	+	-	-	-	-
Curvularia spp.	+	+	+	-	-	-	-
Fusarium spp.	+	+	+	-	-	-	-
Gliocladium spp.	-	-	+	-	-	-	-
Moniliella acetoabutans	+	+	+	-	-	-	-
Mortierella spp.	+	+	+	-	-	-	-
Mucor spp.	+	+	+	-	-	-	+
Penicillium spp.	+	+	+	+	+	+	+
Sporothrix cyanescens	+	-	-	-	+	-	-
Stemphylium spp.	+	-	-	-	+	-	-
Trichophyton terrestre	+	-	-	-	+	-	+
Zygosporium masonii	-	+	+	-	+	-	-
Wangiella spp.	+	-	-	-	+	-	-

The above mentioned scientists (Саттон et al., 2001) have described *Sporothrix cyanescens* as a thermotolerant type with white, velvety colonies and a tannish reverse side. Micromorphologically it was detected that just after every conidiophore separation conidia collections followed.

Wangiella spp. macroscopically were characterised as black, slowly growing colonies, but conidiophore cells were slender, thin, with a typical tapered and elongated end.

Although several researchers (Kwon-Chung et al., 1992; Кузнецов, 2001) have isolated fungi from the internal organs of farm animals, there are no data in literature confirming the isolation of microscopic fungi genera from the tissue of the mink internal organs found in this research.

The agents of systemic mycoses are spread in the environment, and animals are mainly infected by ingestion (Quinn et al., 1994). The results of mycological examination of the ready-mixed mink feed and its components showed evidence of their contamination with fungi (Table 2).

Table 2 shows that the fungi found in cereals and meal (*Alternaria spp., Aspergillus spp., Aureobasidium pullulans, Candida spp., Chaetomium spp., Cladosporium herbarum, Curvularia spp., Coremiella cubispora, Crysonilia sitophila, Fusarium spp., Gliocladium spp., Moniliella acetoabutans, Mortierella spp., Mucor spp., Penicillium spp., Zygosporium masonii*) in literature are described as widely spread environmental contaminants (Kwon-Chung et al., 1992; Кузнецов, 2001; Саттон et al., 2001).

Acremoniella atra were characterised by white cottony colonies. Macromorphologically, one brown conidium on each of the conidiophores was seen. The conidia had a thick, smooth membrane.

From the members of *Cladosporium spp.* in cereals C. *herbarum* and *C. sphaerospermum* were found. *Cladosporium herbarum* had an olive-black colony. The conidiophores were segmented and pigmented, but the conidia were brown, elliptical with a rounded end. The third member of *Cladosporium spp.* - *C. bantianum* - was isolated from offal and redy-mixed feed. *C. bantianum* was isolated also from the kidneys, however, morphologically it was different. *C. bantianum,* found in the mink feedstuffs, colonies had a tree-like conidial structure. The conidia and conidiophores were dark.

Also, *Coremiella cubispora* colonies were black, but very small and solid. It was observed that the characteristic dark square-shaped arthroconidia were changing with colourless cells.

The fungi, found in meal and cereals, were also isolated from the ready-mixed mink feed with the exception of *Acremoniella atra, Alternaria spp., Coremiella cubispora, Crysonilia sitophila,* and *Zygosporium spp.*

The obtained results show evidence that *Aspergillus spp., Aureobasidium pullulans, Candida spp., Chaetomium spp., Cladosporium spp., Curvularia spp., Fusarium spp., Moniliella spp., Mortierella spp., Mucor spp.,* and *Penicillium spp.* were not inactivated during the thermal processing at + 90 °C. So, this research confirms the data, reported by Kuznecov (Кузнецов, 2001), that the fungal spores can survive high environmental temperature.

During this experiment, the fungi of *Aspergillus spp., Candida spp., Cladosporium bantianum, Penicillium spp., Sporothrix cyanescens, Stemphylium spp., Trichophyton terrestre, Zygosporium masonii,* and *Wangiella spp.* were found in the pig meat offal, which confirms the possibility, similarly to Kuznecov's (Кузнецов, 2001) data, of slaughtering to be contaminated with fungi.

Aspergillus spp. and *Penicillium spp.* fungi were found in the fish offal used on the farm. There are no literature data about the above mentioned fungi presence in fish offal, although researchers (Перельдик et al., 1981) have pointed out that contamination with *Aspergillus spp.* and *Penicillium spp.* is possible due to dissatisfactory hygienic conditions of premises and equipment when products get into contact with contaminated surfaces. Whereas Moeller reports on fish contamination with the fungi of *Wangiella spp.* (Moeller, 2006) which was not confirmed by this study.

Among all types of microscopic fungi found as the result of this investigation (see Tables 1 and 2), 39 % consisted of *Dematiaceous* group fungi (*Alternaria spp., Aureobasidium pullulans, Chaetomium spp., Cladosporium spp., Curvularia spp., Fonsecaea spp., Scedosporium spp., Stemphylium spp., Zygosporium spp., Wangiella spp.*) (Ulloa et al., 2000; Kwon-Chung et al., 1992). The common feature of these fungi is the presence of melanin in the cell wall. Other researchers (Жданова et al., 1990) have proved that the presence of melanin pigment affects essentially endurance of these cells against the influence of environmental moisture, temperature and radiation of the sun as well as provides vitality in the surroundings with insufficient amount of nutrients. In addition, when in a body, the dark pigmented microscopic fungi containing melanin has an increased endurance against the body immunity protective factors. It is possible that one of the reasons why in 42 % of cases exactly *Dematiaceous* group fungi were isolated from the mink parenchymatous organs during this investigation.

Conclusions

1. The microscopic fungi such as *Fusarium spp., Aspergillus spp., Aureobasidium pullulans, Sporothrix cyanescens, Mucor spp., Penicillium spp., Chaetomium spp., Trichophyton terrestre, Mortierella spp., Candida spp., Cladosporium spp., Curvularia spp., Wangiella spp., Moniliella spp.*, and *Stemphylium spp.* were found in the ready-mixed mink feed, as obviously the conventional

thermal processing of animal feed at the temperature + 90 °C is unsufficient to inactivate agents causing mycoses.

2. In the mink lung specimens the following fungi were found: *Aspergillus spp., Penicilium spp., Candida spp., Conidiobolus coronatus, Fonsecaea pedrosoi,* and *Mucor spp.*; in the liver specimens there were *Actinomyces israelli, Arthrographis kalrae, Aspergillus spp., Aureobasidium pullulans, Chaetomium spp., Cladosporium sphaerospermum, Emmonsia spp., Penicilium spp., Candida spp., Scedosporium prolificans, Sporothrix cyanescens,* and *Wangiella spp.*, but in the kidney specimens *Acremonium spp., Aspergillus spp, Candida spp., Cladosporium bantianu, Curvularia spp.,* and *Geotrichum candidum* were isolated.

3. The obtained results show evidence that there could be a link between the spread of mycological agents in the mink feed and their presence in the mink parenchimatous organs.

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