THE INFLUENCE OF MYCOTOXINS IN FOOD ON FISH HEALTH STATUS*

ABSTRACT: In our country, there is present extensive, semi-intensive and intensive growing of cyprinid fish species. The quality of food is an essential prerequisite for obtaining optimal production results in fish production.

Fish food is being produced as a complete pellet meal, and raw materials used are of plant, animal, mineral and vitamin origin. Out of plant feed, the most commonly used ones are corn, wheat, barley, oats, soy and others. By applying additional carbohydrate food, energetic needs of an organism are being met.

In this paper, we presented the results of hygienic safety of carbohydrate feed (corn, wheat, barley) investigated in the laboratory of Veterinary Research Institute of Serbia in Belgrade within regular control, or with the aim of establishing the causes of disturbance of health status and decreased production results in the pond.

During 2004 we performed microbiology and mycotoxicology investigations of the total of 43 samples, namely: 31 corn samples, 8 barley samples and 4 wheat samples.

The obtained results point at a high level of mould contamination (Aspergillus, Penicillium, Fusarium, and Rhizopus) and the presence of their secondary mycotoxin metabolites (aflatoxin, ochratoxin, trichothecenes and zearalenone) in feed.

KEY WORDS: cyprinid fish species, food of plant, animal, mineral and vitamin origin, hygienic safety, microbiology and mycotoxicology investigations, mould contamination.

INTRODUCTION

Nutritive requirements for growth, reproduction and all physiology functions of fish are similar to other animals. Everyday intake of proteins, minerals, vitamins, growth factors and energy is necessary.

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Cereals as energy and leguminose as protein feedstuffs are the main part of feed (up to 90%) for all kinds and categories of fish. Usage of complete mixtures without appropriate hygiene qualities leads to decrease of productive results and progressive lowering of health of fish. Cereals — carbohydrate feedstuffs are a good medium for growth of number of fungi. In adequate conditions they multiply and their metabolites cause changes in feed quality. Bad feed can induce consequences on health and productive results of fish (Leiles, W. et al., 2000).

Cereals become contaminated with fungi in the field, during processing, storage, transport and usage. Some fungi cause detrimental effects in feed, either by decomposition of its components or by producing harmful metabolites (Rajić, I., 1993).

Mycotoxins are secondary metabolites of fungi which are formed during consecutive serial enzyme reactions over several biochemically simple intermediary products, from prime metabolism, acetates, mevalonates, malonite and some aminoacids (Mašić, Z., 1993; Mašić et al., 2002).

Their chemical characteristics and biology activities are very wide and able to cause different pathology and pathohistology changes in fish.

Mycotoxins are important contaminants of environment. They enter organisms by ingestion, but also by inhalation according to WHO report in 1979. Quite small amounts of these substances can harm health. High concentrations of mycotoxins are able to provoke acute disorders and can cause cancerogenic, mutagenic and teratogenic effects.

It is proved that mycotoxins production depends on:
- presence of toxin producing fungi
- convenience of substrate for fungal growth
- environmental conditions for fungal growth.

Fungi will produce mycotoxins only if these conditions are complied. Toxin productive strains of fungi are able to produce more than one toxin and also one toxin can be produced by different strains of fungi. But, the presence of fungal strains which are potential toxin producers in feed is indication of possible presence of mycotoxins.

High moisture (20—25%) is important factor for fungal growth in the field and in the raw plant material. Storage fungi are capable to rise in substrates which contain 12—18% of moisture. Contamination of feed with fungi, their growth and mycotoxin production during harvesting, transport, storage and mixing of agricultural products are under the influence of several factors: moisture, temperature, aeration and presence of other microorganisms.

Recent studies show that growth and toxin production of *Aspergillus* (aflatoxin) and *Penicillium* (ochratoxin A, patulin) are under the influence of maximal and minimal water activity and temperature values.

Water activity and temperature are specific for every fungal kind growth and very important for mycotoxin production, e. g. aflatoxin B1 can be produced in such conditions that water activity and temperature are close to minimal for growth of other microorganisms. Patulin, penicillium acid and ochratoxin A are produced at lower water activity and temperature values, mainly mini-
The most important mycotoxicoses in fish are caused by aflatoxins, ochratoxins, zearalenone and trichotecenes. Also recently, due to new methods which are very reliable in quantitative determination of fungi, a great improvement can be noticed in struggle with fungi as fish feed contaminants (Robinson, 1993; Lim and Dominy, 1990).

Experience in analysing feed, which is usually in use in fish feeding, has brought a need of detailed investigation to ensure on time diseases prevention. The aim of this work was testing of mycotoxins presence usually found in fish feed.

**MATERIALS AND METHODS**

During 2004, microbiological and mycotoxicological investigations of total of 43 samples (31 samples of corn, 8 samples of barley and 4 samples of wheat) were done. Samples of carbohydrate feedstuffs from fish farms from all over Republic of Serbia were sent for analyses to laboratories of the Scientific Institute of Veterinary Medicine of Serbia in Belgrade partly as routine control, but mostly in case of suspicion about feed quality and its possible connection with decrease of productive results and health disturbances.

For fungi determination a standard mycology method is implemented. For mycotoxicology examination of aflatoxin, ochratoxin and zearalenone presence in samples ELISA quantitative method, which is based on antigen-antibody reaction, is used. In the wells of microtiter strips, according to the commercial kit guide (R-Biopharm, Deutschland: Aflatoxin total, Ochratoxin A, Zearalenone zearalenone), standards and prepared samples are added. As the reaction has to become visible addition of enzyme and chromogen gives a blue coloured product which changes into yellow after the addition of the stop reagent. Finally, the measurement is made photometrically at 450 nm and the absorption is inversely proportional to the toxin concentration in the sample. Results are interpreted and compared to Rulebook of maximal amounts of detrimental substances in feed (Sl. list 2/90).

**RESULTS AND DISCUSSION**

According to mycology and mycotoxicology examinations of carbohydrate feedstuff samples at the Department for animal nutrition in the Scientific Institute of Veterinary Medicine of Serbia in Belgrade high contamination results are obtained. Almost 100% of samples were infested with fungi (Table 1 and 2).

The most common mycotoxins are zearalenone, ochratoxin A and aflatoxin B₁ and the highest degree of contamination is registered in corn samples (Table 3).
Table 1. Examined feedingstuffs

<table>
<thead>
<tr>
<th>feedingstuffs</th>
<th>N° of samples</th>
<th>N° of samples contaminated with fungi</th>
<th>% of contaminated samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>corn</td>
<td>31</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>wheat</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>barley</td>
<td>8</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>total</td>
<td>43</td>
<td>42</td>
<td>97.67</td>
</tr>
</tbody>
</table>

Table 2. Commonly isolated fungi

<table>
<thead>
<tr>
<th>N° of samples</th>
<th>N° of infested samples</th>
<th>isolated fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>corn</td>
<td>31</td>
<td>Penicillium, Mucor, Fusarium, Aspergillus, Rhisopus</td>
</tr>
<tr>
<td>wheat</td>
<td>4</td>
<td>Penicillium, Mucor, Fusarium, Aspergillus, Rhisopus</td>
</tr>
<tr>
<td>barley</td>
<td>8</td>
<td>Penicillium, Mucor, Fusarium, Aspergillus</td>
</tr>
</tbody>
</table>

Table 3. Mycotoxin content in feedstuffs, [mg/kg]

<table>
<thead>
<tr>
<th></th>
<th>corn</th>
<th>wheat</th>
<th>barley</th>
<th>x ± Sd</th>
<th>iv</th>
</tr>
</thead>
<tbody>
<tr>
<td>zearalenone</td>
<td>5,30</td>
<td>2,06</td>
<td>2,00</td>
<td>3,12</td>
<td>0,80—5,33</td>
</tr>
<tr>
<td>ochratoxin A</td>
<td>0,20</td>
<td>0,22</td>
<td>0,30</td>
<td>0,24</td>
<td>0,16—0,35</td>
</tr>
<tr>
<td>aflatoxin B1</td>
<td>0,04</td>
<td>0,005</td>
<td>0,02</td>
<td>0,02</td>
<td>0,00—0,05</td>
</tr>
</tbody>
</table>

Different organs in fish organism are sensitive to different mycotoxins. According to the characteristics mycotoxicoses are similar to diseases caused by other pathogens or nutritive deficiency and disbalance.

Degree of changes caused by mycotoxins depends on type and the amount of mycotoxins in feed, also on exposure duration, age and species of fish. Mycotoxins induce several disorders in fish organism: biochemical, functional, morphological and in more severe cases mortality. Biochemical alterations and metabolism disturbance lead to changes in nutrient resorption and primary brings to cell and organ alterations.

Toxic effects of certain mycotoxins differ according to age and species of fish. Younger fish are more sensitive.

Aflatoxicoses of salmonids occurs as a consequence of presence of fungi *Aspergillus flavus, Aspergillus parasiticus, Aspergillus versicolor* and *Penicillium*. The major products of these molds are aflatoxins B₁, B₂, G₁, G₂. Other intermediates in the biosintetic pathway of this molds, namely, versicolorin A and sterigmatocystin, can also appear in contaminated feeds. Aflatoxins are hepatotoxins, also well known as carcinogens.

Aflatoxin penetrates into the cells, binds to the DNA molecules, inhibits polymerase enzyme and RNA synthesis, that leads to cell changes. In liver aflatoxin B₁ in presence of enzyme transforms into several metabolites. Aflatoxin metabolites are thought to be the most mutagenic agents which accumulate in hepatic tissue.
First reports about aflatoxin toxicity are made by Halver (1965, 1967) and Bauer et al. (1969). They have noted that aflatoxins are powerful carcinogens in rainbow trout. The hepatocarcinomata usually reach a clinical level after 4—6 month of feeding of the contaminated meal. The amount of contamination can be very small, as little as 0,1 ppb in the total diet.

Pathomorphological changes depend on species and age of fish, as well as on amount of mycotoxins in feed. Aflatoxicoses can manifest in acute or chronic form.

When fed experimentally at high levels, 80 ppb or more, the toxin produces an acute toxic syndrome, severe or even massive focal hepatic necroses, and branchial edema, as well as generalized punctate hemorrhage (Ashley, 1970).

Acute form manifests usually after 12 hours and chronic form after prolonged period of contaminated feed intake. Pathomorphological alterations are mainly located in liver as anaemia (pale liver) with focal hepatic necrosis and hemorrhage and renal inflammatory changes. Chronic form, in older fish, is characterised with invasive malignant trabecular hepatocarcinomata, very obvious because of the focal, darker zones of malignancy.

Ochratoxins (A, B, C, α, β) are products of isocumarin binded to L-phenilalanine.

Ochratoxin A, the most toxic of the metabolites, produced by Aspergillus ochraceus, is a potential fish toxin since it occurs as a natural contaminant of corn and wheat (Shotwell sar., 1969; Scotti sar, 1970). Presence of aflatoxin in fish feed indicates its possible presence in fish tissues. It causes degenerative changes in liver and necrosis of the proximal tubules, hematopoietic tissue, and glomeruli of the kidney. Ochratoxin A was found to be lethal, with an LD$_{50}$ of 4,67 mg/kg.

Ochratoxin B, the dechlorinated form of ochratoxin A, was nonlethal at doses up to 66,7 mg/kg, but the high dose caused some histological changes in the kidneys and liver similar to those caused by low doses of ochratoxin A.

Trichotecenes are secondary metabolites of several fungal genera. Mostly, they are produced by Fusarium species, Trihotecium, Myrhotecium, with 18 species in total, and around 100 compounds are chemically described. The most important natural trichotecens which cause health disturbances are DON-deoxynivalenol or vomitoxin and T-2 toxin.

Vomitoxin is one of the naturally occuring trichotecene mycotoxins produced by genus Fusarium that grow on various cereals grains such as corn, barley, and wheat. Because wheat and the corn products are used frequently in cyprinid diets, vomitoxin is a potential problem for carp culture. Fish receiving low levels (1—12,9 μg/g) of vomitoxin are the diets but demonstrated reduced growth and feed efficiency, neither clinical signs nor mortalities were observed during the 8-weeks study (Woodward et al., 1983).

T-2 toxin is another trichotecene mycotoxin produced by Fusarium species growing on cereal grains. Its detrimental influence is manifested in dosed above 2.5 mg/kg as depressed growth, efficiency of feed use, hematocrit, blood hemoglobin concentration, and feed acceptance. A single acute oral dose (6,5 mg/kg body wt) given to rainbow trout fingerlings caused extensive shed-
The ingestion of the intestinal mucosa, severe edema in body cavities, and eventual death (Marasas, 1967). Long-term (12 month) ingestion of low doses (200—400 µg/g feed) in older fish had no apparent adverse effect and actually promoted better growth than the control.

CONCLUSION

Losses in aquaculture caused by mycotoxins in feed can be significant. Direct loss is a consequence of increased mortality and indirect loss is result of decrease of production and occurring of secondary diseases.

Preventive measures consist of agrotechnical and agrochemical operations which are implemented to inhibit fungal growth in fish feed.

Decrease of grain damaging and moisture, on time application of fungicides and warehouse aeration appeared to be effective in struggle with molds in fish feed. The safest way to avoid problems is not to use mycotoxin contaminated feed in fish nutrition at all.

The most common mycotoxins are described. However, this does not imply that other may not be important. As new feed ingredients are indentified and incorporated in fish diets their mold contaminants will need to be identified and tested for possible deletrious effectious. Whenever general pathological symptoms occur in hatchery fish, the role of mycotoxin should not be overlooked. It is likely that the toxicities of several mold metabolites new to fish remain to be discovered and researchers are encouraged to test the toxicities of potentially important mycotoxins on various species of fish.

REFERENCES


УТИЦАЈ МИКОТОКСИНА НА ЗДРАВЉЕ РИБЕ

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Резиме

У нашој земљи је заступљено екстензивно, полуинтензивно и интензивно гајење ципринидних врста риба. Квалитет хране је битан предуслов за постигање оптималних производних резултата у рибарству.

Храна за рибе производи се као пелетиран комплетан оброк, а сировине које се употребљавају су биљног, животинског, минералног и витаминског порекла. Од биљних храна најчешће се употребљавају кукуруз, пшеница, јачам, овас, соја и др. Додатном угљенохидратном храном се задовољавају енергетске потребе организма.

У раду су приказани резултати хигијенске исправности угљенохидратних хранива (кукуруз, пшеница, јачам) испитиваних у лабораторији Научног института за ветеринарство Србије у Београду у оквиру редовне контроле или у циљу утврђивања узрока поремећања здравственог стања и лоших производних резултата на рибњаку.

У току 2004. године извршена су микробиолошка и микотоксинолошка испитивања укупно 43 узorca: 31 узорак кукуруза, 8 узорака јачме и 4 узорака пшенице.

Добијени резултати указују на висок степен контаминације гљивицама (Aspergillus, Penicillium, Fusarium, Rhizopus) и на присуство њихових секундарних метаболита микотоксина (афлатоксин, охратоксин, трихотеци, зеараленон) у храни-вима.