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MYCOTOXINS IN CEREAL GRAIN AS A RESULT OF INFECTION OF CEREALS BY *FUSARIUM* FUNGI

MYKOTOKSYNY W ZIARNIE ZBÓŻ EFEKTEM PORAŻENIA ZBÓŻ PRZEZ GRZYBY Z RODZAJU *FUSARIUM*

Summary: *Fungal diseases are an important factor limiting the yield of cereals, but also reduce, the quality of the grain obtained. Fungi of the genus Fusarium are among the most important pathogens and cause, among other things, fusarium head blight. Their particular harmfulness lies not only in the reduction of yield, but also in their production of harmful metabolites called mycotoxins. Mycotoxins are defined as harmful substances produced as secondary metabolites by mould fungi. The problem of mycotoxin contamination of cereal grains relates to their high harmfulness to humans and animals. This is due to the fact that cereals are an essential raw material in the production of human food and animal feed. Infection by Fusarium fungi and is determined by a number of factors, the main ones being weather conditions during ear formation, grain formation and harvesting. One of the most important methods of preventing mycotoxin formation is fungicide protection of cereals. The aim of this paper is to discuss the problems associated with fusarium head blight and its effects associated with mycotoxins, the factors determining their synthesis, mechanisms of prevention and the impact of their content in grain and feed on animal health and productivity.*

Keywords: *mycotoxins, fungal diseases of cereals, fusarium head blight, cereal grain, food safety, toxicity*

Streszczenie: *Choroby grzybowe są ważnym czynnikiem ograniczającym plonowanie zbóż, ale także obniżają jakość uzyskiwanego ziarna. Wśród licznych patogenów roślin zbożowych jednymi z ważniejszych są grzyby z rodzaju Fusarium, które powodują m.in. fuzariozę kłosów. Szczególna ich szkodliwość polega nie tylko na obniżeniu plonowania, ale także produkcji przez nie szkodliwych metabolitów nazywanych mykotoksynami. Pod pojęciem mykotoksyny definiuje się szkodliwe substancje, powstające, jako drugorzędne (wtórne) metabolity produkowane przez grzyby pleśniowe. Ważność problematyki dotyczącej skażenia ziarna zbóż mykotoksynami związana jest z ich wieloaspektowym toksycznym wpływem na organizmy ludzi i zwierząt. Wynika to z faktu, że zboża stanowią podstawowy surowiec w produkcji żywności dla ludzi oraz pasz dla zwierząt. Porażenie przez grzyby Fusarium uwarunkowane jest wieloma czynnikami, przy czym za główne uznaje się warunki pogodowe w trakcie wykształcania kłosów i ziarna. Jedną z ważniejszych metod zapobiegającą tworzeniu mikotoksyn jest ochrona fungicydowa zbóż. Celem niniejszej pracy jest omówienie problematyki związanej występowaniem fuzariozy kłosów oraz ich skutków związanych z występowaniem mykotosyn, czynników warunkujących ich syntezę, mechanizmów zapobiegania oraz wpływu ich zawartości w ziarnie i paszy na zdrowie i produkcyjność zwierząt.*

Słowa kluczowe: *mykotoksyny, choroby grzybowe zbóż, fuzarioza kłosów, ziarno zbóż, bezpieczeństwo żywnościowe, toksyczność*

The problem of Fusarium head blight in cereals during field growth

Fusarium head blight is a fungal disease that occurs in many areas of the world and is considered one of the main factors affecting the quantity and quality of the grain yield obtained. It is caused by fungi belonging to the genus *Fusarium* mainly *F.culmorum* and *F. graminearum* [6] Fungi of the genus *Fusarium*, as anamorphic fungi are called mould fungi (they do not form spores). The fungi multiply, resulting in significant yield loss, as well as deterioration in yield quality [54]. They are well adapted to changing soil and atmospheric conditions and have a high tolerance to abiotic factors in the environment, and thrive in a large temperature range, i.e. 0–30°C. Fusarium head blight occurs on all cereal species in our climatic zone (wheat, rye, triticale, oats, barley, maize).

Infestation of ears by *Fusarium* fungi causes a reduction in yield, resulting from a lower weight of 1.000 grains, number of grains per ear and grain weight per ear. The fungi cause a reduction in commercial and consumption value by altering the chemical composition of the grain. *Fungi* of the genus *Fusarium* have the ability to form mycotoxins, resulting in the accumulation of toxins in the grain even before harvest. The most mycotoxigenic species include *F. graminearum* and *F. culmorum*. In Poland, deoxynivalenol, nivalenol and zearalenone are the most common in cereal grain.

The reason for the fairly common occurrence of fusarium head blight is the high proportion of cereal crops in the structure and the limited rotation in the field. In general, cereals are grown in succession and it results in the accumulation of crop residues with the pathogenic pathogen in the soil [3; 40]. Infection of plants by mycotoxigenic fungi and their production of mycotoxins results

in a defence response. As a result of biological transformation, which involves a series of emzymatic reactions, free mycotoxins are converted into less harmful compounds, called modified mycotoxins. Transformed mycotoxins have also been referred to as masked mycotoxins because their altered chemical structure renders them undetectable in standard detection methods for free mycotoxins [35]. They do, however, pose a problem in the feed production chain, as their non-detectability results in grain feed material being approved for use because it meets the requirements of low mycotoxin content. However, they pose a further problem for animal health due to the fact that they are metabolised in the gastrointestinal tract or liver to primary, free forms that are harmful.

The occurrence of fusarium head blight in cereal crops is most often identified by characteristic symptoms. At the earing stage, an early blanching of the husks in the ears and a light pink colouring are observed. These are the first symptoms of *Fusarium* infection. Visual assessment and determination of the ear infestation index have so far been the only methods for assessing the infestation and degree of resistance of individual cereal cultivars to fusarium head blight. Nowadays, attempts are being made to implement modern, more reliable and objective assessments. One of these is remote sensing. The method is based on comparing images of healthy plants with patterns of infested plants and of infested plants with patterns of healthy plants. The resulting images of plant patches are then used as a basis for analysing wavelength histograms, as well as for calculating indicators based on them to assess crop health [33]. The use of remote sensing can be used to create health maps of *Fusarium*-infected cereals [20].

Prevention of *Fusarium* infestation

The severity of fusarium head blight is highly dependent on weather conditions [46] forecrop [29; 52] nitrogen fertilisation and weed control [53]. According to Doohan et al. [17] and Czaban et al. [15], the degree of ear fusarium head blight infection is highly dependent on weather conditions (temperature and humidity). Weather conditions are a factor that determines the infection of wheat grain by *Fusarium* to a greater extent than the variation in tillage systems. Fusarium head blight risk assessment and models to predict the occurrence of this disease are based on weather conditions during the period from flowering to early milk maturity [17]. Also, wheat cultivation technology influences *Fusarium* ear infection. Czaban et al. [15] indicate that ears and grain of wheat from sites with intensive cultivation technology were most severely infested. Inoculation of spelt wheat grain [26] and spring barley grain [5] by *Fusarium* fungi was lower in the organic system compared to the integrated and conventional systems.

Research by other authors [12] confirms that weather conditions were a stronger factor influencing grain infection by *Fusarium* fungi than variation in cultivation systems. Łukanowski and Sadowski [29] showed that winter wheat kernels grown in the organic system were significantly less infested by Fusarium fungi than in the integrated and conventional systems. In other studies [5; 26] the colonisation of cereal grains by *Fusarium* fungi was also lower in the organic system compared to the integrated and conventional systems.

A modern method of counteracting *Fusarium* infestation is the use of preparations containing effective micro-organisms. This is one of the alternative, biological methods of controlling fungal pathogens based on the beneficial action of bacterial strains. Scientific studies have shown that certain bacterial strains can be as effective in reducing fungal diseases as the active substances contained in fungicides. A study by Wachowska et al [49] showed that bacteria of the genus *Sphingomonas* inhibited the growth of fungi of the genus *Fusarium* as effectively as a triazole fungicide. The possibility of using effective microorganisms to protect against *Fusarium* infestation was investigated by Starzyk and Wiśniewska [44]. The researchers, in a field experiment growing spring wheat of the Zebra cultivar susceptible to fusarium head blight, sprayed the plants with a preparation containing effective microorganisms. The results of this study showed that the use of effective microorganisms is an effective method in controlling fusarium head blight. Another biological method is also the use of mycoviruses. These can be used, as potential biocontrol agents for phytopathogenic fungal diseases of cereals including bioprotection against *Fusarium* [42]. Potential viruses multiplying on Fusarium fungi are included in the family *Fusariviridae* [27]. The identification of useful virus strains useful against Fusarium fungi and the possibility of their use in the biological cultivation of cereals is currently underway. Researchers are also trying to determine which viruses can multiply on different strains of *Fusarium* fungi, and which viruses have limited abilities and can only multiply on a specific *Fusarium* species [56].

As awareness of the harmfulness of mycotoxins has increased, measures have begun to be taken to eliminate the threat from these substances. One of the main countermeasures is limiting the possibility of mould growth. This concerns the protection of plants, mainly cereals, from infection during growth in the field, but also the correct storage conditions for grain and its products. An important element of prevention is the prevention of plant infection during the growth period in the field, which prevents the formation of mycotoxins and their accumulation in the grain [2]. Among the species most pathogenic to cereals are species of the genus *Fusarium*, infecting cereal ears and maize cobs. The increase in the degree of contamination of cereal grain with mycotoxins formed by *Fusarium species* is caused by the increasing use of cereal cultivation in monocultures, disregarding the traditional crop rotation. The use of a suitable crop rotation on the site is, after the influence of the weather, one of the most important factors limiting the development of mycotoxigenic fungi [14]. Post-harvest residues of plants infected in the previous season are a reservoir of spores of the mould fungus type *Fusarium graminearum*. Crop rotation is, therefore, one of the most important methods recommended to reduce the risk from *Fusarium sp.* [22]. The cultivation system also influences the degree of plant infestation and mycotoxin content. Several techniques are used during tillage, but ploughing is the most

common. When ploughing is carried out, the surface layer of soil together with crop residues is turned over and ploughed to a depth of 10 to 30 cm. In this way, the possibility of pathogenic fungal growth is reduced. Topsoil cultivation to a depth of 10 to 20 cm, in which part of the harvest residues are mixed with the soil, is also practised. Ploughless cultivation with direct seeding into the stubble is also increasingly used. However, this results in an increased risk of fungal diseases due to the accumulation of fungal spores on harvest residues in the topsoil. It is therefore advisable to remove or burn the crop residues, which can reduce the likelihood of the plant material (grains) becoming infected with *Fusarium* pathogen suspension. However, a number of studies show that ploughless tillage results in increased deoxynivalenol levels in wheat at subsequent harvests [45; 50]. The mycotoxin content is also influenced by the tillage system used. Compared to conventional cultivation, organically grown wheat grain contains more mycotoxins [31]. However, this is also dependent on weather conditions. In the years with higher temperatures and lower rainfall, which creates conditions less favourable for fungal growth, plants grown in the both systems can have comparable and low mycotoxin concentrations [30]. Sowing date and density are also one of the factors determining the degree of *Fusarium* infection. The probability of infection increases when the time of cereal earing is synchronised with the timing of the release of mould spores. Therefore, making changes to the sowing date of cereals or the time of maturity can significantly affect the degree of contamination of cereals by moulds and mycotoxins. In the case of maize, earlier sowing dates in particular areas often result in lower levels of contamination, but seasonal weather changes can reduce this potential benefit [39]. Fertilisation is also one of the factors considered to influence the susceptibility of plants to fungal infections. It is indicated that excess nitrogen in the soil increases the frequency of infection of grains with *Fusarium* fungi. However, this depends on the chemical form of nitrogen used (urea, ammonium nitrate or calcium nitrate) [55]. Crop weed infestation is also considered to be one of the factors influencing the increased degree of mycotoxin content in cereal grains. Some weed species are also believed to be reservoirs of spores of the fungus family *Fusarium sp.* This is, therefore, an explanation for the correlation between a higher degree of weed infestation in crops and fungal infection of wheat ears. Also, the residual green matter of the weeds increases the content of plant residues infested with mycelium and thus creates favourable conditions for propagation with propagules (vegetative form of propagation – propagules). In addition to fungicide protection, one of the most important methods is also the breeding of new cultivars showing higher resistance to infestation, also through the use of biotechnological methods. Genetically modified maize cultivars that show resistance to European corn borer feeding are also less susceptible to fungal infestation and therefore contain fewer mycotoxins. This is confirmed by the results of a study by Tekiela and Grabarkiewicz [47]. In a 2-year experiment, the cited authors found lower mycotoxin content in GM cultivars compared to conventional cultivars. Selvet [39] analysing the average degree of infestation of cobs by fusarium head blight of different cultivars showed varying degrees of infestation. The most severely infested cultivars were Junak and Baca. The lowest concentration of deoxynivalenol was found in 2006 and 2007 in the GMO cultivar MON 810. The highest concentration of deoxynivalenol was found in the Prosna cultivar. In the case of wheat, it was possible to obtain lines that provided cultivars resistant to *Fusarium sp.* but, unfortunately, the quality of the grain obtained and the agronomic properties were reduced, which resulted in these lines not being registered. However, among the already existing breeding lines of many cereal species, there are lines that are more or less susceptible to the aforementioned fungal pathogens. This demonstrates the diversity of traits responsible for resistance to infection and the potential for further selection and breeding work. Additionally, the fact that genes responsible for traits relating to resistance to fusarium infections in wheat have been identified [9; 32; 41] is helpful for this purpose. These traits are often located together with the genes determining morphological traits of the plant. Since there are many methods of preventing Fusarium head blight, but no single effective one, it is worth combining them all to maximise the potentially greatest preventive effect. Weather conditions are the factor most conducive to infestation and which is impossible for the farmer to control. However, it is worth combining those elements that are possible. These include the appropriate selection of cultivars characterised by high resistance, appropriate cultivation and crop rotation applied in the field, and balanced fertilisation can significantly contribute to reducing the risk of mycotoxin infestation [1].

Characteristics of mycotoxins

Each group and species of fungi produces mycotoxin types specific to it. These have varying toxic effects. *Fungi* of the genus *Fusarium* produce mainly zearalenone, fumonisins, deoxynivalenol and trichothecene toxins [7].

In the available scientific literature, the identification of the mycotoxin group of harmful substances dates back to 1711, when the toxic effects of ergot were first identified. Another landmark date was also 1960, when a mass death of turkeys on one farm was found to be caused by feed contaminated with aflatoxin [13]. Subsequently, poisoning was also found in other livestock species such as ducks, pigs, cattle and horses. The cause was the feeding of feed that contained peanut meal contaminated with aflatoxin in its composition [16]. This was when there was an increased scientific interest in mycotoxins. In the following years, numerous papers were published on this group of substances.

Although mycotoxins constitute a numerous group of substances; the same mycotoxin can be produced by many different fungal species, as well as not necessarily by all strains of a given species. It also happens that one species of fungus produces several types of mycotoxins.

What mycotoxins have in common is that they are all produced by mould fungi and have toxic effects on human and animal organisms, but they differ in terms of their chemical structure. This determines the toxicity and effect in the body

of a particular mycotoxin. These toxins pose two risks – acute poisoning and the risk of developing chronic poisoning. Chronic effects develop due to the accumulation of toxins in the body or as a result of the accumulation of minor morphological or biochemical damage within organs [24]. Therefore, a number of scientific studies are devoted to the chemical structure and toxicological characterisation of mycotoxins. Such knowledge is an indispensable prerequisite, the basis of any strategy to combat mycotoxins, both in terms of methods to prevent mould growth and to protect products from contamination. This is a significant problem occurring worldwide [36].

In addition to the free forms of mycotoxins, there are so-called modified mycotoxins [18]. This creates the problem of definitively estimating the total content of mycotoxins, as only their free forms are detected, and thus routine testing methods cause underestimates. Modified mycotoxins are formed as a result of biotransformation of parent forms, among others, in plants by coupling toxins to hydrophilic compounds (e.g. amino acids, sugars) or by bacterial or fungal metabolism (e.g. reduction) [8]. During plant growth, when infestation by *Fusarium* fungi occurs in a defence response, the plant recognises the mycotoxins and starts a defence process. During this process, the free mycotoxins undergo a glycosylation process. This process involves the combination of a free mycotoxin molecule with a glucose molecule by means of the enzyme glycosyltransferase. Another metabolic pathway in plants that neutralises the harmfulness of mycotoxins is the coupling of mycotoxins to a sulphur molecule effectively neutralising their toxic effects [4].

The presence of modified mycotoxins can be of great toxicological importance, as some may exhibit toxicity higher than the basic forms, or they may be released into their parent forms in the gastrointestinal tract of animals and humans. Modified mycotoxins can be formed by plant defence systems (e.g. DON-3-Glc, zearalenone-14-glucoside (ZEN-14-Glc), nivalenol-3-glucoside (NIV-3-Glc), HT-2-glucoside (HT-2-Glc)), bacterial metabolism (deepoxy-DON), fungal metabolism (e.g. 3-acetyl-deoxynivalenol (3-Ac-DON), 15-acetyl-deoxynivalenol (15-Ac-DON)), animals (e.g. formation of aflatoxin M1 from aflatoxin B1). These forms are formed when plants protect themselves from the free forms of mycotoxins by converting them into the form of more polar metabolites that are less toxic to them. This process occurs while plants are still growing in the field when infestation with *Fusarium* fungi occurs; mycotoxins such as DON, ZEN, FB1, FB2, T2, HT-2 and nivalenol (NIV) are the most commonly metabolised by plants. Of all the modified forms of mycotoxins determined to date, the most data exist on the occurrence of DON-3-Glc. The ratio of DON3-Glc to the unmodified form ranges from 20 to 70% [35].

Cereal cultivation has two main purposes - for human food and for livestock feed. Mycotoxin contamination of food and feed is highly dependent on environmental conditions, which can inhibit or accelerate mould formation and growth. Contamination can occur at any stage of production (plant development, harvest, handling, storage and transport). Another problem is that mycotoxins are low molecular weight, weakly polar compounds and are not broken down during the technological processes used in grain processing [43]. They do not decompose at high temperatures. They can enter the human body through the oral route by direct ingestion of contaminated grain products, or by consuming products from animals fed with mycotoxin-contaminated feed. These toxins accumulate in soft tissues such as the liver, kidneys and also in muscle. In detoxification processes taking place in the body, chemical forms of mycotoxins are changed into another chemical form with weaker toxic properties [28]. An example is the discovery that dairy cows fed feed containing aflatoxin B1 excrete its derivative aflatoxin M1 with their milk. Until now, it was thought that milk and milk products from organic production could contain aflatoxin. This view was linked to the belief that restrictions on the use of fungicides resulted in an increase in mycotoxin content in grain for feed. However, some studies contradict this and even show that mycotoxin levels are lower in organic milk [51]. Exposure of livestock to zearelenone leads to urogenital disorders, while acute or chronic poisoning can cause permanent damage to the organs of the reproductive system, such as degenerative changes of the testes, ovarian atrophy, infertility and abortions. The contamination of food of animal origin (mainly milk and meat) with mycotoxins from *Fusarium* is currently low, due to the continuous monitoring of these products for their safety, starting with the feed raw materials through the testing of food products [37].

Mycotoxins are toxic and pose a health risk to all animals. The greatest sensitivity to mycotoxin contamination of feed is found in poultry and pigs [19]. Both these groups of animals also receive complete feeds with a high proportion of cereal grains, which is also important in exposing them to ingestion of high levels of mycotoxins. However, scientific results indicate some species differences in sensitivity to the toxic effects of different mycotoxins. Poultry show less sensitivity to fumonisins in their feed than pigs and horses. This is related to the difference in the degree of absorption of this mycotoxin in the digestive tract [23]. Furthermore, among poultry it is noted that there is also a differential sensitivity to fumonisin. Turkeys and ducks are much more susceptible to poisoning than chickens [48].

Death occurred in horses fed feed that contained aflatoxin-contaminated maize, and extensive liver necrosis was found following autopsy. The chemical analysis of the maize performed showed aflatoxin B1, B2 and M1 at concentrations of 114, 10 and 6 µg/kg, respectively (Vesonder et al., 1991). In contrast, as suggested by the study of Schulz et al. [38] horses are less sensitive lower than 1 mg/kg deoxynivalenol than other animal species. Ruminant animals (cattle as well as goats and sheep), whose diet is based on roughage, mainly hay and green fodder, are less likely to ingest higher amounts of mycotoxime derived from every cereal grain. In addition, they are to some extent protected by the partial ability of the rumen microflora to detoxify mycotoxins [34].

In relation to the issue of the toxicity of mycotoxins to humans and animals, there is a need for preventive mechanisms. As it is inevitable that the risk of these substances is completely eliminated, the most effective method, apart from agrotechnical methods, is the constant control of the level of contamination of

cereals. The multitude of factors influencing mycotoxin content makes it necessary to test each batch of grain to determine the mycotoxin content against the permitted standards.

The detection of batches exceeding the limits allows cereals and cereal preparations to be rapidly withdrawn from the market. The wide variety of mycotoxins and their toxic effects, and their possible synergistic interaction by compounding the harmful effect, is also a significant problem. The synergistic interaction of two or more mycotoxins may cause the sum of their content in cereals, their processing products and feed, to cause even greater harm to humans and animals [11].

One solution to reduce the uptake of mycotoxins into the system is the use of probiotics, which, through their adsorption, reduces their bioavailability in the intestinal lumen, prevents their absorption into the bloodstream and protects organs and tissues. The bound probiotic-microbe complex is excreted, but the effective operation of this mechanism requires a constant supply of probiotic with feed [10]. In an experiment conducted on dairy cows, it was found that the addition of S. *cerevisiae* yeast to feed contaminated with aflatoxin B1, reduced the excretion of its metabolite aflatoxin M1 with milk [21]. A method of protecting animals from the negative effects of feed containing mycotoxins is the use of detoxicants. These are preparations based on aluminosilicates or activated carbon that bind mycotoxins into stable complexes. The binding of mycotoxins prevents them from being absorbed in the intestines into the system and they are eventually excreted in the faeces. However, with significant contamination of feed with mycotoxins, the use of detoxifiers proves ineffective [25].

Conclusions

Mycotoxin content is an important indicator of grain quality. Achieving as low content as possible is the goal in order to protect human and animal health. Measures taken in this direction include breeding cereal cultivars resistant to fusarium head blight, using appropriate fungicide protection and good agricultural practice. The weather pattern during especially earing, which is unpredictable and impossible to modify, is also an important factor. As many factors influence the mycotoxin content of grain, continuous laboratory monitoring of grain batches destined for feed purposes is necessary as before. With the detection of new forms of mycotoxins, so-called modified forms, the development of analytical methods for their detection is a necessary direction. Important methods of protecting animal health against the negative effects of mycotoxins are the use of feed additives. These include probiotic preparations and detoxifiers. As mycotoxins are a constant threat, further research is needed to find effective mycotoxin-binding agents such as sorbents and detoxicants in both in vitro and in vivo studies.

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