Occurrence of mycotoxins in feed grain before and after storage time

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Abstract

The samples of barley and wheat contaminated with mycotoxins produced by *Fussarium* and *Aspergilus* spp. were included in this experiment. The analyzes were carried out in freshly harvested samples in 2019 and after storage of two years in 2021. The samples were collected from all regions of Bulgaria. In the samples, the following mycotoxins were detected such as zearalenone and aflatoxins by Enzyme Linked Immunisorbent Assay method. The highest zearalenone concentration of 156.78 µg/kg was proved in barley samples followed of 143 µg/kg in wheat samples. The highest detected concentration of aflatoxins was 11.55 µg/kg in barley and 10.40 µg/kg in wheat. After two-year storage period, the samples were analyzed again. Zearalenone detected in barley samples was ranging from 90.25–125.30 µg/kg and in wheat samples from 76.58–116.10 µg/kg, while aflatoxins were detected only in wheat samples in the range 3.73–4.63 µg/kg. The established levels of contamination are lower than those referenced in the European regulations. The results have shown that barley and wheat lead to an increase in the concentration of mycotoxins after two years of storage, which could has a direct influence on the feed safety and animal health.

***Key words*:** mycotoxins, barley, wheat, storage

Introduction

Mycotoxins are low molecular weight secondary toxic metabolites of fungi. They are one of the most important groups of anti-nutritional substances found in feed.

Barley and wheat are widely used among the raw feed materials. They are predisposed to the occurrence of mycotoxins particularly produced by *Fussarium* and *Aspergillus* spp.

Fusarium genera fungi produce amongst others, fumonisins and zearalenone (ZEA) mycotoxins and are considered the main cause of feed grains damages (Tanaka, 2001). The ZEA production is related to high temperature and humidity conditions and its formation happens before harvest or during the early drying stage. Thus, the control of the ZEA levels requires more attention in the pre-harvest practices (Munkvold, 2003).

The chemical structures of ZEA and its metabolites are similar to the naturally occurring oestrogens (Gromadzka, et al., 2008) and are described as compounds with oestrogenic properties.

In pigs, for example, the damage caused by ZEA can be high, because its metabolites have oestrogenic and anabolic activities, which may cause hyperestrogenism and affect the animal reproductive system (Hauschild et al., 2007).

ZEA and aflatoxins are recognized as major potential contaminants of the most of feed materials worldwide. Synergistic effects of multiple mycotoxins in the feed (Basso et al., 2013) cause the toxic effects. Their presence depends of many factors some of which are weather and storage conditions. Aflatoxins tipically occur after harvest at storage conditions. Aflatoxins (*Aspergillus flavus* and *Aspergillus parasiticus*) are widely recognized as a major health problem, especially in hot, humid countries. This is a particular serious problem in crops such as barley, wheat, maize, rice, peanuts, tree nuts, and dried fruits. Aflatoxin production occurs normally in high temperatures or during prolonged drying. Aflatoxin-producing molds grow exponentially in conventional multi-month storage as a result of a combination of heat and high humidity (Hell et al., 2010).

Excessive aflatoxin levels can cause death in farm animals such as chickens and turkeys.

Oladele (2014) has shown that aflatoxins cause infertility, abortions, and delayed onset of egg production in birds as well as sudden losses in egg production in actively laying birds. Furthermore, loss of appetite, skin discoloration, or even yellowish pigmentation on skin can be observed in fish.

In addition, the presence of mycotoxins in feed grains can also cause chronic mycotoxicoses (Persi et al., 2014).

The changes of the occurrence of mycotoxins in stored commodities can occur during stored feed (Sobrova et al., 2010).

In Bulgaria some studies have been published related to the ZEA and aflatoxins incidence in feed grains but there is a lack of data about the incidence of these mycotoxins in barley and wheat during the storage period. Thus, due the high toxicity of these mycotoxins, the objective of this study was to assess the incidence of ZEA and aflatoxins before and after the storage time.

Materials and methods

Feed samples: Freshly harvested representative samples of barley (30 samples) and wheat (32 samples) collected from different regions of Bulgaria were analyzed on the individual mycotoxins after harvest in 2019 and after two years storage period.

Freely laid samples were stored in sterile plastic containers at room temperature, in the dark for a period of two years. After this time, the second analysis was conducted to determine the differences between mycotoxins.

Samples analyses: 5 g of ground samples were mixed and processed using 70% methanol (Valerus Ltd, Bulgaria) as solvent for extraction. The filtered (Whatman No 1, Merck) samples were screened for ZEA and aflatoxins by the Enzyme-Linked Immunosorbent Assay (ELISA) method. A commercial kit (R-Biopharm, Germany) was used and the samples were prepared according to the instructions of the manufacturer. The measurement was made at 450 nm. The absorbance was inversely proportional to the ZEA and aflatoxins concentration in the sample. The values calculated for the standards were entered in the Ridawin program, Computer Systems (ELx800 Universal Microplate Reader, BIOTEK® Instruments, Inc., USA).

Results and discussion

Mycotoxicological analysis was performed with barley and wheat samples. The presence of ZEA and aflatoxins was detected in all freshly harvested samples in 2019. In barley samples, the presence of ZEA was detected 20% while in wheat samples it was registered higher, namely 28%. Barley and wheat were the most favorable substrates for ZEA accumulation and namely the higher incidence was registered with wheat samples followed by barley samples.

The highest concentration of ZEA was measured in barley – 156.78 µg/kg and 143 µg/kg in wheat. The both samples originated from Northwestern region. The obtained concentrations of presence of fusarium toxin in the freshly harvested samples did not exceeded the maximum limit adopted by the European Commission, Regulation 2006/576/EC. Similar results were found in the neighboring countries. For instance in Turkey, Bilal et al. (2014) have reported varied ZEA concentrations below the maximum limits in feedstuffs. Likewise, in Romania, Galbenu et al. (2011) have also found low concentrations of ZEA in wheat, barley and maize.

The presence of aflatoxins before storage was detected in all of the studied grain samples, with 13% positive barley samples and 16% positive wheat samples. The lowest detected concentration of 2.18 µg/kg and the highest of 11.55 µg/kg was proven for barley samples. The samples originated from North central region. In wheat samples, the detected high value of aflatoxins was 10.40 µg/kg. The sample originated from southeastern part. The registered values by us for aflatoxins contamination were below 20 µg/kg as referred in Commission Directive 2003/100/EC.

During the storage time, the presence of both mycotoxins was detected in all of the analyzed samples except aflatoxins in barley samples. The levels of individual mycotoxins are shown in Table 1 and Table 2.

The occurence of ZEA was detected in 9 (14.5%) of the total evaluated samples of wheat and barley. Where the concentrations ranged from 76.58 to 125.30 µg/kg, Table 1.

The presence of ZEA was registered mainly in the negative tested samples for 2019 harvest. There was no difference between the levels of ZEA found in the samples measured in 2019 and after the storage time. The only exceptions were two samples of wheat where the concentration increased by factor of two in one and in another one it decreased a little. Reducing the level during the storage period in some cases, may be attributed to the difficulty to obtaining homogeneous sample in this type of storage. The both samples originated from Northwestern region. This funding indicated that ZEA is a mycotoxin that may occurs in the pre-storage period and in the storage too, Table 1.

In our monitoring, we registered the prevalence of ZEA in freshly harvested and stored samples, Figure 1.

The lowest concentration (76.58 µg/kg) of ZEA found in the stored cereal samples was increased compared to those found in the freshly collected samples (50.92 µg/kg), Table 1.

The highest concentration of 125.30 µg/kg was detected in stored barley samples followed by 116.10 µg/kg in stored wheat samples. The samples originated from North central part. Probably the presence of ZEA in the tested samples of storage may have occurred due to contamination previously occurred in the field, once

Table 1. Zearalenone contamination of feed materials in the period 2019–2021

Sample	Year	Number of samples	Number of positive samples	% of positive samples	Range (min-max), (µg/kg)	Average value ± SD, (µg/kg)
Wheat	2019	32	9	28	50.92–143	126.88 ± 28
	2021	32	6	19	76.58–116.10	95.40 ± 12.3
Barley	2019	30	6	20	76.18–156.78	135 ± 29
	2021	30	3	10	90.25–125.30	105.3 ± 14.7

Table 2. Aflatoxins contamination of feed materials in the period 2017–2019

Sample	Year	Number of samples	Number of positive samples	% of positive samples	Range (min-max), (µg/kg)	Average value ± SD, (µg/kg)
Wheat	2019	32	5	16	8.29–10.40	9.3 ± 0.83
	2021	32	2	6	3.73–4.63	4.18 ± 0.45
Barley	2019	30	4	13	2.18–11.55	7.23 ± 3.38
	2021	30	N/D	N/D	N/D	N/D

**N/D – not detected*

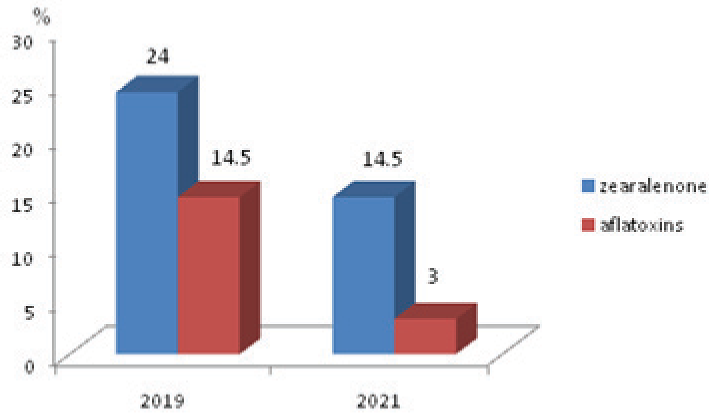

Fig. 1. Mycotoxins contamination before and after storage time

this mycotoxin is produced by *Fusarium* spp., a typical field fungus.

The risk of mycotoxins is increased in agricultural commodities for long periods of storage. The content of important nutrients is also reduced (Toth et al., 2013). Mycotoxins, in turn, affect the digestion and metabolism of nutrients in animal production, resulting in nutritional and physiological disorders, besides a negative effect on the immune system (Bünzen and Haese, 2006).

The contamination with aflatoxins during the storage time is a little different. Production of aflatoxins was found only in two wheat samples with concentrations lower than measured before in freshly harvested grains, Table 2. The both samples originated from south central part. Regarding contamination with aflatoxins, it should be mentioned that wheat is a favorable substrate for the development of toxic fungi of the genus *Aspergillus* spp.

Most of the stored seeds were free of contamination. This could be because the seeds were in good quality, e.g. they were not damaged and discolored which is an effective way to prevent further contamination. Moreover, taking into account that aflatoxins are storage mycotoxins, the dry containers are the basic elements of aflatoxin prevention. In addition, we have an access to drying and storage equipment too.

Clean, dry, insect and rodent free storage conditions are critical to prevent aflatoxin growth (Negash, 2018).

Aflatoxins are just one of many mycotoxins that can adversely affect animal health and productivity. Care regarding animal feed must be extended not only to the nutritional and economic value, but also to food quality (Gonçalez et al., 2004).

From another side Chulze (2010) has showed that the important part is the elimination of insect disrupting plant tissues, which simplifies the penetration of pathogens.

The protective atmosphere CO_2 can be also used to prevent the occurrence of pathogenic fungi (Prange et al., 2005).

Another way to effectively reduce the occurrence of fungal infestation is a fungicide applications in stored commodities. These substances can effectively protect against the occurrence of pathogenic fungi leading to reduction of mycotoxin production (Horky, 2014).

Conclusions

ZEA and aflatoxins were found as natural contaminants for barley and wheat samples before and after storage time. The highest concentration of ZEA was measured in barley – 156.78 µg/kg and 143 µg/kg in wheat.

In the stored samples, the occurrence of ZEA ranged from 76.58 to 125.30 µg/kg.

The highest detected concentration of 11.55 µg/kg for aflatoxins was proven for freshly harvested barley samples while in the stored samples aflatoxins were not detected. The obtained concentrations of presence of the both mycotoxins in the freshly harvested samples and in the storage period did not exceede the permissible levels recommended by the European Commission for animal feed.

Analyzing the results according to European regulations, it can be concluded that contamination with ZEA and aflatoxins does not pose a potential risk to the health and productivity of farm animals. Nonetheless, it is recommended to continuously monitor the seeds in order to protect them against the risk of mycotoxins contamination.

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