UDC 636.598.15:619:616:615.

Survey of poultry feeds contamination with mycotoxins

Trufanova V.
Trufanov O.
Candidates of Biological Sciences
Horbenko Z.
Chorna G.
State poultry research station, NAAS of Ukraine

The purpose. To evaluate the contamination of poultry feeds in Ukraine with mycotoxins and other toxic substances. **Methods**. ELISA, TLC-bioautography, *Lebistis reticulatus* bioassay. **Results**. A quarter of the tested feed samples were shown to be contaminated with at least one mycotoxin, and a third — by substances toxic for fish. Only few samples contained mycotoxins in concentrations, which either exceeded or were similar to the permissible levels in poultry feeds. The contamination was least frequent during 2016, and the most — during 2015. The frequency of wheat and maize contamination was lower than that of compound feeds. **Conclusions**. Generally, the tested feed samples met existing sanitary requirements. However, prevalence and variability of mycotoxin contamination create a necessity for systematic monitoring of feeds quality and corresponding application of prophylactic measures.
Keywords: *mycotoxin contamination survey, feed quality, poultry.*

Poultry farming plays a critical role in food security, since its products are among the cheapest traditional sources of complete animal protein, many essential and functional nutrients in the human diet. In Ukraine, volumes of poultry meat production greatly exceed those of beef or pork, and production of eggs has almost doubled since 2001.

However, poultry health and productivity, as well as the quality of production obtained from it, strongly depend on the sanitary condition of feeds, defined as the level of their contamination with microorganisms, harmful substances of anthropogenic and natural origin. Mycotoxins are among the most dangerous and ubiquitous grain and feed contaminants. They are produced by mold fungi of *Aspergillus, Penicillum*, *Fusarium, Claviceps, Alternaria* genera that invade crops and products of crops processing. It is believed, that among few hundred known mycotoxins, of the greatest concern, due to their high toxicity and prevalence, are aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, T-2 and HT-2 toxins), fumonisins and zearalenone [1].

Monitoring must be an integral element of the system intended to ensure the quality of feeds. Therefore, the objective of the present work was to survey the contamination of poultry feeds with mycotoxins and other toxic substances.

The work was conducted at the State Poultry Reseach Station of NAASU from 2014 to 2016. The content of aflatoxin B_1, deoxynivalenol, and zearalenone was determined by ELISA method («Ridascreen» test-kits, R-Biopharm AG); of T-2 and HT-2 toxins — by TLC-bioautography [2], and the toxicity of feed extracts — by *Lebistis reticulatus* bioassay [3].

In total, 436 samples were analyzed, namely 124 samples of compound feeds, 125 — of maize, 111 — of wheat, and 76 other samples (gluten, sunflower press cake, grain mixtures, feed grade yeast), which resulted in 1856 determinations. The samples were obtained from more than thirty grain processing enterprises and poultry farms.

The frequency of the analyzed samples contamination was not constant during the study — in 2015 it had been much higher, than in 2014 or 2016 (image 1).

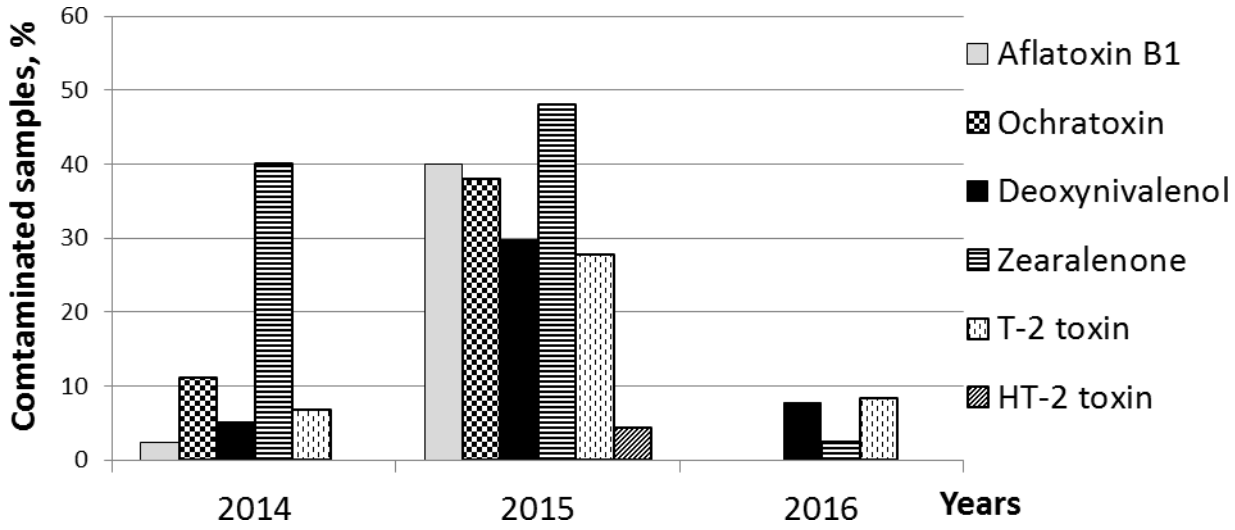

Image 1. Mean frequencies of the analyzed samples contamination with mycotoxins by years

The observed unevenness of mycotoxin contamination is consistent with the works published by SPRS researchers, as well as by other authors. For example, according to [4], the frequency of T-2 toxin detection amounted to 55% in 2006 and only to 1.5 in 2010. Also, the outbreaks of corn contamination with toxin-producing fungi and of mycotoxicoses in poultry farms are known to be periodical [5, 3]. However, the exact cause of this is hard to determine, since the frequency of mycotoxin contamination depends on numerous natural and anthropogenic factors.

Throughout the survey, zearalenone was found to be the most frequent contaminant, and HT-2 toxin — the least (respectively, 30 and 2% of the analyzed samples) (table 1).

1. The fraction of contaminated feeds

Contamination	Analyzed samples / Contaminated samples			
	Compound feed	Maize	Wheat	Other samples
Aflatoxin B_1	74/22	90/11	94/5	25/8
Ochratoxin	57/24	84/10	92/5	16/4
T-2 toxin	96/21	102/20	102/10	30/3
HT-2 toxin	96/5	102/1	102/1	30/0
Deoxynivalenol	64/24	86/6	92/5	20/5
Zearalenone	51/24	85/15	91/10	19/8
Toxic by bioassay	76/29	22/6	13/4	45/20

Low frequency of HT-2 toxin detection, taking into consideration its comparatively high concentrations, found in feeds (up to 0.24 mg/kg, table 3), might be explained by relatively low sensitivity of the method used for its determination — 0.1 mg/kg (sensitivity of the methods, used for zearalenone, deoxynivalenol and T-2 toxin determination amounted to 50, 20 and 10 mkg/kg respectively). So, the existence of considerably greater frequencies of HT-2 toxin contamination, than found in present work, cannot be excluded.

The frequencies of other mycotoxins detection in wheat and maize samples varied from 5 to 20, and in compound feed and other samples — from 10 to 40%. Obtained result is in certain contradiction with the popular notion that aflatoxins do not occur in countries with temperate climates. However, widespread distribution of aflatoxins was also demonstrated in other surveys [6]. This, in part, might be explained by the high sensitivity of the modern methods used for aflatoxins determination (<1 mkg/kg).

Approximately a quarter of the analyzed samples appeared to be contaminated by at least one mycotoxin, and a third contained substances toxic for fish (tables 1 & 2).

2. Co-contamination of the analyzed samples with mycotoxins

Contamination with mycotoxins	Contaminated samples, % of analyzed			
	Compound feed	Maize	Wheat	Other samples
One	43	15	14	37
Two	31	14	5	37
More than two	24	6	2	12

The frequency of maize co-contamination was 2-3, and of wheat — up to 9 times lower, than that of compound feeds or other samples. Also, they less frequently appeared to be toxic for fish (table 1). Among possible reasons for the lower frequency of wheat and maize contamination, in comparison to the products of their processing (compound feeds, gluten, grain distillery dregs used for yeast cultivation), might be that grains were more likely to be analyzed shortly after harvest. Consequently, strengthening of the measures, aimed at prevention of mycotoxin contamination during storage, might have the potential for improving the safety of feeds.

The highest levels of contaminations were observed with deoxynivalenol (up to 1 mg/kg). Maximum concentrations of zearalenone, HT-2 and T-2 toxins were more than three times lower — 0.1-0.3, and for aflatoxin B_1 and ochratoxin, they amounted only to 0.01-0.06 mg/kg (table 3).

3. Mycotoxin concentrations in samples, mkg/kg

Mycotoxins	Feed samples			
	Compound feed	Maize	Wheat	Other samples
Maximum concentrations				
Aflatoxin B_1	10	10	10	12
Ochratoxin	14	60	7	43
T-2 toxin	70	60	96	80
HT-2 toxin	180	120	240	-
Deoxynivalenol	1000	200	100	700
Zearalenone	300	210	40	450
Mean concentrations				
Aflatoxin B_1	3	4	5	6
Ochratoxin	6	9	3	14
T-2 toxin	17	20	37	40
HT-2 toxin	70	-	-	-
Deoxynivalenol	200	60	30	270
Zearalenone	50	40	20	180

Just a small fraction of the analyzed feed samples (less than two percent) contained mycotoxins in concentrations that amounted to more than a half of the maximum permissible levels (MPL), established in Ukraine for poultry feeds. Among all determinations performed, only contamination of a maize sample with ochratoxin was found to exceed the MPL (image 2).

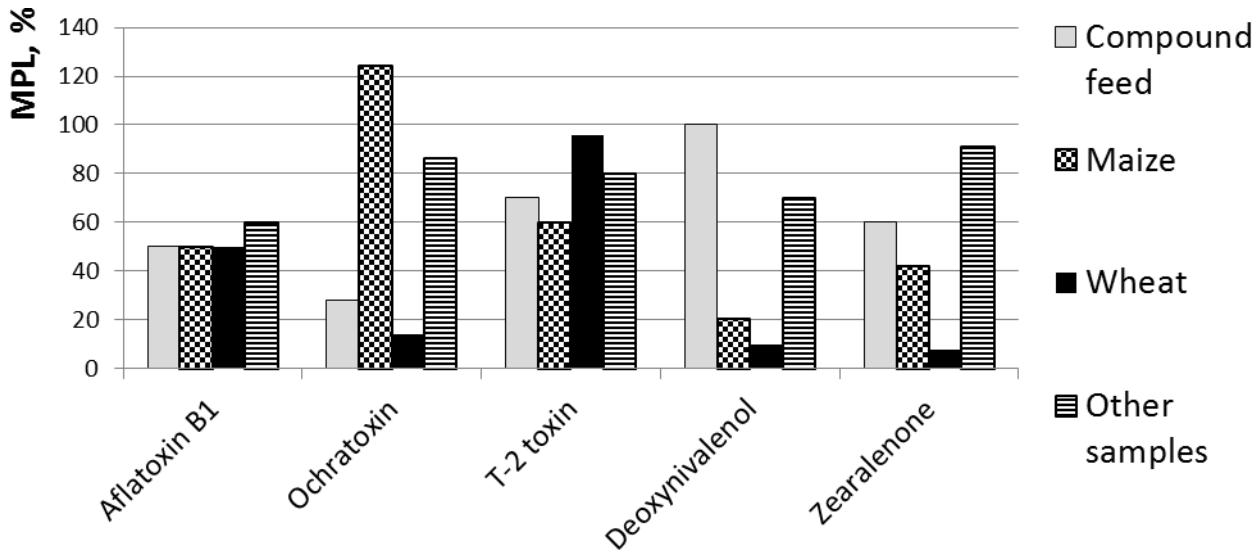

Image 2. The ratio of the maximum mycotoxin concentrations, found in the analyzed feed samples, to corresponding MPL

Note: MPL of poultry feed contamination with mycotoxins amount to: for aflatoxin B1 — 0.02, ochratoxin — 0.05, T-2 toxin — 0.1, deoxynivalenol — 1, and zearalenone — 0.5 mg/kg (HT-2 toxin is not regulated)[7].

Observed concentrations of mycotoxins in feeds are unlikely to cause any marked symptoms of mycotoxicoses. For example, the maximum concentrations of T-2 toxin were dozens, and of other mycotoxins — about a hundred times lower than necessary to cause a 10% weight gains reduction in chickens (image 3).

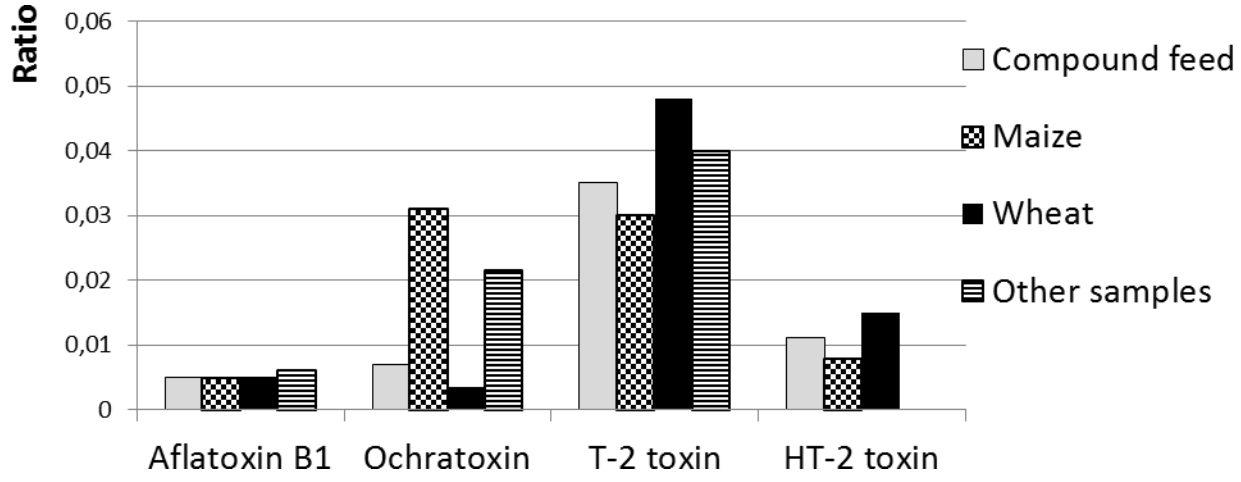

Image 3. Ratio of the maximal mycotoxin concentrations, found in samples, to such, that cause a 10% weight gains reduction in chickens

Note: specified weight gain reduction can be caused by addition to feeds 2 mg/kg of aflatoxin B_1, ochratoxin or T-2 toxin. The concentration of HT-2 toxin, required for this, amounts to 16 mg/kg, while purified deoxynivalenol and zearalenone have virtually no effect on weight gains in chickens [8-13].

However, under “field conditions”, adverse influence on poultry health was reported even in cases of feed contamination with trichothecene mycotoxins in concentrations similar to the observed. For example, a case of decreased productivity of hens, associated with feeds contamination by less than 0.1 mg/kg of T-2 of toxin has been reported [5]. Deoxynivalenol, to effects of feed contamination by which poultry is considered almost insensitive, at concentration of 1 mg/kg was shown to decrease glucose transport in

broiler enterocytes [14]. Synergistic interactions with other mycotoxins are believed to be the reason for comparatively greater toxicity of trichothecenes, when fed in the form of naturally contaminated feeds [15, 16]. This, taking into consideration the high prevalence of mycotoxin co-contamination, might create certain risks of enteritis, immunity suppression, and other adverse effects, especially in young birds.

Conclusions

The levels of contamination, found in present work, are much lower than those, which can cause marked symptoms of mycotoxicoses. However, the maximal concentrations of trichothecenes, found in the analyzed samples, along with the high prevalence of co-contamination, pose a risk of their synergistic interactions that might lead to adverse effects like enteric inflammatory diseases, immunity suppression. That, along with variability of mycotoxin contamination, creates a necessity for systematic monitoring of feed quality and corresponding application of prophylactic measures.

References

1. Bhat R. Mycotoxins in food and feed: present status and future concerns / R. Bhat, R.V. Ral, A.A. Karim // Comprehensive reviews in food science and food safety. — 2010. — Vol.9. — P. 57-81.
2. Котик А.М., Труфанова В.О., Труфанов О.В. та ін. Визначення Т-2 і НТ-2 токсинів в зерні та комбікормах: Метод. рек.- Бірки, 2006.-8 с.
3. Санитария кормов / Г.А. Таланов, Б.Н. Хмелевский // М.: Агропромиздат, 1991. — 303 с.
4. Труфанов О.В. Мониторинг загрязненности микотоксинами зерна и кормов в Украине в 2005-2010 гг. / О.В. Труфанов // Сучасні проблеми токсикології. — 2011. –№1-2. — С. 35-39.
5. Котик А.Н. Случаи микотоксикозов сельскохозяйственніх птиц в Украине в 1974-96 г.г. / А.Н. Котик, Труфанова В.А. // Птахівництво (Харків) Міжвідомчий тематичний науковий збірник. — 1997. — № 47. — С. 92-100.
6. Rodrigues I. A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed / I. Rodrigues, K. Naehrer // Toxins. — 2012. — Vol.4. — P. 663-675.
7. Наказ міністерства аграрної політики та продовольства України № 131 «Про затвердження переліку максимально допустимих рівнів небажаних речовин у кормах та кормовій сировині для тварин» від 19.03.2012.
8. Yueming D.L. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry / D.L.Yueming, Verstegen M.V.A, Gerrits W.J.J. // Nutrition research reviews. — 2003. — №16. — P. 223-239.
9. Battacone G. Effects of Ochratoxin A on Livestock Production / G. Battacone, A. Nudda, G. Pulina // Toxins. — 2010. — №2. — P. 1796-1824.
10. Nesic V. Efficacy of T-2 toxin detoxifying agent in broiler chickens / V. Nesic, R. Resanovic, D. Marinivic [et al.] // Acta Veterinaria. — 2012. — №2-3, Vol. 62. — P. 171-182.
11. Труфанов О.В. НТ-2 токсин: мікробіологічний метод визначення, розповсюдженість, токсичність та застосування препаратів Bacillus Subtilis при НТ-2 токсикозі курей / Автореферат дисертації на здобуття наукового ступеня кандидата біологічних наук 2009.
12. Pestka J.J. Deoxynivalenol: Toxicity, mechanisms and animal health risks // Animal feed science and technology. — 2007. — №137. — P. 283-298.
13. Allen N.K. Effects of Dietary Zearalenone on Finishing Broiler Chickens and Young Turkey Poults / N.K. Allen, C.J. Mirocha, G. Weaver / Poultry Science. — 1981. — №1. Vol.60. — P. 124-131.
14. Awad W.A. A diet naturally contaminated with the Fusarium mycotoxin deoxynivalenol downregulates gene expression of glucose transporters in the intestine of broiler chickens / W.A. Awad, W. Vahjen, J.R. Aschenbach, J. Zentek // Livest. Sci. — 2011. — №140. — P. 72-79.
15. Emetic and refusal activity of deoxynivalenol in swine / D.M. Forsyth, T. Yoshizawa, N. Morooka, J. Tuite // Applied and environmental microbiology. — 1977. — Vol. 34, №5. — P. 547-522.
16. Smith T.K. Recent advances in the understanding of Fusarium Trichothecene mycotoxicoses / T.K. Smith // Journal of animal science. — 1992. — №70. — P. 3989-3993.