EFFECTS OF USING A DETOXIFYING AGENT IN MIXED FEED, ON THE HAEMATOLOGICAL, IMMUNOLOGICAL PARAMETERS AND HISTOPATOLOGICAL ALTERATIONS IN CHICKEN BROILERS

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Abstract

Fight against mycotoxicosis could be carried on through prophylaxis measures, such as well drying the cereals presenting high humidity values, prior to storage. If they are already contaminated with mycotoxins, they could be annihilated through absorption inhibition or by usage of some bacteria which consume mycotoxins. The purpose of this paper was to assess the effect of ochratoxine A (OTA) and deoxynivalenol (DON) on the haematological, immunological an histological response of the chicken broilers, protected or not protected against mycotoxins through feeding or not a detoxifying-mycotoxin inhibitor additive, meaning Mycofix MTV product, from Biomin, at dietary inclusion rates of 1‰ and 3 ‰. The biological material was represented by 111678 ROSS-308 chicken broilers, reared industrially, in deep litter system, till slaughter at 40 days old. Prior to slaughtering, blood samples were taken; digesta samples and internal organ tissues were sampled from slaughtered broilers, in order to assess the influence of the used feed additive on the following parameters: concentration of DON in digesta, concentration of OTA in gall, white blood cells formula (%), properdine (µg/ml), serum lysosime (µg/ml), histopatological alterations in ingluvies, ileum, caecum, liver, kidneys and spleen. Mycotoxicological exam revealed rapid inactivation of DON, since the ingluvies, with: 55.52% at 1‰ Mycofix MTV and 64.41% at 3‰ Mycofix MTV. In caecum, compared to control group, DON decreased by: 11.12 at 1‰ Mycofix MTV and 30.56% at 3‰ Mycofix MTV. Histopatologically, in ingluvies, 3‰ Mycofix MTV, mucosa lesions were reduced. In ileum and caecum, necrosis, ulcers and flattening of villus drawing supported the protective effect of Mycofix MTV against DON, no matter the dietary dosage. Mycofix MTV diminished the liver excretion of OTA, through gall, with: 15.93% at 1‰ Mycofix MTV and 21.24% at 3‰ Mycofix MTV, as a consequence of OTA inactivation and detoxification in the digestive tract.

Key words: broilers, mycotoxins, properdine, lysosime, histopatology

INTRODUCTION

In our country and worldwide, the frequency of pollution occurrence in feedstuffs and especially in cereals is quite high, in order to become a priority for animals protection [2, 3]. Fight against mycotoxicosis could be carried on through prophylaxis measures, such as well drying the cereals presenting high humidity values, prior to storage [4, 8]. If they are already contaminated with mycotoxins, they could be annihilated through absorption inhibition or by usage of some bacteria which consume mycotoxins. There have been used several methods to prevent or treat mycotoxicosis in animals, but the usage of certain special feed additives, known as mycotoxins adsorbents of detoxifying agents is the most common one [1, 7]. There have been published other researches on the same topic, like those focused on the detoxifying action of Mycofix Plus product on the feed contaminated with aflatoxin, T-2 and ochratoxine. Close performance values were found between the negative control group and the one contaminated with mycotoxins and protected by adding 1‰ Mycofix Plus in feed. Same situation occurred for the feed conversion ratio. The necropsy exam revealed

maximal extending of the histopatologic lesions in the positive control group, while the usage of the detoxifying agent practically reduced the wounds amplitude by half [5]. Other experiment [6], revealed the productive advantages of using a mycotoxin inhibitor in broilers feeding, when the feedstuffs were contaminated. The detoxifying agent contributed in increasing body immunity and protecting the chickens that received 0.3‰ feed additive. Comparing with the unprotected group, the chickens exteriorised better performances, generating higher revenues (+13.3%), even the cost of feed recipe was more expensive, due to the feed additive inclusion.

The purpose of this paper was to assess the effect of ochratoxinei A (OTA) and deoxynivalenol (DON) on the haematological, immunological an histological response of the chicken broilers, protected or not protected against mycotoxins through feeding or not a detoxifying-mycotoxin inhibitor additive.

MATERIAL AND METHOD

The feedstuffs used in producing mixed feed for chicken broilers in the experiment were contaminated naturally with DON and OTA, as follows:

* corn DON –> 150 – 377 µg/kg;
* wheat DON –> 150 µg/kg;
* soymeal DON –> 369 – 525 µg/kg
* soymeal OTA –> 14 µg/kg

In mixed feed, DON uptake was 550 µg/kg, while OTA uptake reached 35 µg/kg.

Zearalenone (ZEA) content did not exceed 25 µg/kg in corn.

Quality conditions from the mixed feed were in accordance with the nutritional requirements established by broiler producer.

In order to fight the unpleasant effect of feed contamination with DON and OTA, the Mycofix MTV feed additive, produced by Biomin GmbH, Austria, was used in feed.

The Mycofix MTV product is designed to increase mycotoxins inactivation and detoxification of feed contaminated with deoxynivalenol (DON), ochratoxine (OTA) and zearalenone („ZON"). Apart from other products in Mycofix group, the MTV version comprises a yeast (*T. mycotoxinivorans*) which consumes and digests mycotoxins (feed detoxification). The product is a complex of 4 elements: mixture of synergic minerals for selective mycotoxins adsorption; BBSH 797, involved in molecular disassembling of mycotoxins, fitogenics for liver protection; fito-fitic compounds for immune response stimulation. It inactivates mycotoxins through biotransforming and adsorption; it reduces the wounds in the intestinal tract mucosa, caused by trychotecenes; it stimulates the immune system activity, which is commonly inhibited by mycotoxins; does not interact with drugs and other compounds existing in feed.

The biological material was represented by 111678 ROSS-308 chicken broilers, reared industrially, in deep litter system and allocated in 3 experimental groups, as related to Mycofix MTV inclusion rate in feed: Lc – 37226 chickens, feed additive=not present; L1exp. - 37226 chickens, feed additive=1‰; L2exp. - 37226 chickens, feed additive=3 ‰.

The chickens were slaughtered at 40 days old. Prior to slaughtering, blood samples were taken; digesta samples and internal organ tissues were sampled from slaughtered broilers, in order to assess the influence of the used feed additive on the following parameters:

Mycotoxicological traits:

- concentration of DON in digesta from ingluvies, jejunum and caecum (ppm)
- concentration of OTA in gall (ppm)

Haematological traits:

- white blood cells formula (%)

Immunological traits:

- properdine (µg/ml)
- serum lysosime (µg/ml)

Histopatological alterations in ingluvies, ileum, caecum, liver, kidneys and spleen.

Lisosyme was assessed by diffusimetric method, in *Micrococcus lysodeicticus* cultures.

Properdine was dosed via colorimetry.

Mycotoxicologic assessments were run through liquid chromatography, in accordance with the AOAC 26.100-26-125 standard. RP-HPLC analysis of DON was run on a SHIMADZU-20DAD

LiqCromatographer, with two quaternary pumps, autosampler and UV-VIS detector, using a column C18 150 × 4.6 µm. In OTA analysis, we used the Lichrospher 100RP 18 125 × 4.5 µm column.

Histological samples have been processed through paraffin inclusion technique and hematoxilin-eosine staining, then analysed at the photonic microscope. Statistical computation was applied, through ANOVA and Mann-Withney TEST

RESULTS AND DISCUSSIONS

Haematologic and immunologic exams

On the self-natural defence of the chickens, it was found that white blood cells formula was altered in experimental groups: polymorphonuclear neutrophil cells increased by 25.45 % in L1exp. group and by 7.25 % in L2exp. group. Proportion of eosinophils was 20 % higher than the control group, but still within physiologic range of the chicken broilers aged 6 weeks (tab. 1).

Table 1 – White blood cells formula in the chicken broilers intoxicated chronically with DON and OTA and supplementary fed with Mycofix MTV

Notice		Neutrophils	Eosinophils	Basophils	Monocites	Lymphocites
Lc		51.28±7.96	2.00±0.57	0.43±0.57	2.14±0.77	44.15±8.73
L1exp.		64.33±5.66	2.00±0.33	0.50±0.50	2.33±0.66	30.84±6.55
L2exp.		55.00±9.20	2.40±0.48	0.60±0.40	2.4±0.48	39.60±9.92
% from Lc	L1exp.	**125.45**	**100**	**116.28**	**108.88**	**69,85**
	L1exp.	**107.25**	**120**	**139.53**	**112.15**	**89,69**
Reference values		13-49 (30)	2-14 (7)	1-17 (9)	1-4 (2)	5-28 (15)

On the serum lysosime it was found a stimulating effect of Mycofix MTV, at inclusion of 1‰ in feed (167.33 %, compared to control), while for 3‰ Mycofix MTV, lysosime level decreased by 14.21 %. The properdine level was decreased with 3.99% in L1exp. group and with 16.09% in L2exp. group, compared to control (tab. 2).

Table 2-Average values of serum properdine (µg/ml) and lysosime (µg/ml) in chickens intoxicated chronically with DON and OTA and supplementary fed with Mycofix MTV

Notice	Lc	L1exp	L2exp
Properdine (µg/ml)	49.84±3.20	47.85±5.78	41.82±2.93
% of Lc	**100**	**96.01**	**83.91**
Serum lysosime (µg/ml)	21.46±12.43	35.91±23.27	18.41±10.55
% of Lc	**100**	**167.33**	**85.79**

Mycotoxicologic exam

Mycotoxicological investigations on the chickens digesta revealed the inactivating and detoxifying effect of Mycofix MTV product (tab. 3).

Table 3-Concentration of DON (µg/g) in ingesta from ingluvies, jejunum and caecum, in chickens fed with feed contaminated with DON and supplemented with 1‰ and 3‰ "Mycofix MTV"

Notice	Lc	L1exp	L2exp.
Ingluvies	562±34	250	200
Jejunum	205±18	200	140
Caecum	180	160	125

From tab. 3 it results a decrease of DON level in digesta, in experimental groups L1exp. and L2exp., compared to control group Lc, as follows: in ingluvies: 55,52% less in L1exp. and 64.41% less in L2exp.; in jejunum: 2.44% less in L1exp. and 31.71% less in L2exp.; in caecum: 11.12% less in L1exp. and 30.56% less in L2exp., revealing thus the inactivation and detoxification effect of Mycofix MTV product, proportionally with the dietary inclusion rate.

OTA concentration in gall support the same detoxifying effect of Mycofix MTV product, through its decrease in liver secretion (tab. 4).

Table 4 – OTA concentration (µg/g) in gall, at the chicken broilers from experimental groups

Notice	Lc	L1exp.	L2exp.
OTA (µg/ml) in gall	1.13 ±0.009	0.95 ±0.007	0.89 ±0.013
%	100	84.07	78.76

Mycofix MTV reduced OTA excretion in gall, by 15.93%, at 1‰ Mycofix MTV in feed and by 21.24% at 3‰ feed inclusion.

Histopatologic exam

In ingluvies, at Lc group were found: inequal thickening of mucosa epithelium, ortoparakeratosis and balonising dystrophy of keratinocites, as well as hialinisation of lamina propria. Vacuolar degenrerescence of keratinocites was also observed in L1exp., but not in L2exp.

Same lesions were present in ileum, at the nthree groups, expressed by: mucosa fibrosis, ulcers, chronic inflammatory infiltrations, necrosis and flattening of villus imprint (fig. 1-3).

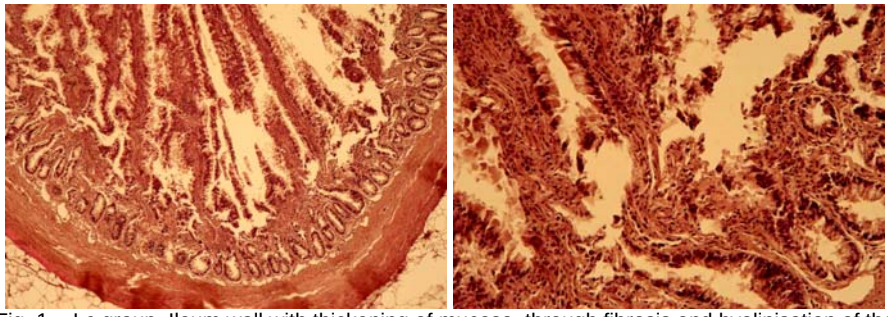

Fig. 1. - Lc group. Ileum wall with thickening of mucosa, through fibrosis and hyalinisation of the villus connective axis and exulcerations of epithelium (coll. HE).

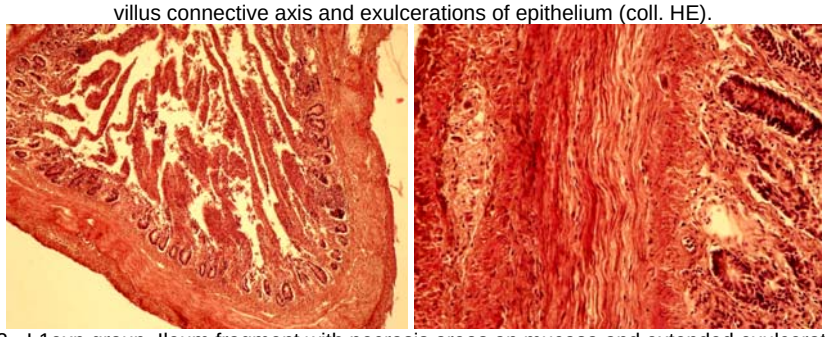

Fig. 2 - L1exp group. Ileum fragment with necrosis areas on mucosa and extended exulcerations; flattened villus, hyalinisation of lamina propria and moderate lympho-plasmocitary infiltrate; stasis in villus axis and periglandular (col. HE)

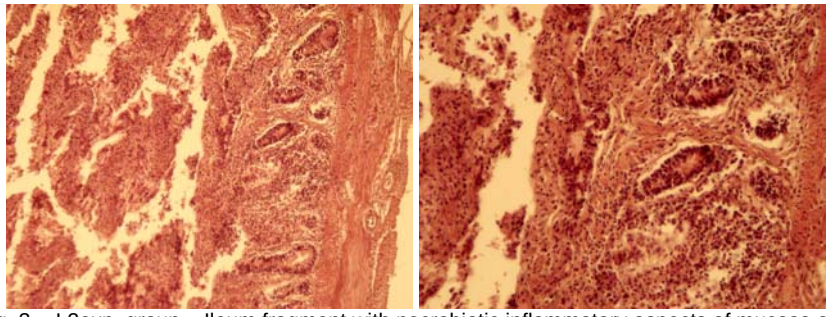

Fig. 3. - L2exp. group – Ileum fragment with necrobiotic inflammatory aspects of mucosa and villus relief lack; villus morphology replaced by fibrous connective tissue, lympho-plasmocitary infiltrate and macrocites/macrophages; on the analysed mucosa there are found glandular fundus with inflammatory exudates, fibrosis of submucosa and musculosa (col. HE).

In caecum, there were observed necroses and mucosa ulcers, lesions similar to those in ileum. Therefore, it is possible that Mycofix MTV had a reduced effect on DON, although that in mycotoxicologic exam, Mycofix MTV detoxification was better than other products, such as Mycofix Plus, previously studied. It is necessary to complete these investigations to better elucidate the phenomenon.

In liver, it was found fibrosis in Lc group and hyalinisation in portal spaces and turbid intumescence of liver cells. In L1exp. and L2exp. chickens, the lesions comprised mostly intumescence, while the portal inflammatory processus was discrete.

In kidneys, the Lc lesions included necrosis and ischemia necrobiosis in the corcticomedula; nephrocites presented, proximally and distally, turbid intumescence. In L1exp. and L2exp. groups, the corticomedular drawing was well presented, existing though slight lesions of glomerular stasis, which led to scattered lacks of glomerular filtration spaces.

In spleen, in Lc group samples, we found the atrophy of certain lymphoid follicles, with destruction of some connective-muscular networks. Conversely, in L1exp. and L2exp. groups, it occurred the hyperplasia of lymphoid periosteolar sheats and od lymphoid follicles.

Overall, there were found more beneficial effects of Mycofix MTV over OTA inactivation, through the decrease of liver and kidney lesions and, possible, though a stimulation of spleen follicles, aspects correlated with the diminution of OTA excretion through gall.

CONCLUSIONS

Mycotoxicological exam revealed rapid inactivation of DON, since the ingluvies, with: 55.52% at 1‰ Mycofix MTV and 64.41% at 3‰ Mycofix MTV. In caecum, compared to control group, DON decreased by: 11.12 at 1‰ Mycofix MTV and 30.56% at 3‰ Mycofix MTV.

Histopatologically, the findings were controversial, except in ingluvies: at 3‰ Mycofix MTV, mucosa lesions were reduced. Despite this, in ileum and caecum, necrosis, ulcers and flattening of villus drawing supported the protective effect of Mycofix MTV against DON, no matter the dietary dosage.

Mycofix MTV diminished the liver excretion of OTA, through gall, with: 15.93% at 1‰ Mycofix MTV and 21.24% at 3‰ Mycofix MTV, as a consequence of OTA inactivation and detoxification in the digestive tract; the same aspect is confirmed by histopatologic exam of liver, which revealed less lesions in the groups supplemented with Mycofix MTV.

In spleen, OTA a produced atrophia of lymphoid follicles in Lc group, while in the chickens supplemented with Mycofix MTV, it was found follicles and lymphoid periarteriolar sheets hyperplasia.

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