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**PhD DISSERTATION**

*“Contributions on the knowledge of the productive effect of  
using certain additives for feed mycotoxins inactivation and  
detoxifying, in chicken broilers”*

**Dissertation for the achievement of PhD degree in Sciences  
Animal Science field**

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## **PhD DISSERTATION ABSTRACT**

Modern poultry raising is an industrial activity, run in large units, specialized, with mechanized and automated technologies. In these units, the birds are fed with compound feed, based on specific recipes.

Of the total world production of compound feed, over 35% is feed for poultry, from which 19 % is for meat production and 16% for eggs.

The exceptional progress made in genetics and in the technology providing best rearing conditions (environment) birds were accompanied by similar progress in the field of nutrition and feeding.

Finding new sources of food, the introduction of feed additives, re-evaluation of inclusion rates of new or conventional feedstuffs, to better fulfill the physiological requirements of the new highly productive hybrids, represent some of the key levers powered by nutritionists in an effort to increase efficiency of livestock production systems for poultry meat and eggs.

For over 60 years, the field of feed additives is explored through many researches, during which they were developed and tested a variety of experimental and commercial products. From simple substances, such as organic acids to systems with more than 10 incorporated active substances, this new class of feed ingredients has become one of the main ways to improve performance and efficiency in livestock.

Development of fungus in cereal crops as well as in storehouses produces nutritional and physical loss and leads to mycotoxins productions, that could become extremely dangerous for animals and mankind. Hence, the huge economic impact on the performance of animals, given by the development of fungus.

Control of moulds development became essential for preserving the nutritional values of cereals and avoiding the mycotoxins contaminations of feedstuffs. Despite all prevention measures, the raw matters used in mixed feed manufacturing could be contaminated with mycotoxins.

Within this context, in order to prevent animals intoxications, a series of feed additives, so called mycotoxins inhibitors, mycotoxins ligands and detoxification agents, could be successfully used

to counterbalance the unpleasant effects generated by the interaction between mycotoxins-immune system-production-food safety.

According to the main experimental design, there were studied two (2) feed additives for inactivation and detoxification of mycotoxins from feed given to broiler chickens, respectively: "**Mycofix Plus**" and "**Mycofix MTV**", produced by the company "BIOMIN" in Austria.

Our researches comprised 5 (five) experimental series, as following:

- **experiment no. 1:** „Contributions on the inactivation and detoxifying of mycotoxins from the mixed feed used in broilers feeding (naturally contaminated with “DON” and “OTA”), using the „Mycofix Plus” product, at inclusion rates of 0.5‰ and 2,0‰”.
- **experiment no. 2:** „Contributions on the inactivation and detoxifying of mycotoxins from the mixed feed used in broilers feeding (artificially contaminated with 1000 ppm “DON” and 500 ppm “OTA”), using the „Mycofix Plus” product, at inclusion rates of 0.5‰ and 2,0‰”.
- **experiment no. 3:** „Contributions on the inactivation and detoxifying of mycotoxins from the mixed feed used in broilers feeding (naturally contaminated with “DON” and “OTA”), using the „Mycofix MTV” product, at inclusion rates of 1‰ and 3‰”.
- **experiment no. 4:** „Contributions on the inactivation and detoxifying of mycotoxins from the mixed feed used in broilers feeding (artificially contaminated with 1000 ppm “DON” and 500 ppm “OTA”), using the „Mycofix Plus” product, at inclusion rates of 2‰ and 3‰”.
- **experiment no. 5:** „Contributions on the inactivation and detoxifying of mycotoxins from the mixed feed used in broilers feeding (naturally contaminated with 5.63 ppm „OTA” and 574 ppm „DON” and also artificially, with 20,35 ppm “OTA”), using the „Mycofix MTV” product, at inclusion rates of 2‰ and 3‰”.

The 5 (five) experiments differed among them through the investigated experimental factors:

- absence or occurrence of mycotoxins in used feed (natural contamination, within/under detection limits);
- artificial contamination and mixed contamination (natural and artificial);
- usage of certain additives for inactivation and detoxification of mycotoxins in the feed of chickens („**Mycofix Plus**” and „**Mycofix MTV**”), at different inclusion rates („**Mycofix Plus**” – 0.5‰; 2‰ and 3‰; „**Mycofix MTV**” – 1‰; 2‰ and 3‰).

Main achieved results presented below:

- **for experiment no. 1:**

- the protective effect of „**Mycofix Plus**” product, against the mycotoxic action of deoxynivalenol („DON”) and ochratoxine („OTA”) was proportional with the feed inclusion rate, leading to body mass increase from 3.5 % at 0.5 ‰ „**Mycofix Plus**” to 8.15 % at 2 ‰ „**Mycofix Plus**”. Maximal effect was obtained during the first week of chickens life, especially at the rate of 2 ‰ „**Mycofix Plus**”; at that age, broilers were more sensitive to „DON” and „OTA”;

- feed conversion ratio(kg feed/kg gain) reached 1.95 in Lc group, compared to 1.92 in L1exp. (-1.54%) or to 1.83 in L2exp. (-6.16%) groups;

- throughout the entire experimental period, mortality in experimental groups decreased by 70.28 %, compared with control-Lc, at rate of 0.5 ‰ „**Mycofix Plus**” and by 64.68 %, at an inclusion of 2 ‰ „**Mycofix Plus**”;

- „**Mycofix Plus**” product decreased uricemia with 12.88 %, at inclusion rate of 0.5‰ and with 18.00 %, at rate of 2 ‰;

- the studied product („**Mycofix Plus**”) had an immunomodulative effect through the stimulation of serum lysosime production, meaning 2.48 times compared to control-Lc, at the rate of 0.5 ‰, respectively 3.64 times, at a rate of 2‰;

- concentration of serum properdine increased by 38.88 % at 2 ‰ „**Mycofix Plus**”, the value of this umoral immunity factor being relevant for immunological chickens reactivity;

- the histopathological exam of the internal organs of chickens revealed discrete lesions in experimental groups (L1exp. and L2exp.) and intense lesions in control group – Lc, especially into the ingluvies.

- **for experiment no. 2:**

- dietary inclusion of „**Mycofix Plus**” in proportion of 2‰, in the contaminated feeds with „DON” (500 ppm) and „OTA” (1000 ppm), led to a significant increase ( $P < 0,05$ ) of the average daily gain in L4exp broilers;

- mycotoxins „OTA” and „DON” generated the decrease of the average daily gain and the increase of feed conversion rate (kg feed/kg gain); these effects were significantly improved by the feed inclusion of „**Mycofix Plus**”, especially at a rate of 2 ‰;

- increase of „**Mycofix Plus**” proportion, from 0.5‰ to 2‰ in the feed contaminated with 1000 ppm OTA and 500 ppm DON led to a significantly decrease of feed conversion rate (kg feed/kg gain) in chicken broiler, during their first week of life;

- „OTA” and „DON” significantly reduced the cellular body defence ( $P < 0,05$ ), while the addition of 2‰ „**Mycofix Plus**” in feed surpassed any negative effect on the fagocytosis cell function;

- chronic intoxication of broilers with 500 ppm „DON” and 1000 ppm „OTA” seriously affected the natural umoral immunity of the organism. Lysosime production was more influenced than the properdine secretion. Inclusion of „**Mycofix Plus**”, at rate of 2‰ in feedstuffs contaminated with „DON” and „OTA”, induced normal values of properdine and significantly affected the mycotoxins effect ( $p < 0,05$ ) on the serum lysosime;

- natural cellular defence of the organism was significantly depressed by „OTA” and „DON” in feed; dietary „**Mycofix Plus**” 2‰ allowed to avoid the negative effects on the fagocitar functions.

- **for experiment no. 3:**

- body weight was significantly different among groups; thus, at slaughter age (37 days), in control group (Lc), average body weight was 2000.00 g, less than that found in experimental groups (2089 g in L1exp., with 1‰ „**Mycofix MTV**” and 2188 g in L2exp., with 3‰ „**Mycofix MTV**”). Correlated with the body weight dynamics was found the feed conversion ratio– FCR (kg feed/kg gain) (FCR=1.973 in Lc; FCR=1.924 in L1exp.; FCR=1.810 in L2exp.);

- mycotoxicologic exam revealed the quick reduction, through inactivation and detoxification, since the ingluvies, of „DON”, with: 55.52% at 1‰ „**Mycofix MTV**” and 64.41% at 3‰ „**Mycofix MTV**”. In caecum, compared to control group – Lc, „DON” was decreased with 11.12 at 1‰ „**Mycofix MTV**” and 30.56% at 3‰ „**Mycofix MTV**”;

- the histopatologic aspects were somehow controversial, except for the ingluvies, at the group fed with 3‰ „**Mycofix MTV**”, whose lesions were reduced. However, into the ileon and caecums, the necroses, ulcers and the loose of villus drawing did not prove the protective effect against „DON” of „**Mycofix MTV**”, no matter the dietary inclusion rate;

- „**Mycofix MTV**” decreased the hepatic excretion of „OTA” through gall, with: 15.93% for 1‰ „**Mycofix MTV**” and 21,24% for 3‰ „**Mycofix MTV**”, as consequence of inactivation and detoxification of „OTA” in the digestive tract; this fact is supported also by the histopatologic liver exam, which revealed discrete lesions in the groups supplemented with „**Mycofix MTV**”;

- in kidneys, histopathological exam proved the „**Mycofix MTV**” protective effect on the renal architecture, through the decrease of „OTA” absorbed in the gut;

- in spleen, „OTA” induced atrophy of lymphoid follicles in Lc group, while in groups supplemented with „**Mycofix MTV**” (L1exp. and L2exp.) was found the hyperplasia of lymphoid follicles and of periarteriolar lymphoid sheaths.

- **for experiment no. 4:**

- the „**Mycofix Plus**” product determined in chickens from experimental groups (L1exp.; L2exp. and L3exp.) the achievement at 42 days old, of different body weight values, compared with those from control group (Lc), which did not receive the additive; thus, body weight was 8.25% less in L1exp. and 7.97% less in L2exp. group; conversely, in L3exp. group, the average body weight at slaughter (42 days) was 4.20% higher than in reference group (Lc);

- greater body mass differences, as reported to control group (Lc) existed in the groups artificially contaminated with „DON” and „OTA” (L1exp. and L2exp.), during the first weeks of chickens life, when they were very sensitive to the mycotoxins aggression;

- feed conversion ratio (kg feed/kg gain) was lesser in L3exp. (uncontaminated artificially with „DON” and „OTA”, inclusion of 3‰ „**Mycofix Plus**”), and higher, increasingly, in the groups Lc, L1exp. and L2exp. Compared to group Lc, feed conversion ratio was 7.27% less in L3exp. and 2.20-2.79% higher in groups L1exp. and L2exp;

- mycotoxins quantity („DON” and „OTA”) from the dry matter of digesta decreased across the digestive tract, from ingluvies to caecums, revealing thus the inactivating and detoxifying effect in digesta, proportionally with the „**Mycofix Plus**” feed inclusion rate;

- „**Mycofix Plus**”, used in 3‰ rate, led to an increasing with statistical signification ( $p < 0.05$ ) of the serum properdine and lysosime, revealing thus a stimulating effect for the umoral non-specific defence of the broilers from groups L2exp. and L3exp;

- in the dry matter of digesta in jejunum, „**Mycofix Plus**” 3‰ reduced „DON” concentration at 63,81%, while in caecums, at 9.76% from the ingested quantities;

- „OTA” concentration in the dry matter of caecums was 46.38% reduced, compared to the initial one, through the increase of „**Mycofix Plus**” feed inclusion, from 2‰ to 3‰ inc chicken broilers chronically intoxicated with 500 ppm „OTA”;

- histopathological, the lesions of the digestive tract mucosa occurred in all experimental groups, with slight differences in groups L1exp. and L2exp., in which the apical necrosis and the

epithelia ulcerations in villuses were more obvious; lesions importance decreased from duodenum toward caecums in all groups, the effect of „DON” being considered as minor and associated to other toxins in the digesta.

- **for experiment no. 5:**

- occurrence of mycotoxins „OTA and „DON” in mixed feed used in chickens broilers feeding decreased their rearing performances. Supplementing „**Mycofix MTV**”, in rates of 2‰ and 3‰ in feed significantly decreased the effect of „OTA”, directly proportional with the inclusion rate, while the „DON” effects were still present, but at lower intensity;

- usage in L3exp. of „**Mycofix MTV**” product, at increased rate of 3 ‰ led to the achievement of an average body weight at slaughter age (42 days), with 31.46 % higher than in control group (Lc2), at the same age;

- best feed conversion ratio (FCR = kg feed/kg gain) was achieved by the chickens in the L3exp. group, which received 3 ‰ „**Mycofix MTV**” (FCR = 1.85);

- it was found that approximately 80 % of ingested mycotoxin „DON” was kept into the proximal digestive tract, revealing therefore its massive absorption into ingluvies and gizzard, while almost 19.5 % was absorbed in the distal digestive tract (gut and caecums). The „**Mycofix MTV**” product did not totally protect the studied chicken broilers against the damage of „DON” mycotoxin;

- non-specific defence, expressed through properdine value, did not reveal significant differences between groups ( $p > 0,05$ ); however, when the groups with low level of „OTA” (Lc1; Lc2 and L3exp.) were compared, there were found certain differences for properdine level; thus, in L3exp. group, assessed properdine was 22,14 % more than in group, as a consequence of feed supplementation with 3‰ „**Mycofix MTV**”. In groups L1exp. and L2exp., which had greater „OTA” contamination (natural – 5.63 ppm and artificial – 20.35 ppm), increase of inclusion rate of „**Mycofix MTV**” from 2‰ (L1exp.) to 3‰ (L2exp.) led to an increase of properdine value by 6,93%; thus, it was observed the beneficial effect of „**Mycofix MTV**” product, within the nonspecific protection of the studied chickens bodies;

- comparative histopatological exam of mucosa in the digestive tract as well as in liver and kidneys of the chickens from experimented groups (Lc1; Lc2; L1exp.; L2exp. and L3exp.), contaminates, more or less, through the consumed feed with „DON” and „OTA”, revealed that the product „**Mycofix MTV**” did not provide enough protection against „DON”; however, the protection was quite good against „OTA”.

The experiments we run scientifically proved that the products „**Mycofix Plus**” and „**Mycofix MTV**”, used in chicken broilers feeding exert their inactivating and detoxifying role against some mycotoxins („DON” – deoxynivalenol and „OTA” – ochratoxin), leading to improvements in average daily gain and feed conversion ratio, therefore better meat yields. These feed additives significantly reduced the negative effects of „DON” and „OTA” and especially of „OTA”. Therefore, we recommend the usage of these additives in nutrition and feeding practice for chicken broilers, as follows:

- „**Mycofix Plus**”, at feed inclusion rates of 2‰ și 3‰, as related to feed contamination degree with „OTA” and DON”;
- „**Mycofix MTV**”, at dietary rates of 2‰ și 3‰, as related to feed contamination degree with „OTA” and DON”.