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SHORT COMMUNICATION

Mitigating T-2 Toxin-Induced Immunosuppression In Ducks: Effects Of Mycofix® Plus 5.0 And Silymarin On Innate Immunity

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A B S T R A C T

This experiment investigates the effects of T-2 toxin on innate immune function of ducks and explores the protective capacity of Mycofix® Plus 5.0 and silymarin. Six groups of ducks (10 birds per group) received a diet with different combinations of the three substances for 6 weeks, where the levels of the alternative pathway of complement activation (APCA), lysozyme, and beta-lysine activity were checked on 21 and 42 days of challenge. Exposure to T-2 toxin (1mg/kg feed) initiated early immune activation, followed by dramatic decrease in all parameters by day 42. The applied mycotoxin detoxifier (15g/kg feed) demonstrated great potential to moderate the impact of the T-2 toxin on APCA and lysozyme levels but lacked a dedicated immune stimulation effect. The application of silymarin (20g/kg feed) displayed limited protection against T-2 toxin but exhibited great potential to boost innate immunity when applied alone. The experiment confirmed the immunosuppressive effect of T-2 toxin and highlighted the need for integration of mycotoxin detoxifiers in poultry diets.

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INTRODUCTION

The T-2 toxin belongs to a group of mycotoxins, known as a type A trichothecenes. The latter are produced by different *Fusarium* species and affect almost all cereal crops used for animal feed (Wu *et al.,* 2013). Although the biggest concerns are usually focused on the productivity reduction, the toxin also poses immunosuppression capacity (Chen *et al.,* 2019), where the influence on the innate immune parameters such as the alternative pathway of complement activation (APCA), lysozyme, and beta-lysine could be critical for animals' survival (Li *et al.,* 2022).

Due to its antibody-independent mechanisms, the APCA is a key component of the early innate immune response against various pathogens (Pestka *et al.,* 2004). The presence of T-2 toxin is usually accompanied by reduction of protein synthesis, which impairs the production of valuable components of pathway's cascade (Li *et al.,* 2022; Girish and Devegowda, 2006). An additional layer of protection is provided by the serum lysozyme and beta-lysine which facilitate cell lysis of Gram-positive bacteria and stimulate phagocytosis (Karakolev *et al.,* 2024).

A modern-day approach to reduce mycotoxin negative impact utilizes the use of different adsorbents and biologically active substances. Mycofix® Plus 5.0 is promoted as an all-in-one feed additive and is based on a combination of FUMzyme®, a unique enzyme allowing the degradation of fumonisins, and Biomin® BBSH® 797, a microorganism able to transform trichothecenes into harmless metabolites. It is known for its hepatoprotective and immunomodulatory effects (Li *et al.,* 2022; Shareef and Sito, 2019). On the other hand, silymarin is a commercial product composed of flavonolignans like silibinin, silidianin, silicristin and isosilibinin, extracted from milk thistle (*Silybum marianum*) seeds. Its hepatoprotective and antioxidant properties are well documented in various species (Vargas-Mendoza *et al.,* 2021; Zhao *et al.,* 2021; Celep and Gedikli, 2023).

To date, there is no data demonstrating the impact of T-2 toxin exposure on the innate immune markers like APCA, lysozyme, and beta-lysine in aquatic birds. Although both Mycofix® Plus 5.0 and silymarin have been widely explored for their respective detoxifying and hepatoprotective properties, data on their specific immunomodulatory effects is also limited. The present study aims to evaluate the immunosuppressive effects of T-2 toxin in ducks and investigates the protective roles of Mycofix® Plus 5.0 and silymarin on APCA, lysozyme, and beta-lysine activity.

MATERIALS AND METHODS

A total of 60, day-old ducks were randomly split into six groups of 10 birds. All groups were reared under the same environmental conditions for a period of 6 weeks but received different diets. The control (group 1) received standard commercially available duck feed without any additives. The basal diet contained approximately 180g/kg crude protein, 2764.39kcal/kg metabolizable energy, 59.16g/kg crude fiber, and 30.88g/kg crude fat. Group 2 received feed supplemented with 1mg/kg T-2 toxin (*Fusarium sp.,* Sigma-Aldrich, USA; Product number: T4887-25MG), allowing evaluation of T-2 toxin's immunosuppressive effect. Group 3 was fed with feed supplemented with 1mg/kg T-2 toxin and 2% silymarin (Phytoviv Ltd, Bulgaria), while group 4 received feed supplemented with 1mg/kg T-2 toxin and 1.5% Mycofix® Plus 5.0 (DSM-Firmenich, Netherlands). These two groups allowed assessment of the protective properties of both additives. The immunomodulation effect of the additives used was explored in groups 5 and 6, where the feed of group 5 was supplemented with 2% Silymarin, whilst the feed of group 6 was supplemented with 1.5% Mycofix® Plus 5.0.

T-2 toxin and the feed additives used were homogenized with the feed using a standard animal feed mixer. The feeds for groups 5 and 6 were produced by mixing the appropriate quantity of supplement with the total amount of feed required for the entire trial (approximately 50kg per group). The extremely small dose of the T-2 toxin (1mg/kg feed) required a two-step process. Firstly, 50mg of the toxin were manually premixed with 5kg of feed to ensure initial uniformity. The premix was then combined with the remaining 45kg of feed in the mixer to achieve homogeneous distribution. This approach was used for all groups receiving T-2 toxin (groups 2, 3, and 4), with silymarin or Mycofix® Plus 5.0 added simultaneously where applicable.

Blood samples for analyses were collected in the middle and at the end of the project (days 21 and 42, respectively), via venipuncture of the medial metatarsal vein, using plain vacutainers and blood collection needles size 22G. Once collected, samples were transferred to the lab in cool bags, where sera were extracted by centrifugation at 3,000rpm and stored at -20°C until used. The experiment evaluated the effect of each treatment by the variations in the alternative pathway of complement activation (APCA), serum lysozyme concentration, and Beta-lysine activity. The APCA activity was assessed by the method of Sotirov *et al.* (2021), the lysozyme levels were analyzed following the procedure explained by Sotirov *et al.* (2011), and the Beta-lysine activity was measured by the process described by Karakolev and Nikolov (2015).

Statistical analysis: Acquired data was subjected to statistical analyses using one-way analysis of variance (ANOVA) in IBM SPSS v29, with a significance level set at $P<0.05$.

Ethical approval and animal welfare compliance: The experiment followed the regulations of the Bulgarian animal welfare law and was approved by the Bulgarian Food Safety Agency by Order No. 406/04.10.2024.

RESULTS AND DISCUSSION

Alternative pathway of complement activation (APCA): Results regarding the influence of T-2 toxin on APCA activity are presented in Table 1. Groups 1 and 6 exhibited similar APCA activity throughout the experiment, confirming the absence of toxin-induced suppression. In groups 2 and 3, T-2 toxin exposure triggered an initial boost in APCA activity, followed by a sharp decline by day 42 ($P<0.001$). These findings indicate a quick exhaustion of the complement system due to impaired production of proteins, essential for complement activation (Pestka *et al.,* 2004). Regardless of being exposed to the same dose of T-2 toxin, group 4 maintained APCA levels comparable to the controls for both test periods. The positive result could be attributed to the supplementation of animals' diet with Mycofix® Plus 5.0, which contained several de-epoxidase, esterase enzymes and Biomin® BBSH® 797, a bacteria capable of converting the T-2 toxin into non-toxic metabolites (Omar, 2010; Li *et al.,* 2022). The observed effect could also be attributed to the gastrointestinal adsorption capacity of Mycofix® Plus 5.0. The mineral adsorbents in its composition (e.g., EU-authorized bentonite) allow binding of mycotoxins, thereby reducing toxin absorption and systemic circulation (Shareef and Sito, 2019). The combination of its biotransformation and selective adsorption capabilities likely play a synergistic role in mitigating the immunosuppressive effects of T-2 toxin. Although known for its hepatoprotective properties, silymarin (group 3) failed to provide a dedicated detoxifying effect. This observation coincides with the findings of Zhao *et al.,* (2021) and Vargas-Mendoza *et al.,* (2021) and indicates the limited capacity of this additive to provide protection against severe toxin exposure. When applied alone (group 5) silymarin demonstrated slightly elevated levels of APCA activity compared to controls, which could be a result of its antioxidant function.

Blood serum lysozyme: Results for this parameter exhibited a marked decrease between the two test periods across all groups (Table 2). The most significant declines were spotted among groups 2 and 3, where lysozyme concentrations dropped by more than 180% ($P<0.001$). Such a drastic decrease implies a T-2 toxin associated impairment of lysozyme synthesis, likely due to its ribosomal inhibition abilities (Pestka *et al.,* 2004). Although a lot more moderate, reduction in lysozyme levels between days 21 and 42 was also observed among the control ducks, suggesting an age-related variation of this parameter (Chen *et al.,* 2019). Mycofix® Plus 5.0 demonstrated great potential to reduce the negative influence of the T-2 toxin to lysozyme levels. The positive effect of the commercial detoxifier is evident when comparing the results of groups 4 and 2 on the 42 days of challenge. Although challenged with the same dose of toxin, ducks receiving Mycofix® Plus 5.0 (group 4) exhibited significantly higher ($P<0.01$) lysozyme levels compared with the group challenged with the toxin alone (group 2). The positive effect of the additive is probably due to its ability to reduce mycotoxins' viability and reduce their toxicity (Shareef and Sito, 2019). Silymarin's effects

Table 1: APCA activity (CH50) in ducks exposed to T2 toxin, detoxifying and hepatoprotective agents over a 42-day period.

Group	Treatment	Challenged for 21 days	Challenged for 42 days	Variation between 21st and 42nd day of challenge in %	P value between 21st and 42nd day of challenge
		Mean ± SE	Mean ± SE		
Group 1	Control feed	645,87±4,90^{cghi}	631,12±4,95^{eqrs}	-2,34%	0,048
Group 2	T2 toxin	841,41±6,09^{gjkl}	510,34±4,19^{atwx}	-64,87%	0,001
Group 3	T2 toxin + Silymarin	835,43±7,14^{hmno}	526,04±3,98^{aruyz}	-58,81%	0,001
Group 4	T2 toxin + Mycofix® Plus 5.0	682,60±4,79^{ijmp}	660,19±7,34^{befwy}	-3,39%	0,020
Group 5	Control feed + Silymarin	671,05±5,73^{cdkn}	678,81±4,77^{bstuv}	1,14%	0,312
Group 6	Control feed + Mycofix® Plus 5.0	643,11±7,02^{dlop}	629,80±7,24^{fvxz}	-2,11%	0,203

$^{a-b}$ – P<0.05, $^{c-f}$ – P<0.01, $^{g-z}$ – P<0.001

Table 2: Serum lysozyme concentration (mg/L) in ducks exposed to T2 toxin, detoxifying and hepatoprotective agents over a 42-day period.

Group	Treatment	Challenged for 21 days	Challenged for 42 days	Variation between 21st and 42nd day of challenge in %	P value between 21st and 42nd day of challenge
		Mean ± SE	Mean ± SE		
Group 1	Control feed	3,72±0,19^{ef}	2,17±0,22^{d}	-71,28%	0,000047
Group 2	T2 toxin	4,94±0,18^{eghi}	1,75±0,04^{kl}	-182,26%	0,000001
Group 3	T2 toxin + Silymarin	4,87±0,19^{bcfj}	1,70±0,09^{amn}	-187,03%	0,000001
Group 4	T2 toxin + Mycofix® Plus 5.0	3,93±0,17^{bg}	2,28±0,11^{kmo}	-72,90%	0,000001
Group 5	Control feed + Silymarin	3,92±0,14c^{h}	2,96±0,05^{dlnop}	-32,34%	0,000139
Group 6	Control feed + Mycofix® Plus 5.0	3,70±0,16^{ij}	2,12±0,18^{ap}	-74,49%	0,000001

^{a} – P<0.05, $^{b-d}$ – P<0.01, $^{e-p}$ – P<0.001

Table 3: Beta-Lysine activity (%) in ducks exposed to T2 toxin, detoxifying and hepatoprotective agents over a 42-day period.

Group	Treatment	Challenged for 21 days	Challenged for 42 days	Variation between 21 and 42 days of challenge in %	P value between 21 and 42 days of challenge
		Mean ± SE	Mean ± SE		
Group 1	Control feed	38,63±0,40^{bcde}	42,29±0,86^{aopq}	8,66%	0,001149
Group 2	T2 toxin	21,18±0,94^{cfgh}	16,06±0,47^{orstu}	-31,83%	0,000125
Group 3	T2 toxin + Silymarin	23,07±0,81^{dijk}	19,71±0,77^{prvwx}	-17,09%	0,007593
Group 4	T2 toxin + Mycofix® Plus 5.0	31,60±0,66^{efilm}	37,60±0,57^{qsvy}	15,94%	0,000001
Group 5	Control feed + Silymarin	41,07±0,76^{bgjln}	44,24±0,81^{twyz}	7,15%	0,010469
Group 6	Control feed + Mycofix® Plus 5.0	37,75±0,35^{hkmn}	39,55±0,82^{auxz}	4,54%	0,055473

^{a} – P<0.05, ^{b} – P<0.01, $^{c-z}$ – P<0.001. Results followed by the same letter indicate significance level between respected groups within the same period of challenge (21st or 42nd day).

appeared to be context dependent. In toxin-free birds (group 5), the supplement preserved the serum lysozyme levels with a minor reduction between the two test periods. These results support the hypothesis for positive influence of the flavonoid's antioxidant properties to lysozyme production (Vargas-Mendoza *et al.,* 2021). When co-administered with T-2 toxin (group 3), the supplement failed to preserve the lysozyme levels which demonstrates its limited capacity to mitigate a toxin-induced immunosuppression (Zhao *et al.,* 2021).

Beta-lysine: Apart from groups 2 and 3, all other groups exhibited an increasing tendency of beta-lysine activity towards the end of the experiment (Table 3). The highest rise in beta-lysine levels between the two sampling periods was observed in group 4, implying that the detoxification of animal feed by Mycofix® Plus 5.0 promotes increased synthesis of beta-lysine. Moderate elevations were also observed for the controls and silymarin-only groups (P<0.01), indicating an age-linked activation of this immune parameter (Karakolev *et al.,* 2024). T-2 toxin's harmful impact was most evident among groups 2 and 3 where beta-lysine activity for both time periods was significantly lower than the results of controls (P<0.001). Ducks challenged with T-2 toxin (group 2) showed the lowest beta-lysine activity, pointing out toxin's devastating effect on this parameter. Ducks treated with silymarin (group 5) showed the highest beta-lysine levels in both sampling periods. Such results indicate the flavonoid's supportive role in beta-lysine synthesis and is probably linked to silymarin's ability to modulate inflammatory signaling and oxidative stress responses (Celep and Gedikli, 2023). The observed beta-lysine levels among group 6 were comparable to the controls (P>0.05), indicating that Mycofix® Plus 5.0's primary function is delegated to detoxification rather than direct immune stimulation.

Conclusions: T-2 toxin exposure has a dramatic effect on the analyzed innate immune parameters in ducks which indicates the need for introduction of proper detoxifying agents to poultry diets to mitigate the risk of immunosuppression. The Mycofix® Plus 5.0 demonstrated a strong detoxifying potential but lacked dedicated immunomodulation properties. Conversely, the silymarin supplementation displayed a great influence on innate immune factors but offered limited toxin protection. The data obtained suggests that the introduction of targeted detoxifiers and immunostimulant agents in poultry diets would facilitate better immune resilience under mycotoxin exposure.

Authors contribution: TK conducted the immune assays, analyzed data, and co-wrote the manuscript with IV. IV and DN designed the study. TH, NN, and NK handled animal treatment and sampling. RB reviewed the manuscript. All authors revised and approved the final version.

REFERENCES

Celep NA and Gedikli S, 2023. Protective effect of silymarin on liver in experimental sepsis model of rats. Acta Histochem Cytochem 56:9-19.

Chen Y, Han S, Wang Y, *et al.,* 2019. Oxidative stress and apoptotic changes in broiler chicken splenocytes exposed to T-2 toxin. Biomed Res Int 2019:5493870.

Girish CK and Devegowda G, 2006. Efficacy of glucomannan-containing yeast product (Mycosorb®) and hydrated sodium calcium aluminosilicate in preventing the individual and combined toxicity of aflatoxin and T-2 toxin in commercial broilers. Asian-Australas J Anim Sci 19:877-83.

Karakolev R, Koynarski T, Sotirov L, *et al.,* 2024. Opportunities to improve the immune status of broiler chickens. J Hellenic Vet Med Soc 75:6771-8.

Karakolev R and Nikolov D, 2015. Fluctuations in serum beta-lysine activity in laying hens depending on age, hybrid affiliation, and rearing. Poult Farming 3:18-9.

Li S, Zhang G, Xue B, *et al.,* 2022. Toxicity and detoxification of T-2 toxin in poultry. Food Chem Toxicol 169:113-22.

Omar EKS, 2010. Effect of Mycofix and Synertox adsorbents on the health and performance of broiler chicks fed aflatoxin and T-2 toxin. MSc Thesis, Mosul Univ, Iraq, pp:17-29.

Pestka JJ, Zhou HR, Moon Y, *et al.,* 2004. Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: Unraveling a paradox. Toxicol Lett 153:61-73.

Shareef AM and Sito EO, 2019. Effect of Mycofix® Plus 5.0 and aflatoxin on health and performance of broiler chickens. Basrah J Vet Res 18:283-7.

Sotirov L, Denev S, Chobanova S, *et al.,* 2021. Effects of dietary marine microalgae *Schizochytrium limacinum* on natural humoral immunity of broiler chickens. Bulg J Agric Sci 27:194-9.

Sotirov L, Koynarski T, Semerdjiev V, *et al.,* 2011. Effect of breed upon blood lysozyme and complement activity in different sheep breeds. Agric Sci Technol 3:302-5.

Vargas-Mendoza N, Angeles-Valencia M, Morales-González Á, *et al.,* 2021. Effect of silymarin supplementation in lung and liver histological modifications during exercise training in a rodent model. J Funct Morphol Kinesiol 6:72-80.

Wu Q, Dohnal V, Kuca K, *et al.,* 2013. Trichothecenes: Structure-toxic activity relationships. Curr Drug Metab 14:641-60.

Zhao X, Wang H, Yang Y, *et al.,* 2021. Protective effects of silymarin against d-gal/lps-induced organ damage and inflammation in mice. Drug Des Devel Ther 15:1903-14.