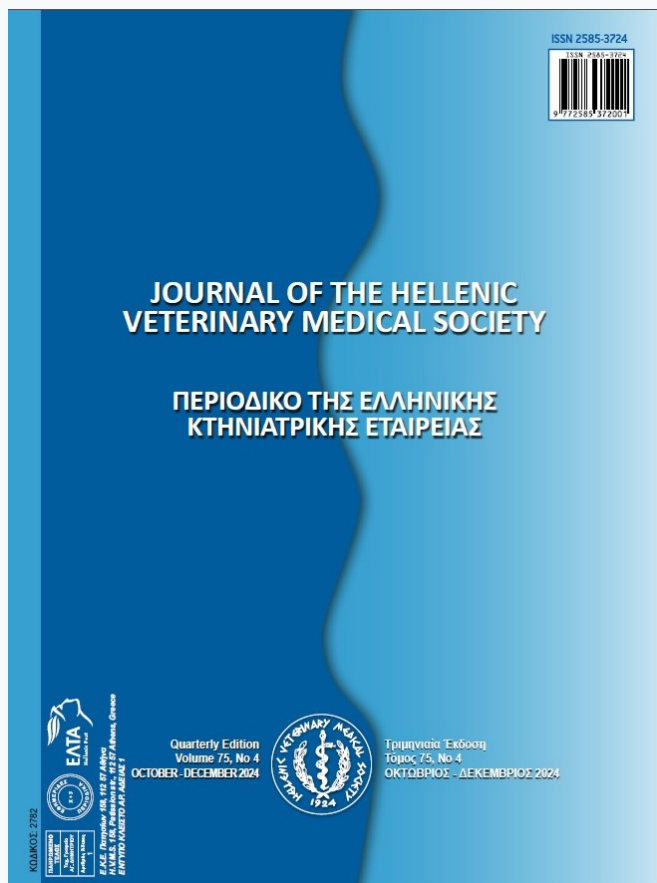


Journal of the Hellenic Veterinary Medical Society

Vol 75, No 4 (2024)



Influence of Mycostop Mycotoxin Adsorbents on Production, Oxidative Stress, and Economic Cost of Laying Hens Intoxicated with T-2 Toxin

N Puvača, T Shtylla Kika, I Brkić, D Ljubojević Pelić, V Tufarelli, N Nikolova, B Kostić, I Radojičić, S Brkanlić, V Bursić

doi: [10.12681/jhvms.37778](https://doi.org/10.12681/jhvms.37778)

Copyright © 2025, N Puvača, T Shtylla Kika, I Brkić, D Ljubojević Pelić, V Tufarelli, N Nikolova, B Kostić, I Radojičić, S Brkanlić, V Bursić



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

To cite this article:

Puvača, N., Shtylla Kika, T., Brkić, I., Ljubojević Pelić, D., Tufarelli, V., Nikolova, N., Kostić, B., Radojičić, I., Brkanlić, S., & Bursić, V. (2025). Influence of Mycostop Mycotoxin Adsorbents on Production, Oxidative Stress, and Economic Cost of Laying Hens Intoxicated with T-2 Toxin. *Journal of the Hellenic Veterinary Medical Society*, 75(4), 8445–8456. <https://doi.org/10.12681/jhvms.37778>

Influence of Mycostop Mycotoxin Adsorbents on Production, Oxidative Stress, and Economic Cost of Laying Hens Intoxicated with T-2 Toxin

N. Puvača^{1*}, T. Shtylla Kika², I. Brkić¹, D. Ljubojević Pelić³, V. Tufarelli⁴,
N. Nikolova⁵, B. Kostić^{1,6}, I. Radojičić⁷, S. Brkanlić¹, V. Bursić⁸

¹Faculty of Economics and Engineering Management in Novi Sad, University Business Academy in Novi Sad, Cvečarska 2, 21000 Novi Sad, Serbia

²Faculty of Veterinary Medicine, Agricultural University of Tirana, Koder Kamez, 1029 Tirana, Albania

³Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia

⁴Department of Precision and Regenerative Medicine and Jonian Area (DiMePRE-J), Section of Veterinary Science and Animal Production, University of Bari Aldo Moro, 70010 Valenzano, Italy

⁵Institute of Animal Science, Skopje, University "Ss. Cyril and Methodius", North Macedonia

⁶Ministry of Agriculture, Forestry and Water Management, Belgrade, Omladinskih brigada 1, 11070 Serbia

⁷INBERG d.o.o., Braće Iker 74, 11275 Boljevci, Serbia

⁸Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

ABSTRACT: Mycotoxin contamination in poultry feed poses a significant threat to both production performance and animal health. Among various mycotoxins, the T-2 toxin stands out as particularly detrimental to laying hens, leading to decreased productivity and increased oxidative stress. This study aimed to evaluate the efficacy of two mycotoxin adsorbents, Mycostop Premium 2.0 and Mycostop Supreme, at a concentration of 2 kg/t of feed, in mitigating the adverse effects of T-2 toxin on laying hens' production performance, nutritive and physical egg quality, oxidative stress levels, the occurrence of oral and tongue lesions, and economic cost of production. A total of 200 laying hens were randomly allocated to four dietary treatments: control T1 (basal diet without T-2 toxin or mycotoxin adsorbents), T2 (basal diet contaminated with 8 mg/kg of T-2 toxin), T3 (basal diet contaminated with 8 mg/kg of T-2 toxin and 2kg/t of feed Mycostop Premium 2.0), and T4 (basal diet contaminated with 8 mg/kg of T-2 toxin and 2 kg/t of feed Mycostop Supreme). The trial lasted for 56 days, during which production performance parameters, including feed intake, egg production, egg weight, and feed conversion ratio, were monitored. Additionally, nutritive and physical egg quality traits, such as shell strength, yolk color, albumen height, and Haugh unit, were assessed. Oxidative stress biomarkers, including malondialdehyde (MDA) levels were measured to evaluate the impact of mycotoxin adsorbents on oxidative stress levels in laying hens. Furthermore, the occurrence and severity of oral and tongue lesions were recorded to assess the potential protective effects of Mycostop Premium 2.0 and Mycostop Supreme against mycotoxin-induced oral health complications. In conclusion, findings of this research highlight the potential of mycotoxin adsorbents as valuable tools for safeguarding poultry health and optimizing productivity in mycotoxin-challenged environments.

Keywords: hens; table eggs; mycotoxins; T-2; stress; adsorbent; nutrition; poultry

Corresponding Author:

Nikola Puvača, Department of Engineering Management in Biotechnology, Faculty of Economics and Engineering Management Novi Sad, University Business Academy, 8 Cvečarska 2, 21000, Novi Sad, Serbia.
E-mail address: nikola.puvaca@fimek.edu.rs

Date of initial submission: 16-4-2024
Date of acceptance: 24-5-2024

INTRODUCTION

Mycotoxins, toxic metabolites produced by fungi, pose a significant threat to animal health and productivity, particularly in the poultry industry (Haque et al., 2020). Among various mycotoxins, the T-2 toxin stands out as one of the most detrimental to poultry production due to its potent cytotoxicity and immunosuppressive effects. Laying hens, being highly susceptible to mycotoxin exposure, experience compromised performance and health challenges when exposed to T-2 toxin-contaminated feed. In response to this threat, the utilization of mycotoxin adsorbents has gained attention as a potential strategy to mitigate the adverse effects of mycotoxins on poultry health and productivity (Kępińska-Pacelik and Biel, 2021).

Production performance metrics, including feed intake, egg production, egg weight, and feed conversion ratio, serve as key indicators of laying hen health and productivity (Puvača et al., 2023). Mycotoxin contamination, particularly by T-2 toxin, can disrupt these parameters, leading to decreased feed utilization efficiency and impaired egg production. Egg quality attributes such as shell strength, yolk color, albumen height, and Haugh unit are essential determinants of egg marketability and consumer acceptance. Mycotoxin exposure has been associated with alterations in egg quality parameters, posing challenges for egg producers (Zhao et al., 2021). Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms within the organism. Mycotoxins, including T-2 toxin, have been implicated in the generation of ROS and subsequent oxidative damage in various tissues, including those of laying hens (Adhikari et al., 2017). The assessment of oxidative stress biomarkers such as malondialdehyde (MDA) levels, antioxidant enzyme activities, and total antioxidant capacity provides insights into the extent of oxidative damage and the potential ameliorative effects of mycotoxin adsorbents in mitigating oxidative stress-induced poultry health complications (Mavrommatis et al., 2021).

Mycotoxin-induced oral and tongue lesions represent a significant welfare concern in poultry production systems (EFSA, 2012). These lesions not only cause discomfort and pain to affected birds but also compromise feed intake and nutrient utilization, thereby impacting production performance. By examining the incidence and severity of oral and tongue lesions in laying hens exposed to T-2 toxin and supplemented

with mycotoxin adsorbents, it is possible to assess the potential protective effects of these interventions on oral health and welfare outcomes in poultry (Diaz et al., 2016).

This paper explores the influence of two commercial mycotoxin adsorbents Mycostop Premium 2.0, and Mycostop Supreme, respectively, on laying hens' production performance, nutritive and physical egg quality, oxidative stress levels, and the occurrence of oral and tongue lesions in the context of T-2 toxin intoxication. Understanding the impact of mycotoxin adsorbents on these parameters is crucial for devising effective strategies to safeguard poultry health and optimize productivity in the face of mycotoxin challenges.

MATERIALS AND METHODS

The protocol for the experiment was performed following the EU legislation and principle of the Three Rs within Directive 2010/63/EU. A total of 200 Lohmann Brown hens aged 42 weeks (initial average body weight 1810 ± 56 g) was used for a 56-day feeding study. The birds were randomly assigned to 4 treatments (5 replicates, 10 birds/replicate) according to the dietary treatments. The birds were housed in a 2-sided, 3-tier battery cage system with one bird per cage [50 cm × 46 cm × 54 cm (l, w, h)] for 8 weeks. Feed was restricted to 110 g per bird per day, while water was provided *ad libitum* according to feedstuffs and nutritive composition (Table 1), and experimental design (Table 2). Temperature and humidity in the laying house were controlled by the automatic ventilation system. A light/dark cycle of 14 h light/10 h dark was used during the experimental period.

All eggs were collected and weighed daily at the same time for calculating hen day egg production (HDEP), average egg weight (AEW), and egg mass (EM). Feed intake was recorded weekly. Average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Haugh unit was calculated by using the tripod micrometer to measure albumen height and followed the equation proposed by Haugh. The eggshell and yolk color were measured by using a Roche color fan. Shell thickness was measured using a thickness meter. Shell hardness was measured using a hardness tester.

For tissue lipid peroxidation evaluation, at the end of the experiment, a total of 12 hens were sacrificed, 3 hens from each treatment, respectively. After 24h

Table 1. Feedstuffs and nutritive composition of laying hens diets.

Feedstuffs, %	Basic hens diets
Corn	60.0
Soybean meal	20.0
Sunflower meal	2.0
Corn gluten	2.0
Soy oil	1.0
Limestone	9.60
Sodium chloride	0.40
Premix	5.0
Total	100
Nutritive composition, %	
Crude protein	18.0
Crude fat	3.7
Crude fiber	5.0
Ash	11.3
Lysine	0.85
Methionine	0.70
Ca	4.0
P _(available)	0.42
Metabolic energy (MJ/kg)	11.4

Table 2. Experimental design with laying hens.

Treatments	Diets		
	T-2 toxin, mg/kg	Mycostop Premium 2.0, kg/t	Mycostop Supreme, kg/t
T1	-	-	-
T2	8	-	-
T3	8	2	-
T4	8	-	2

T1 - control treatment without T-2 toxin and adsorbents; T2 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin; T3 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Premium 2.0 in concentration of 2 kg/t of feed; T4 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Supreme in concentration of 2 kg/t of feed. T-2 toxin and dietary mycotoxin binders Mycostop Premium 2.0, and Mycostop Supreme are added on top of the basic diets.

post-mortem, samples of breast (*Musculus pectoralis*) and thigh with drumstick (*Tibialis anterior* and *Biceps femoris*), and liver (right lobe) were analyzed for lipid peroxidation. Tissue lipid peroxidation was performed by thiobarbituric acid reactive substances (TBARS) assay as previously described by (Puvaca et al., 2016). A total of 1 g each of the chicken's breast, thigh with a drumstick, and liver samples were minced with scissors and homogenized with an ultraturax in 3 volumes of isotonic buffer (0.05 mol/L tris-HCl, 0.25 mol/L sucrose, pH = 7.5). The homogenates were filtered through gauze into ice-cold tubes and collected for further analysis. Tissue homogenates (0.2 mL) were transferred into the tube and 2 mL of a 2,4,6-tribromoanisole and 2,4,6-trichloroanisole solution was added. The mixture was further mixed with the addition of 1.05 mL of HCl. Subsequently, the tube was filled with distilled water up to 50 mL.

The mixture was incubated in a boiling water bath for 15 minutes to develop color. After color development, the samples were cooled in cold water for 10 minutes and then centrifuged for 10 minutes at 3000g. Spectrophotometric measurement was performed at 536 nm. The TBARS concentration was expressed in mg malondialdehyde (MDA) per kg of tissue.

For gas emission in the facility with hens, the Aeroqual Series 200 Monitor with Sensor Head a portable gas monitor that is capable of measuring a wide range of gases was used. Aeroqual sensors were installed vertically and exposed directly to the hen's position in the facility and gases such as ammonia (NH₃), methane (CH₄), nitrogen dioxide (NO₂), and carbon dioxide (CO₂), were monitored in several series.

Statistical analyses were conducted using the sta-

tistical software program Statistica 13 for Windows to verify whether variables differed between different dietary treatments. Significant effects were further explored using analysis of variance (ANOVA), and Duncans post-hoc test to ascertain differences among treatment means. A level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The results presented in Figure 1, show a significant influence of the T-2 toxin in laying hens on all productive parameters. Starting with hen day egg production (HDEP) in treatment T2 with the lowest value of 77%, following average egg weight (AEW) of 53.2 g/egg, then egg mass (EM) of 42.1 g/day/hen, reduced average daily feed intake (ADFI) of 96 g/day/hen to significantly increased feed conversion ratio (FCR) of 2.5. In our study, T-2 toxin exposure in laying hens on treatment T-2 with basal diet and 8 mg/kg addition of T-2 toxin without the addition of mycotoxin binders led to decreased hen day egg production, reduced average egg weight, diminished egg mass, lower feed intake, and an increased feed conversion ratio, collectively posing significant challenges to the productivity and profitability of egg production operations.

Additionally, in other research (Guo et al., 2024) laying hens exposed to T-2 toxin have experienced a reduction in average egg weight, leading to smaller

and potentially less valuable eggs, which is in accordance with the results obtained in our study. Also, other investigations show that T-2 toxin exposure induces a decrease in average daily feed intake among laying hens (Bócsai et al., 2015; Rezar et al., 2007). This reduced feed intake led to inadequate nutrition and compromised hen health, exacerbating the negative effects on egg production parameters. Additionally, laying hens exposed to T-2 toxin often exhibit an increased feed conversion ratio, indicating that more feed is required to produce a unit of egg mass. This inefficiency in feed utilization elevates production costs and diminishes the economic viability of egg production enterprises (Meneely et al., 2023). Regarding other obtained results in our study, the best results without any statistically significant differences ($p > 0.05$) were recorded for control treatment T1, and experimental treatment T4 as follows for AEW (63.0 and 61.5 g/egg), EM (56.0 and 53.0 g/day/hen), respectively. Results of ADFI were highest for T1 treatment (107.0 g/day/hen), followed by T3 (101.0 g/day/hen), and T4 (100.5 g/day/hen) treatment without any statistical significance ($p > 0.05$).

All treatments recorded statistically significant differences ($p < 0.05$) when compared to treatment T2 the treatment where hens were fed with a basal diet and 8 mg/kg addition of T-2 toxin without any mycotoxin adsorbent supplementation. The lowest FCR of 1.90,

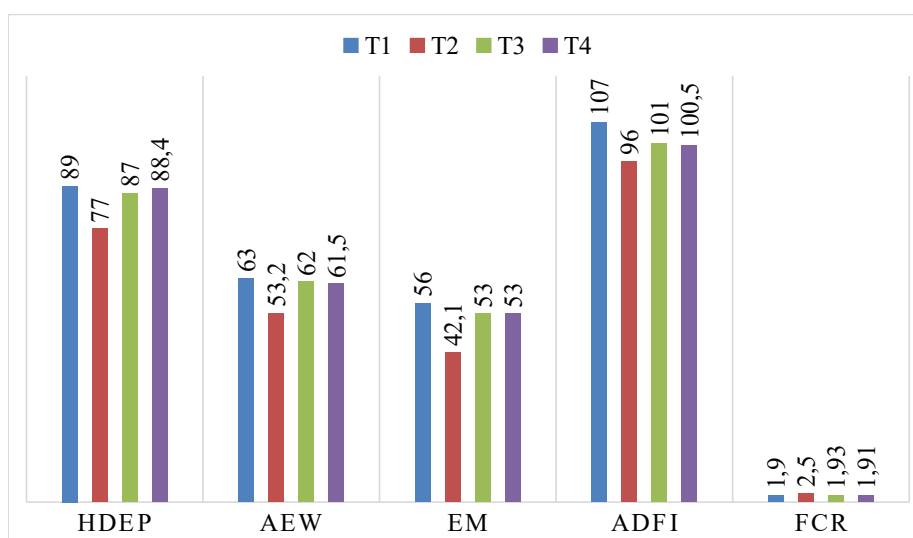


Figure 1. Performance of laying hens in the experiment.

T1 - control treatment without T-2 toxin and adsorbents; T2 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin; T3 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Premium 2.0 in concentration of 2 kg/t of feed; T4 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Supreme in concentration of 2 kg/t of feed; HDEP - hen day egg production (%); AEW - average egg weight (g/egg); EM - egg mass (g/day/hen); ADFI - average daily feed intake (g/day/hen); FCR - feed conversion ratio (weekly feed intake ÷ weekly egg mass output).

and 1.91 was recorded in treatments T1 and T4 without statistically significant differences ($p>0.05$), while the highest FCR of 2.5, with statistically significant differences ($p<0.05$) compared to all treatments was recorded in laying hens on treatment T2, respectively.

Investigations have shown that T-2 toxin exposure in laying hens can have profound negative effects on both external and internal egg quality (Guo et al., 2024). Externally, eggs laid by hens exposed to T-2 toxin may exhibit abnormalities such as thin or irregular eggshells, which can increase the susceptibility of eggs to breakage during handling and transportation. Additionally, the color and texture of the eggshell may be altered, leading to a decrease in visual

appeal and market value (de Oliveira et al., 2017).

Our obtained results for external egg quality (Figure 2) show a similar tendency to the performance results of laying hens. The highest shell thickness of 0.45 and 0.43 mm was recorded in treatments T1 and T4, without statistically significant differences between themselves ($p>0.05$), but with statistically significant differences ($p<0.05$) compared to T2 treatment. The same tendency was observed regarding the shell hardness where the dietary addition of mycotoxin binder Mycostop Premium 2.0 in treatment T3, and Mycostop Supreme in treatment T4 at a concentration of 2 kg/t significantly ($p<0.05$) increased shell hardness (4.34 and 4.51 kg/cm²) compared to treatment T2

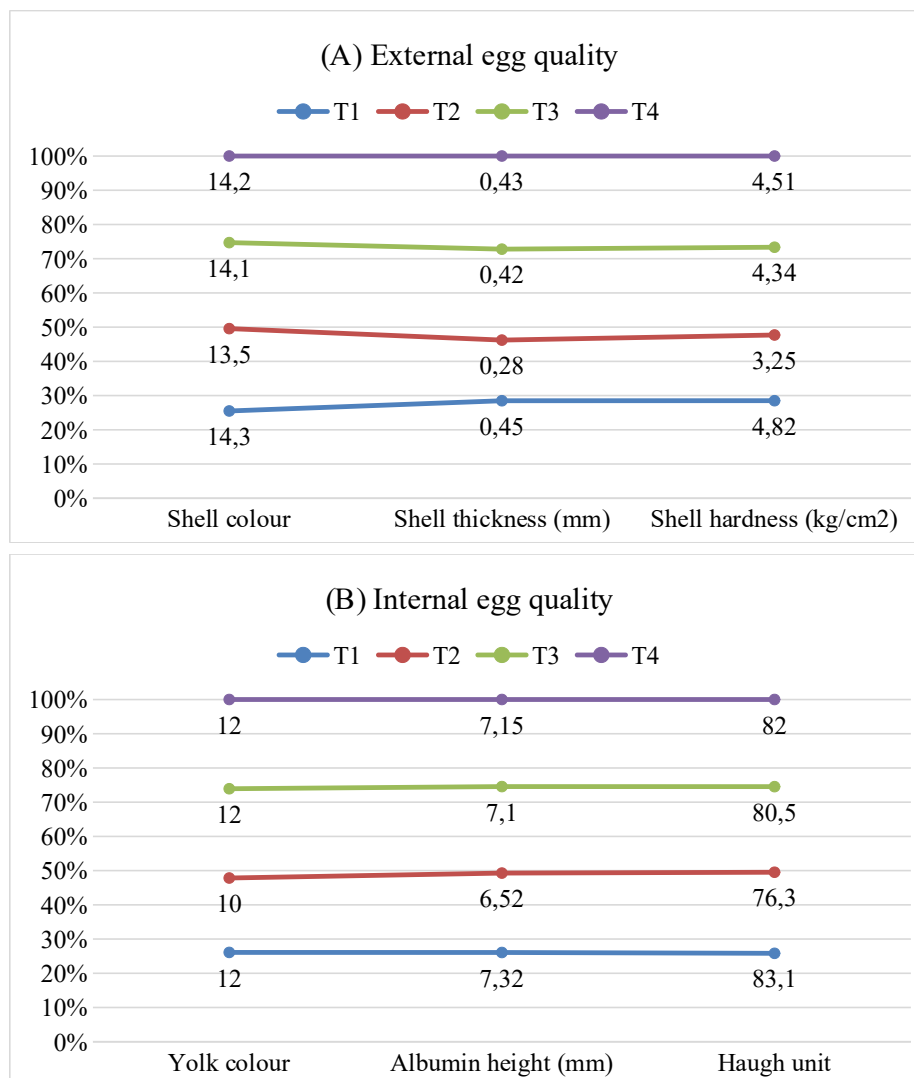


Figure 2. External (A) and internal (B) egg quality of laying hens.

T1 - control treatment without T-2 toxin and adsorbents; T2 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin; T3 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Premium 2.0 in concentration of 2 kg/t of feed; T4 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Supreme in concentration of 2 kg/t of feed.

(3.25 kg/cm²), while statistically significant differences with control treatment T1 (4.82 kg/cm²) was absent ($p > 0.05$).

Research has shown that internally, the T-2 toxin can compromise the integrity of the egg contents. It may disrupt the formation of egg white proteins, resulting in decreased albumen quality and an increased likelihood of watery or runny egg whites (Chukwuka et al., 2010). This deterioration in albumen quality can diminish the overall nutritional value of the eggs. Furthermore, T-2 toxin exposure affects the internal structure of the egg, leading to abnormalities such as double yolks or misshapen eggs (Meerpoel et al., 2020). These defects not only reduce the marketability of the eggs but also indicate disturbances in the reproductive physiology of the laying hens (EL-Deep, 2016).

Regarding the internal egg quality (Figure 2), it can be seen that the addition of both mycotoxin adsorbents didn't have any effect on yolk color. The presence of T-2 toxin has slightly lightened the yolk color from 12 to 10 compared with other treatments but with statistically significant differences ($p < 0.05$). Other research demonstrates that T-2 toxin exposure in laying hens impacts the color of egg yolks, typically resulting in paler yolks compared to those from unaffected hens, which is in accordance with our findings in this experiment. The authors also point out that this alteration in yolk color is attributed to disturbances in the deposition of pigments such as carotenoids, which are responsible for the characteristic yellow-orange hue of egg yolks. As a result, eggs laid by hens exposed to T-2 toxin may exhibit a less vibrant or desirable yolk color (Guo et al., 2024). On the other hand, consumer preference for egg yolk color varies widely and is influenced by cultural, regional, and individual factors. In many regions, consumers associate deeper, more

intense yolk colors with freshness, nutritional quality, and flavor richness. As such, eggs with vibrant, golden yolks are often perceived as more appealing and are preferred over those with paler yolks (Altmann et al., 2023). However, preferences for egg yolk color can also be influenced by marketing and advertising efforts, as well as misconceptions regarding egg quality. Some consumers may mistakenly believe that paler yolks indicate higher quality or healthier eggs, leading to a preference for lighter-colored yolks. The same tendency was observed regarding the albumin high and Haugh units. Only treatment with T-2 toxin in feed expressed negative effects while control treatment T1 showed the best results in both parameters following the treatment T4, and T3, without any statistically significant differences ($p > 0.05$) between themselves but with significant differences ($p < 0.05$) compared to T2 treatment. According to other research T-2 toxin exposure in laying hens adversely affects the egg Haugh units, which is a measure of egg freshness and albumen quality (Yuan et al., 2022). When laying hens are exposed to T-2 toxin, the quality of the albumen is compromised, resulting in a decrease in Haugh units. This decline is indicative of reduced albumen viscosity and structural integrity, which lead to eggs with poorer functional properties, such as decreased ability to retain their shape during cooking or baking (Chowdhury and Smith, 2004).

Overall, T-2 toxin exposure in laying hens results in external abnormalities such as thin or irregular eggshells, as well as internal issues including poor albumen quality and structural deformities. These negative effects collectively undermine the external appearance, nutritional quality, and market value of eggs produced by affected hens.

Results of tissue lipid peroxidation are presented in Table 3. It can be noticed that the dietary supple-

Table 3. Concentration of thiobarbituric acid reactive substances (TBARS) (mg MDA/kg tissue).

Treatments	TBARS		
	Breast meat	Thigh meat	Liver
T1	0.22 ± 0.04 ^c	0.30 ± 0.03 ^c	0.39 ± 0.05 ^c
T2	0.70 ± 0.12 ^a	1.48 ± 0.23 ^a	2.06 ± 0.56 ^a
T3	0.68 ± 0.14 ^a	1.32 ± 0.19 ^a	1.86 ± 0.14 ^a
T4	0.44 ± 0.01 ^b	0.94 ± 0.01 ^b	1.28 ± 0.01 ^b
Pooled <i>p</i>	0.03	0.01	0.02

T1 - control treatment without T-2 toxin and adsorbents; T2 - experimental treatment with basal diet and 8ppm addition of T-2 toxin; T3 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Premium 2.0 in a concentration of 2 kg/t of feed; T4 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Supreme in the concentration of 2 kg/t of feed; TBARS - thiobarbituric acid reactive substances; column with the different letters in the superscript are considered significantly different ($p < 0.05$).

mentation of both mycotoxin adsorbents significantly ($p < 0.05$) influenced the malondialdehyde (MDA) concentrations in all investigated tissues when compared to treatment T2, while significant differences between control treatment T1 and experimental T4 were not present ($p > 0.05$).

Based on the obtained results in this experiment, lipid peroxidation in tissues of laying hens supplemented with dietary mycotoxin adsorbents was significantly lower than in tissues of laying hens in control treatment T1. The malondialdehyde concentration increased significantly ($p < 0.05$) in treatment with T-2 toxin compared to all treatments in the experiment. Results presented in Table 3, show the highest MDA concentration of 2.06 per kg tissue in the liver in treatment T2, and the lowest of 0.22 per kg tissue in breast meat of hens in treatment T1. Generally, T-2 toxin exposure in laying hens leads to increased levels of malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS) in their tissues or biological fluids (Kulcsár et al., 2023). MDA and TBARS are commonly used as biomarkers of lipid peroxidation, a process in which reactive oxygen species (ROS) attack and degrade lipid molecules, resulting in the formation of lipid peroxidation products such as MDA and TBARS. Elevated levels of MDA or TBARS in laying hens exposed to T-2 toxin indicate increased oxidative stress and lipid damage (Hoehler and Marquardt, 1996), which was the case in our experiment as well. This oxidative damage can have widespread negative effects on cellular membranes, proteins, and DNA, ultimately compromising cellular function and integrity.

T-2 toxin exposure in laying hens also influences the emission of gases in their excreta, potentially

leading to environmental and health concerns. Mycotoxins like T-2 toxins disrupt the gastrointestinal tract and alter microbial populations within the digestive system of laying hens. These disruptions affect the fermentation processes that occur in the hindgut, leading to changes in the composition and volume of gases emitted in the excreta. From the results presented in Table 4, it can be seen that the presence of T-2 toxin in the feed of laying hens significantly ($p < 0.05$) increased the concentration of all measured gasses with the highest concentration of CO_2 (144.3 g/d/bird), followed by NH_3 (2.33 g/d/bird), and other measured gasses. One of the primary concerns is the increased production of NH_3 gas. Ammonia is a by-product of microbial fermentation of nitrogen-containing compounds in the excreta and is released into the environment. Elevated levels of ammonia emissions contribute to air pollution, particularly in confined poultry production facilities, and pose respiratory health risks to both animals and workers (Guo et al., 2022). Furthermore, changes in gas emissions from excreta can indicate alterations in nutrient utilization and microbial activity within the digestive tract of laying hens. These alterations affect the efficiency of feed conversion and nutrient absorption, ultimately impacting the overall health and productivity of the birds (Brink et al., 2022). The addition of both mycotoxin binders significantly decreased the concentration of all measured gasses, showing that mycotoxin binders can present useful tools in reducing environmental pollution and may have beneficial effects on air quality and human health.

T-2 toxin exposure in laying hens often leads to the development of black tongues or tongue necrosis, a condition characterized by dark discoloration, lesions, and tissue damage in the oral cavity. This condi-

Table 4. Emission of gasses in the excreta of laying hens.

Gasses, g/d/bird	Treatments				Pooled <i>p</i>
	T1	T2	T3	T4	
Ammonia (NH_3)	0.70 ± 0.32 ^c	2.33 ± 0.42 ^a	1.02 ± 0.34 ^b	0.81 ± 0.12 ^c	0.03
Methane (CH_4)	0.08 ± 0.02 ^c	0.89 ± 0.01 ^a	0.45 ± 0.38 ^b	0.12 ± 0.01 ^c	0.04
Nitrogen dioxide (NO_2)	0.04 ± 0.01 ^c	0.73 ± 0.04 ^a	0.48 ± 0.02 ^b	0.23 ± 0.07 ^c	0.02
Carbon dioxide (CO_2)	92.2 ± 2.06 ^c	144.3 ± 5.15 ^a	98.2 ± 2.19 ^b	101.4 ± 3.05 ^b	0.05

T1 - control treatment without T-2 toxin and adsorbents; T2 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin; T3 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Premium 2.0 in concentration of 2 kg/t of feed; T4 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Supreme in concentration of 2 kg/t of feed. T-2 toxin and dietary mycotoxin binders Mycostop Premium 2.0, and Mycostop Supreme are added on top of the basic diets; raw with the different letters in the superscript are considered significantly different ($p < 0.05$).

tion is often associated with the consumption of feed contaminated with mycotoxins, including T-2 toxin. The presence of T-2 toxin in the feed causes irritation and inflammation of the oral mucosa in laying hens. Continued exposure to the toxin results in the formation of necrotic lesions, particularly on the tongue, which may appear black or darkened in color. These lesions can vary in severity, ranging from mild inflammation to extensive tissue damage and ulceration. From the obtained results in our study, Figure 3 shows clinical signs of T-2 toxin, and mycotoxin binder influence. From the presented figure it can be noticed that the hens on treatment T2 showed extensive tissue damage, while the addition of mycotoxin binder in treatment T3 prevented extensive inflammation and led to mild inflammation and slight discoloration of the laying hens' tongue. According to observations in our study control treatment T1, and experimental tre-

atment T4 didn't express any signs of mycotoxicosis.

Many investigations have shown that black tongues or tongue necrosis can adversely affect the feeding behavior and overall health of laying hens, which was in accordance with our findings as well. The presence of painful lesions in the oral cavity can impair the birds' ability to consume feed and water, leading to reduced nutrient intake and weight loss. In severe cases, tongue necrosis can also compromise the birds' ability to preen, vocalize, and engage in normal behaviors, affecting their welfare and productivity. Likewise, black tongues or tongue necrosis can serve as indicators of systemic mycotoxin exposure and may be accompanied by other clinical signs of mycotoxicosis, such as gastrointestinal disturbances, immunosuppression, and reproductive problems (Jones and Orosz, 1996).



Figure 3. Clinical signs of T-2 toxicity in laying hens.

T1 - control treatment without T-2 toxin and adsorbents; T2 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin; T3 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Premium 2.0 in concentration of 2 kg/t of feed; T4 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Supreme in concentration of 2 kg/t of feed.

T-2 toxin contamination in laying hen production can impose significant economic costs on poultry producers. The negative effects of T-2 toxin on hen health, productivity, and egg quality can translate into various expenses and losses throughout the production cycle. As already previously mentioned, decreased egg production and lower egg quality resulting from T-2 toxin exposure lead to reduced revenue for poultry producers. Eggs with abnormalities such as thin shells, reduced size, and poor yolk color may fetch lower prices in the market or be rejected altogether, impacting overall sales and profitability. Moreover, T-2 toxin-induced health issues such as black tongues, tongue necrosis, and other mycotoxicosis-related conditions may necessitate veterinary intervention, medication, and treatments, increasing production costs. Additionally, measures to mitigate mycotoxin contamination in feed, such as testing, sourcing uncontaminated feed ingredients, or applying feed additives, can further add to production expenses. Furthermore, the impact of T-2 toxin on feed conversion efficiency, nutrient utilization, and growth performance of laying hens can result in higher feed costs per unit of egg produced. Reduced feed intake, impaired nutrient absorption, and decreased egg mass contribute to inefficiencies in feed utilization, elevating the cost of egg production. Additionally, the presence of T-2 toxin in laying hen facilities can pose risks to worker health and safety, potentially leading to absenteeism, medical expenses, and legal liabilities for employers.

Results presented in Table 5 show the economic cost of laying hens production with the main accent on egg production efficiency index (EPEI) as the main indicator. From the presented results it can be seen that the lowest EPEI was recorded in treatment T2 (137), while the highest EPEI was recorded in experimental treatment with the addition of mycotoxin

adsorbent in treatment T4 (183), following treatment T1 (169), and T3 (158), respectively.

Mycotoxin binders, also known as mycotoxin adsorbents or detoxifiers, play a crucial role in mitigating the negative impacts of mycotoxin contamination on laying hen production, thereby offering several potential economic benefits to poultry producers (Puvača and Ljubojević Pelić, 2024; Wielogórska et al., 2016). One significant positive effect is the reduction in the incidence and severity of mycotoxicosis-related health issues in laying hens. By binding to mycotoxins in the gastrointestinal tract, mycotoxin binders prevent their absorption into the bloodstream and subsequent distribution to organs and tissues. This helps minimize the occurrence of conditions such as black tongues, tongue necrosis, immunosuppression, and reproductive problems, which would otherwise require veterinary intervention, medication, and treatment expenses (Čolović et al., 2019; Zhu et al., 2016). Mycotoxin binders contribute to maintaining or improving the productivity and performance of laying hens, which can be seen from the EPEI presented in Table 4 of our study. By mitigating the adverse effects of mycotoxin exposure on feed intake, nutrient utilization, egg production, and egg quality, binders help sustain optimal levels of hen day egg production, egg weight, egg mass, and feed conversion efficiency. This results in higher yields of marketable eggs and improved profitability for poultry producers. The use of mycotoxin binders can help reduce the need for costly feed disposal or replacement in the event of mycotoxin contamination. Instead of discarding contaminated feed, which would incur additional expenses, binders allow producers to salvage usable feed by effectively neutralizing mycotoxins and preventing their harmful effects on laying hens. Mycotoxin binders contribute to enhancing the overall safety and quality of eggs produced (Lee et al., 2023; Puvača and Vapa, 2024).

Table 5. Economic cost of laying hens production.

Cost per laying hen	Treatments			
	T1	T2	T3	T4
Feed consumption (kg)	5.99	5.37	5.65	5.62
Total feed cost (€)	1.67	1.50	1.58	1.56
Medication cost (€)	1.75	1.75	1.75	1.75
Mycotoxin adsorbent cost (€/kg)	0	0	0.80	1.40
Egg production efficiency index (EPEI)	169	137	158	183

T1 - control treatment without T-2 toxin and adsorbents; T2 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin; T3 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Premium 2.0 in concentration of 2 kg/t of feed; T4 - experimental treatment with basal diet and 8 mg/kg addition of T-2 toxin and adsorbent Mycostop Supreme in concentration of 2 kg/t of feed. T-2 toxin and dietary mycotoxin binders Mycostop Premium 2.0, and Mycostop Supreme are added on top of the basic diets.

Incorporating mycotoxin binders into feed management practices represents a cost-effective strategy for mitigating the economic impacts of mycotoxin contamination and maintaining the sustainability of laying hen operations.

CONCLUSION

In conclusion, the investigation into the influence of mycotoxin adsorbents on laying hens' production performance, egg quality, oxidative stress levels, and oral health in the context of T-2 toxin intoxication holds significant implications for poultry producers seeking to mitigate the adverse effects of mycotoxin contamination on flock health and productivity. By elucidating the efficacy of mycotoxin adsorbents in alleviating T-2 toxin-induced deleterious effects, this study contributes to the development of evidence-based strategies for safeguarding poultry welfare and optimizing production efficiency in

mycotoxin-challenged environments. Incorporating mycotoxin binders into feed management practices represents a cost-effective strategy for mitigating the economic impacts of mycotoxin contamination and maintaining the sustainability of laying hen operations. Results of this study show the positive effects of mycotoxin binders on laying hen production including improved hen health and performance, increased productivity and profitability, reduced feed wastage, and enhanced egg safety and quality.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Education, Science, and Technological Development of the Republic of Serbia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Adhikari, M., Negi, B., Kaushik, N., Adhikari, A., Al-Khedhairi, A. A., Kaushik, N. K., & Choi, E. H. (2017). T-2 mycotoxin: Toxicological effects and decontamination strategies. *Oncotarget*, *8*(20), 33933-33952. <https://doi.org/10.18632/oncotarget.15422>
- Altmann, B. A., Trinks, A., & Mörlein, D. (2023). Consumer preferences for the color of unprocessed animal foods. *Journal of Food Science*, *88*(3), 909-925. <https://doi.org/10.1111/1750-3841.16485>
- Bócsai, A., Ancsin, Zs., Fernye, Cs., Zándoki, E., Szabó-Fodor, J., Erdélyi, M., Mézes, M., & Balogh, K. (2015). Dose-dependent short-term effects of T-2 toxin exposure on lipid peroxidation and antioxidant parameters of laying hens. *European Poultry Science (EPS)*, *79*. <https://doi.org/10.1399/eps.2015.115>
- Brink, M., Janssens, G. P. J., Demeyer, P., Bağci, Ö., & Delezie, E. (2022). Reduction of dietary crude protein and feed form: Impact on broiler litter quality, ammonia concentrations, excreta composition, performance, welfare, and meat quality. *Animal Nutrition*, *9*, 291-303. <https://doi.org/10.1016/j.aninu.2021.12.009>
- Chowdhury, S. R., & Smith, T. K. (2004). Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of laying hens. *Poultry Science*, *83*(11), 1849-1856. <https://doi.org/10.1093/ps/83.11.1849>
- Chukwuka, O. K., Okoli, I. C., Okeudo, N. J., Udedibie, A. B. I., Ogbuewu, I. P., Aladi, N. O., Iheshiulor, O. O. M., & Omede, A. A. (2010). Egg Quality Defects in Poultry Management and Food Safety. *Asian Journal of Agricultural Research*, *5*(1), 1-16. <https://doi.org/10.3923/ajar.2011.1.16>
- Čolović, R., Puvača, N., Cheli, F., Avantaggiato, G., Greco, D., Đuragić, O., Kos, J., & Pinotti, L. (2019). Decontamination of Mycotoxin-Contaminated Feedstuffs and Compound Feed. *Toxins*, *11*(11), 617. <https://doi.org/10.3390/toxins11110617>
- de Oliveira, C. A. F., de Neef, D. V., de Pinho Carão, Á. C., & Corassin, C. H. (2017). Mycotoxin Impact on Egg Production. In P. Y. Hester (Ed.), *Egg Innovations and Strategies for Improvements* (pp. 581-596). Academic Press. <https://doi.org/10.1016/B978-0-12-800879-9.00054-8>
- Diaz, G. J., Vargas, M. L., & Cortés, A. (2016). Evaluation of the supplementation of a feed additive as a potential protector against the adverse effects of 2.5 ppm T-2 toxin on growing broiler chickens. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *68*, 709-715. <https://doi.org/10.1590/1678-4162-8416>
- EFSA. (2012). Scientific Opinion on the welfare of cattle kept for beef production and the welfare in intensive calf farming systems. *EFSA Journal*, *10*(5), 2669. <https://doi.org/10.2903/j.efsa.2012.2669>
- EL-Deep, M. H. (2016). POSSIBLE PROTECTIVE EFFECTS OF GLUTATHIONE IN PREVENTING AFLATOXICOSIS IN EGYPTIAN LAYING HENS. *Egyptian Poultry Science Journal*, *36*(2), 543-560. <https://doi.org/10.21608/epsj.2016.168796>
- Guo, H., Wan, H., Lou, W., Khan, R. U., You, J., Huang, B., Hao, S., Li, G., & Dai, S. (2024). Deoxynivalenol and T-2 toxin cause liver damage and egg quality degradation through endoplasmic reticulum stress in summer laying hens. *International Journal of Biometeorology*. <https://doi.org/10.1007/s00484-024-02674-w>
- Guo, L., Zhao, B., Jia, Y., He, F., & Chen, W. (2022). Mitigation Strategies of Air Pollutants for Mechanical Ventilated Livestock and Poultry Housing—A Review. *Atmosphere*, *13*(3), Article 3. <https://doi.org/10.3390/atmos13030452>
- Haque, M. A., Wang, Y., Shen, Z., Li, X., Saleemi, M. K., & He, C. (2020). Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review. *Microbial Pathogenesis*, *142*, 104095. <https://doi.org/10.1016/j.micpath.2020.104095>
- Hoehler, D., & Marquardt, R. R. (1996). Influence of Vitamins E and C on the Toxic Effects of Ochratoxin A and T-2 Toxin in Chicks. *Poultry Science*, *75*(12), 1508-1515. <https://doi.org/10.3382/ps.0751508>
- Jones, M. P., & Orosz, S. E. (1996). Overview of avian neurology and neurological diseases. *Seminars in Avian and Exotic Pet Medicine*, *5*(3), 150-164. [https://doi.org/10.1016/S1055-937X\(96\)80004-1](https://doi.org/10.1016/S1055-937X(96)80004-1)
- Keřińska-Pacelik, J., & Biel, W. (2021). Mycotoxins—Prevention, Detection, Impact on Animal Health. *Processes*, *9*(11), Article 11. <https://doi.org/10.3390/pr9112035>
- Kulcsár, S., Kövesi, B., Balogh, K., Zándoki, E., Ancsin, Z., Erdélyi, M., & Mézes, M. (2023). The Co-Occurrence of T-2 Toxin, Deoxynivalenol, and Fumonisin B1 Activated the Glutathione Redox System in the EU-Limiting Doses in Laying Hens. *Toxins*, *15*(5), Article 5. <https://doi.org/10.3390/toxins15050305>
- Lee, J., Cho, H., Song, D., Chang, S., An, J., Nam, J., Lee, B., Kim, S., Kim, W. K., & Cho, J. (2023). Effects of Combinations of Toxin Binders with or without Natural Components on Broiler Breeders Exposed to Ochratoxin A. *Animals*, *13*(14), Article 14. <https://doi.org/10.3390/ani13142266>
- Mavrommatis, A., Giamouri, E., Tavrizelou, S., Zacharioudaki, M., Danezis, G., Simitzis, P. E., Zoidis, E., Tsiplakou, E., Pappas, A. C., Georgiou, C. A., & Feggeros, K. (2021). Impact of Mycotoxins on Animals' Oxidative Status. *Antioxidants*, *10*(2), Article 2. <https://doi.org/10.3390/antiox10020214>
- Meerpoel, C., Vidal, A., Tangni, E. K., Huybrechts, B., Couck, L., De Rycke, R., De Bels, L., De Saeger, S., Van den Broeck, W., Devreese, M., & Croubels, S. (2020). A Study of Carry-Over and Histopathological Effects after Chronic Dietary Intake of Citrinin in Pigs, Broiler Chickens and Laying Hens. *Toxins*, *12*(11), Article 11. <https://doi.org/10.3390/toxins12110719>
- Meneely, J., Greer, B., Kolawole, O., & Elliott, C. (2023). T-2 and HT-2 Toxins: Toxicity, Occurrence and Analysis: A Review. *Toxins*, *15*(8), Article 8. <https://doi.org/10.3390/toxins15080481>
- Puvača, N., Kostadinović, Lj., Popović, S., Lević, J., Ljubojević, D., Tufarelli, V., Jovanović, R., Tasić, T., Ikončić, P., & Lukač, D. (2016). Proximate composition, cholesterol concentration and lipid oxidation of meat from chickens fed dietary spice addition (*Allium sativum*, *Piper nigrum*, *Capsicum annum*). *Animal Production Science*, *56*(11), 1920-1927. <https://doi.org/10.1071/AN15115>
- Puvača, N., & Ljubojević Pelić, D. (2024). Problems and Mitigation Strategies of Trichothecenes Mycotoxins in Laying Hens Production. *Journal of Agronomy, Technology and Engineering Management*, *7*(2), 1074-1087. <https://doi.org/10.55817/ISAD5453>
- Puvača, N., Ljubojević Pelić, D., & Tufarelli, V. (2023). Mycotoxins Adsorbents in Food Animal Production. *Journal of Agronomy, Technology and Engineering Management*, *6*(5), 944-952. <https://doi.org/10.55817/GYIC7602>
- Puvača, N., & Vapa, B. (2024). Implementation of food safety policy in

- the European Union: Guidance, variety, and resolution of challenges. *Pravo - Teorija i Praksa*, 41(1), 18-34. <https://doi.org/10.5937/ptp2401018P>
- Rezar, V., Frankič, T., Narat, M., Levart, A., & Salobir, J. (2007). Dose-Dependent Effects of T-2 Toxin on Performance, Lipid Peroxidation, and Genotoxicity in Broiler Chickens. *Poultry Science*, 86(6), 1155-1160. <https://doi.org/10.1093/ps/86.6.1155>
- Wielogórska, E., MacDonald, S., & Elliott, C. t. (2016). A review of the efficacy of mycotoxin detoxifying agents used in feed in light of changing global environment and legislation. *World Mycotoxin Journal*, 9(3), 419-433. <https://doi.org/10.3920/WMJ2015.1919>
- Yuan, T., Li, J., Wang, Y., Li, M., Yang, A., Ren, C., Qi, D., & Zhang, N. (2022). Effects of Zearalenone on Production Performance, Egg Quality, Ovarian Function and Gut Microbiota of Laying Hens. *Toxins*, 14(10), Article 10. <https://doi.org/10.3390/toxins14100653>
- Zhao, L., Feng, Y., Wei, J.-T., Zhu, M.-X., Zhang, L., Zhang, J.-C., Karrow, N. A., Han, Y.-M., Wu, Y.-Y., Guo, Y.-M., & Sun, L.-H. (2021). Mitigation Effects of Bentonite and Yeast Cell Wall Binders on AFB1, DON, and OTA Induced Changes in Laying Hen Performance, Egg Quality, and Health. *Toxins*, 13(2), Article 2. <https://doi.org/10.3390/toxins13020156>
- Zhu, Y., Hassan, Y. I., Watts, C., & Zhou, T. (2016). Innovative technologies for the mitigation of mycotoxins in animal feed and ingredients—A review of recent patents. *Animal Feed Science and Technology*, 216, 19-29. <https://doi.org/10.1016/j.anifeedsci.2016.03.030>