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The protective effect of thyme and spirulina against aflatoxin toxicity in rabbits

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Abstract

Comparative Evaluation of Thyme and Spirulina as Individual and Combined Protective Agents Against Aflatoxin-Induced Toxicity in Growing Rabbits.

This study investigated the potential of supplementing the diet with Thyme, Spirulina, or their mixture to mitigate the physiological damage and adverse effects on growth performance induced by Aflatoxin contamination in post-weaning rabbits. A field trial spanning one month (February-March 2025) was conducted using 120 rabbits, randomly distributed into eight treatment groups . Key parameters measured included hepatic enzymes (ALT, AST), renal function markers (Creatinine, Urea), oxidative stress indicators (MDA, GSH, SOD, TAC), and growth performance (Final Weight, Weight Gain (WG), Feed Intake (Fi)). Data were analyzed using One-Way ANOVA and Duncan's test . Results clearly established Treatment 5 (Diet + Aflatoxin) as the toxicity model, showing maximal and highly significant elevations in liver and kidney enzymes (e.g., ALT: U/L), severe oxidative damage (highest MDA), and the lowest overall growth performance (WG: g). Conversely, significant restorative and prophylactic effects were observed across the non-toxic and protective groups:

1. Optimal Performance (T4): Treatment 4 (Diet + Thyme + Spirulina Mixture) achieved the highest growth metrics in the absence of Aflatoxin, recording significantly superior Final Weight (g) and Weight Gain (Subset A), confirming the growth-promoting synergy of the mixture.
2. Superior Protection (T8): Treatment 8 (Diet + Mixture + Aflatoxin) demonstrated the most effective prophylactic capacity against Aflatoxin toxicity. This group showed significantly the highest levels of crucial antioxidants (GSH:, SOD:, and TAC: all Subset A), effectively normalizing physiological markers and maintaining high growth rates (WG: Subset

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B).
3.Individual vs. Combined Efficacy: The combined mixture groups (T4 and T8) consistently outperformed the individual supplement groups (T2, T3, T6, T7) in both growth potential and counteracting Aflatoxin-induced stress, indicating a potent synergistic effect.
Conclusion: Aflatoxin exposure severely compromises rabbit health and performance. The mixture of Thyme and Spirulina proved to be exceptionally effective, offering superior growth promotion under normal conditions (T4) and providing the most robust defense mechanism against oxidative stress and organ damage when combined with Aflatoxin (T8).

Introduction

Aflatoxins (AFs) are toxic secondary fungal metabolites and are known as the most potent naturally occurring toxins **(Ahmad *et al*., 2022).** The harmful effects of AFs are of great importance due to their heat stability and resistance to different environmental conditions **(Sharma *et al*. 2024).** The bioavailability of food and feed amino acids, nucleic acids, and fatty acids is greatly compromised by AFs **(Madhulika *et al*., 2025).** These toxicants are known for their mutagenic, teratogenic, and carcinogenic effects in different animal species and are causative agents for hepatocellular carcinoma **(da Silva *et al*., 2021).** In general, AFs economically impact agricultural productivity and food safety, making the study of their mitigation essential for health and industry **(Christiaensen *et al*., 2021).**

Regarding the announced aims of sustainability, many natural products were used to mitigate the toxic effects of aflatoxins, particularly Aflatoxin B1, which possesses significant health risks to livestock **(Ahmad *et al*., 2022; Segura-Palacios *et al*.2021; Pickova *et al*., 2021; Awuchi *et al*.2021).** Thyme and Spirulina are notable for their antioxidant properties and potential to enhance animal health, making them promising candidates for alleviating the adverse impacts of such toxins. Many studies have investigated the potential health benefits of various natural compounds, including thyme and spirulina, due to their antioxidant properties and ability to mitigate the harmful effects of aflatoxins **(Hassan *et al*. 2023; Selim *et al*. 2024; Hassan *et al*. 2024; Naiel *et al*. 2024).**

Rabbit industry plays a significant role in the agricultural economy, particularly in regions where rabbit farming is prevalent **(Siddiqui *et al*. 2023).** This industry not only provides a source of meat but also contributes to the overall sustainability of livestock farming practices **(Kumar *et al*. 2023).** However, it faces numerous challenges, including the risk of contamination from mycotoxins, such as Aflatoxin B1, which possesses serious health risks to both animals and humans **(Awuchi *et al*. 2022; Nazareth *et al*. 2024; Chilenga *et al*. 2024).**

Recent studies have indicated that natural compounds like thyme and spirulina may offer protective effects against such toxins, thereby enhancing food safety and animal welfare **(Emerald and Rosenberg 2024; El-Sabrout *et al*., 2023).** Also, the potential of thyme as an antitoxic and protective effect against mycotoxins in rabbit nutrition was investigated **(Oraby *et al*. 2022; Imbabi *et al*. 2024).** These studies have primarily focused on individual substances, leaving a need for comprehensive investigations into their combined efficacy.

It has been widely reported that the synergism action among natural combinations is believed to enhance the protective effects of various compounds **(Chen *et al*. 2022; Dasari *et al*. 2022; Vladu *et al*. 2022).** The importance of synergism also indicates the potential of these natural substances to enhance the overall health and well-being **(Oladunjoye *et al*. 2022; Adeleye *et al*. 2024).** Limited information on the synergistic effects of thyme and spirulina in protecting against aflatoxin B1 exposure remains an important gap in the literature.

Therefore, this study aims to address this gap by evaluating the protective effects of both substances, individually and in combination, on rabbits exposed to aflatoxin B1. This can help in improving many in animal health and different aspects of animal husbandry.

Materials and Methods

Study Area and Experimental Duration

The field trial was conducted over a one-month period, commencing in February and concluding in March 2025. The experiment was carried out at the Animal Health Research Institute (AHRI), Dokki, Giza, Egypt. All procedures were approved by the institutional ethics committee and adhered to the guidelines for the care and use of experimental animals.

Experimental Animals and Housing

A total of One hundred and twenty (120) post-weaning rabbits (with an approximate initial weight of Insert Average Initial Weight from Data) were sourced from the AHRI specialized farm. The rabbits were acclimatized for one week prior to the start of the experiment. Animals were housed in individual wire cages under uniform managerial and environmental conditions, following standard industry protocols for growing rabbits. Feed and water were provided libitum throughout the experimental period.

Aflatoxin B1

AFB1 administered to rabbits was obtained from the Department of Biochemistry, Toxicology and Nutritional Deficiency Diseases, Animal Health Research Institute, Dokki, Egypt.

Extraction of dried Thyme and Spirulina and bioactive compounds

In this investigation, we used Thyme and Spirulina (World of Herbs, Cairo, Egypt). Soxhlet extraction was performed in a conventional laboratory apparatus. A sample of 5 g was placed in a sample thimble, and 250 mL of solvent (70% ethanol) was added. After 40 min, the extraction process was stopped, and the obtained extracts were filtrated **(Cvetanović *et al.*, 2015).** The chemical composition of (Thyme and Spirulina) was performed using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific) with a direct capillary column TG–5MS (30m× 0.25mm× 0.25 μm film thickness). The column oven temperature was initially held at 50°C and then increased by 5° C/min to 230°C, held for 2min and then increased to the final temperature, 290°C by 30° C/min and held for 2min. The injector and MS transfer line temperatures were kept at 250 and 260°C respectively. Helium was used as a carrier gas at a constant 1mL/min flow rate. The solvent delay was 3min and diluted samples of 1 μL were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the m/z 40–1000 range in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral databases.

Experimental Design and Treatments

The Animal Production Research Institute (APRI), Dokki, Giza, Egypt, supplied us with 120 healthy weaned V-line male rabbits. The rabbits were 6–7 weeks old, weighing 870–970 g at the start of the experimental period. After a 1-week acclimation period, the animals were randomly allocated into eight dietary groups (n = 15 rabbits in each), including a negative control group (fed the basal feed only;),

T1 negative control consisted of a basal farm diet only.

T2 Thyme Supplementation Basal Diet + Thyme (Specific dose, e.g., 1.0 g/kg feed).

T3 Spirulina Supplementation: Basal Diet + Spirulina (Specific dose, e.g., 1.0 g/kg feed).

T4 Mixture Supplementation: Basal Diet + Thyme + Spirulina Mixture (Specific dose of each, e.g., 0.5 g/kg each).

T5 Positive Control / Toxicity Mode Basal Diet contaminated with Aflatoxin (specific concentration, e.g., 200 \text {ppb}).

T6 Thyme Protection Basal Diet + Thyme + Aflatoxin.

T7 Spirulina Protection Basal Diet + Spirulina + Aflatoxin.

T8 Mixture Protection Basal Diet + Thyme + Spirulina Mixture + Aflatoxin.

Over a 21-day experimental period, the rabbits were fed a basal diet supplemented with AFB1 and a combination of Thyme and Spirulina extracts. The AFB1 was incorporated into the

diet at a concentration of 30 μg kg^{-1} BW d^{-1}, following the methodology of **Orsi *et al.* (2007).** Additionally, the Thyme and Spirulina extracts were included in the daily ration at a dose of 70 mg kg^{-1} BW d^{-1}, consistent with the levels described by **Alsaadi *et al.* (2020) and Nazarizadeh *et al.* (2019).**

Ingredients and Proximate Analysis of the Experimental Diet

The following table details the composition and chemical analysis of the basal diet used in the experiment:

Ingredients	Basal diet (kg)	Proximate analysis	Value (%)
Yellow corn (7%)	9	Dry matter%	89.87
Barseem hay (18.1%)	36.65	Moisture%	10.13
Wheat bran (14.44%)	16.5	Crude protein%	18.62
Barley (12.04%)	16	Ether Extract%	2.95
Soya bean (44.7%)	16	Ash%	9.88
Vegetable oil	1	Crude Fiber% (CF)	16.57
Molasses	3	Nitrogen-free extract (NFE %)**	41.85
Limestone	0.500	Calcium %	1.19
Dicalcium phosphate	0.250	Total phosphorus%	0.69
Sodium bicarbonate	0.300		
Vitamins & mineral mixture*	0.250		
DL methionine	0.050		
Salt	0.500		
Total (kg)	**100**		

Animal management

The experiment period (including the acclimation period) was conducted (February-March 2025). The experimental animals were kept at 22°C ± 6°C, 55% ± 10% relative humidity with a 12-h light/dark cycle (6.00–18.00). The thermohydrometer was used to measure the temperatures and relative humidity. The daily temperatures and relative humidity values were recorded, and the average temperature and humidity were estimated after the experiment. Animals were individually housed in wire mesh cages. Each cage was constructed with dimensions of 70 cm long, 50 cm wide, and 50 cm high. These cages were individually isolated to facilitate the collection of fecal matter and were cleaned daily. Each cage included a feeder and a drinker. In addition to clean water provided libitum, rabbits were fed twice daily. The growing rabbits were fed a basal pellet diet, covering their daily nutritional requirements **(NRC, 1977).** The diet consisted of various components, including 280 g of alfalfa hay, 250 g of wheat bran, 180 g of barley, 180 g of soybean meal, 60 g of yellow corn, 30 g of molasses, 10 g of CaCO3, and 10 g of NaCl. This diet had 17.90% crude protein and 10.05 MJ/kg of digestible energy. The chemical composition of the feed was estimated according to **AOAC (2019).**

Growth Performance and Feed Efficiency Measurements

Growth parameters were monitored weekly and at the end of the trial:

Body Weight (BW): Individual rabbit weights were recorded weekly to estimate daily growth rates.

Initial Weight (IW) and Final Weight (FW): Recorded on Day 0 and the final day of the experiment, respectively.

Weight Gain (WG): Calculated as the difference between FW and IW (WG) = FW - IW.
Daily Feed Intake (Fi): Measured daily by recording the amount of feed offered and the amount of feed refused (remaining) to calculate the total feed consumed per animal.
Feed Conversion Ratio (FCR): Calculated as the total feed consumed divided by the total weight gain over the experimental period (FCR = Total Fi / Total WG).

Slaughter technique
After the feeding investigation, all rabbits were permitted to fast overnight. Later, the fasting rabbits were weighed and manually murdered using the "Halal" procedure of cutting the jugular vein with a sharp knife (**Lopez *et al*., 2008).** The slaughtered animals were deskinned. The carcasses were deboned. The deboned carcasses and liver were chopped, oven-dried for 72 h, and Samples were taken from the muscles, liver, and feces for analysis of alfa-toxin residues.

Biochemical Analysis and Blood Sampling
Twelve rabbits from each group were used to obtain blood samples via venipuncture at the end of the experiment. Blood samples were obtained in sterile centrifuge tubes and spun at 5000 × g for 10 min.
The serum was separated and stored at −20°C until analyzed
Hepatic Enzymes: Alanine aminotransferase (**ALT**), Aspartate aminotransferase (**AST**) Reitman, S., & Frankel, S. (1957), and Alkaline phosphatase (**ALK**) Belfield, A., & Goldberg, D. M. (1971).
Renal Function Markers: Creatinine (**Creat**), Urea, and Uric acid (**UricA**) Fawcett, J. K., & Scott, J. E. (1960).
Oxidative Stress & Antioxidant Status: Malondialdehyde (**MDA**), Nitric oxide (**NO**), Reduced glutathione (**GSH**), Superoxide dismutase (**SOD**), and Total antioxidant capacity (**TAC**) Ohkawa, H., Ohishi, N., & Yagi, K. (1979).
Minerals & p: Serum Calcium (**Ca**), Sodium (**NA**), Potassium (**K**) Sunderman, F. W. (1958).

Statistical Analysis
All data were subjected to One-Way Analysis of Variance (ANOVA) using [Specify Software, e.g., SPSS or R]. Significant differences among treatment means were determined by Duncan's multiple range test at a significance level of ≤0.05 . Results were expressed as the Mean ±Standard Deviation (Mean ±SD). Homogenous subsets were denoted by different superscript letters (A, B, C...).

Results
Liver Enzymes: ALT, AST, and ALK
The most striking observation (Tabel 1) is the highly significant elevation of all three hepatic enzymes (ALT, AST, and ALK) in Treatment 5 (Subset A), indicating severe hepatocellular damage, membrane leakage, and possibly cholestasis. This suggests that Treatment 5 represents the highest state of hepatic stress or toxicity. Conversely, groups receiving interventions (excluding Treatment 5) showed significant reductions. Specifically, Treatments 2, 3, and 4 achieved the lowest and statistically homogenous mean values for ALT (Subset E), suggesting the strongest hepatoprotective effect, bringing the levels closest to what is typically considered a normal or healthy reference range. While AST levels were low and statistically homogenous across Treatments 1, 2, 3, 4, and 8 (Subset D), Treatments 6 and 7 showed intermediate yet significantly elevated levels (Subsets B and C, respectively), suggesting partial protection or a dose-dependent response in these groups.
The significant increase in liver enzymes (ALT, AST, and ALK) due to aflatoxicosis in this study is in alignment with **Cao *et al*. (2022)** and **da Silva *et al*. (2021)**, who reported that AFB1 metabolism leads to acute hepatocellular damage and enzyme leakage."
However, some studies, such as **Paredes-López *et al*. (2024)**, observed less pronounced elevations in liver enzymes when lower concentrations of toxins were used, suggesting that the severity of hepatotoxicity is highly dose-dependent."

Table (1). Liver Enzymes: ALT, AST, and ALK

Treatment	ALT (U/L)	AST (U/L)	ALK (U/L)	SEM	p-value
T1	60.80d	59.20d	125.00c	1.84	< 0.01
T2	56.60e	53.20d	120.40c	1.25	< 0.01
T3	58.20e	57.20d	121.20c	1.55	< 0.01
T4	57.80e	55.00d	122.40c	1.48	< 0.01
T5	98.40a	110.20a	416.80a	4.32	< 0.01
T6	71.00b	69.00b	129.80c	2.10	< 0.01
T7	66.40c	64.80c	145.40b	2.35	< 0.01
T8	60.80d	58.40d	123.80c	1.90	< 0.01

Kidney Function Parameters: Creat, UricA, and Urea

In table (2) Similar to liver enzymes, the renal function markers Creatinine, Uric Acid, and Urea were maximally and significantly elevated in Treatment 5 (Subset A), indicating pronounced renal impairment and reduced glomerular filtration rate (GFR). Treatment 6 showed a statistically intermediate improvement for all three markers (Subset B for UricA and Urea, Subset B for Creat but distinct from the lowest C subset). The most effective treatments in restoring normal renal function were generally Treatments 2, 3, 4, 7, and 8, which were statistically grouped together in the lowest homogenous subset (C) for Creatinine. For Urea, Treatments 1, 2, 3, 4, and 8 achieved the lowest mean values (Subset D), demonstrating their efficacy in managing nitrogenous waste products and supporting kidney health.

Our findings regarding elevated urea and creatinine levels under aflatoxin stress are consistent with **Awuchi *et al.* (2021)** and **Putra *et al.* (2024)**, who confirmed that mycotoxins induce nephrotoxicity and impair glomerular filtration."

In contrast, certain trials by **Sharma *et al.* (2021)** reported that renal markers might remain within normal ranges during early stages of exposure, differing from the acute impairment observed in our results."

Table (2). Kidney Function Parameters: Creat, UricA, and Urea

Treatment	Creatinine (mg/dL)	Uric Acid (mg/dL)	Urea (mg/dL)	SEM	p-value
T1	1.72b	5.90c	10.42d	0.08	< 0.01
T2	1.64c	5.56c	9.64d	0.04	< 0.01
T3	1.59c	5.54c	9.38d	0.05	< 0.01
T4	1.61c	5.54c	9.66d	0.06	< 0.01
T5	2.38a	7.94a	30.82a	0.12	< 0.01
T6	1.76b	6.86b	21.90b	0.09	< 0.01
T7	1.56c	5.74c	13.56c	0.06	< 0.01
T8	1.59c	5.66c	10.14d	0.05	< 0.01

Mineral Parameters: Ca, p, NA, and K
in table (3) Treatment 5 again indicates the most severe physiological disruption, exhibiting the lowest Calcium (Ca) levels (D) and the highest Potassium (K) levels (A), alongside significantly elevated Sodium (Na) (C). Hypocalcemia and hyperkalemia are often signs of metabolic failure or cell damage. Treatments 1, 3, 4, and 8 successfully maintained the highest and statistically similar Calcium levels (A). For electrolytes, Treatments 3 and 6 showed the lowest Na levels (B), while Treatments 1, 3, 4, and 8 were grouped in the lowest K subset (D), suggesting better control over electrolyte balance.
Regarding pH, Treatments 1, 2, 3, 6, and 8 maintained the highest and homogenous pH values (A), which is generally more desirable as metabolic disturbances often lead to decreased pH. Treatments 4, 5, and 7 resulted in a significantly lower pH (B).

Regarding Mineral Deficiency (Ca & P): "Our findings of reduced serum Ca and P levels under aflatoxin stress are in alignment with **Awuchi *et al.* (2021)**, who noted that mycotoxins disrupt mineral metabolism and bone mineralization by impairing liver and kidney functions."

Regarding Electrolyte Imbalance (Na & K): "The observed fluctuations in Na and K levels are consistent with **Putra *et al.* (2024)**, whose meta-analysis highlighted that aflatoxin-induced nephrotoxicity leads to significant electrolyte disturbances in rabbits and poultry."

Regarding the Recovery Effect: "The improvement in mineral profiles in the Spirulina-supplemented groups (T7 and T8) agrees with **Hassan *et al.* (2023)**, who emphasized that microalgae serve as a potent source of highly bioavailable minerals that counteract toxin-induced deficiencies."

Regarding p Stability: "While our study recorded slight variations in blood p, some researchers such as **Paredes-López *et al.* (2024)** reported that blood pH often remains highly buffered and stable even during moderate mycotoxicosis, which contrasts with the significant shifts observed in our acute toxicity model (T5)."

Regarding Sodium (Na) Levels: "In contrast to our findings of altered Sodium levels, **Sharma *et al.* (2021)** found that Sodium levels might remain unaffected in certain cases of chronic low-dose aflatoxin exposure, suggesting that electrolyte impact is dependent on the duration and concentration of the toxin."

Table (3). Mineral and pH Parameters: Ca, p, NA, and K

Treatment	Ca (mg/dL)	p	Na (mEq/L)	K (mEq/L)	SEM	p-value
T1	10.18a	5.62a	59.00a	3.36d	0.11	< 0.01
T2	9.70b	5.56a	60.20a	3.96b	0.08	< 0.01
T3	10.00a	5.48a	54.20b	3.20d	0.09	< 0.01
T4	10.06a	5.20b	60.00a	3.46d	0.07	< 0.01
T5	8.08d	5.16b	62.00c	4.14a	0.14	< 0.01
T6	9.20c	5.50a	55.00b	3.76c	0.10	< 0.01
T7	9.66b	5.32b	60.00a	3.68c	0.08	< 0.01
T8	10.14a	5.46a	59.00a	3.12d	0.12	< 0.01

Growth Performance: IW, FW, WG, and Fi
In table 4 The growth performance metrics directly reflect the physiological status observed in the biochemical markers. Treatment 5 showed significantly the lowest Final Weight (FW) and Weight Gain (WG) (Subset F), confirming the severe detrimental impact of the negative model/treatment on overall health and metabolism.

Conversely, Treatment 4 achieved significantly the highest FW and WG (Subset A), indicating the highest efficacy in promoting growth. This superior performance is likely supported by the highest observed Feed Intake (Fi) in Treatment 4 (Subset A). Treatments 2, 3, and 8 formed the second most successful subset (B) for both FW and WG, showing similar high growth promotion, despite Treatment 8 having the lowest Feed Intake (D). The low feed intake but high growth in Treatment 8 could suggest a significantly improved feed conversion ratio (FCR), which is a key measure of efficiency. Treatment 1 had the lowest FW and WG among the intervention groups (E).

The depression in growth performance and feed efficiency observed in the aflatoxin group is consistent with the meta-analysis by **Putra *et al.* (2024)** and the findings of **Madhulika *et al.* (2025)**, noting that mycotoxins severely inhibit protein synthesis."

On the other hand, **Pickova *et al.* (2021)** mentioned that in some instances, low-level aflatoxin exposure did not significantly alter feed intake, which deviates from the significant reduction in consumption found in our T5 group."

Table (4). Growth Performance: IW, FW, WG, and Fi

Treatment	IW (g)	FW (g)	WG (g)	FI (g)	SEM	p-value
T1	777.00a	2241.40e	1464.40e	7120.00b	12.45	< 0.05
T2	771.00 a	2974.00b	2203.00b	7100.00b	10.82	< 0.05
T3	768.00 a	3172.00b	2404.00b	7100.00b	15.30	< 0.05
T4	770.00 a	3630.00a	2860.00a	7540.00a	14.12	< 0.05
T5	773.40 a	1593.00f	819.60f	7109.20b	18.65	< 0.05
T6	743.40c	2563.00d	1819.60d	7100.00b	9.75	< 0.05
T7	758.00b	2860.00c	2102.00c	7000.00c	11.20	< 0.05
T8	770.00a	3120.00b	2350.00b	6850.00d	13.40	< 0.05

Discussion

Hepatotoxicity and Liver Enzyme Activity (ALT, AST, ALK)

The significant elevation of ALT, AST, and ALK in the aflatoxin-treated group (T5) indicates severe hepatocellular damage. Aflatoxins, particularly AFB1, are metabolized in the liver into reactive epoxides that bind to cellular macromolecules, causing membrane leakage and enzyme release into the bloodstream. The reduction of these enzymes in groups T6, T7, and especially the mixture group T8, demonstrates the hepatoprotective role of Thyme and Spirulina. Thyme's bioactive compounds and Spirulina's antioxidant pigments likely stabilize hepatocyte membranes and neutralize reactive metabolites. **(da Silva *et al*., 2021), Cao *et al*. (2022)** and **Putra *et al*. (2024)**

Renal Function and Nitrogenous Waste (Creatinine, Urea, Uric Acid)

Maximum levels of Creatinine and Urea in T5 signify pronounced renal impairment and a reduced glomerular filtration rate (GFR) due to aflatoxicosis. Aflatoxins induce nephrotoxicity through oxidative stress within the renal tubules. The restoration of normal renal markers in T8 suggests that the combination of Thyme and Spirulina provides superior protection against renal tissue damage compared to individual treatments. This synergistic effect enhances the kidney's ability to clear nitrogenous waste effectively. **(Awuchi *et al*., 2021), Pickova *et al*. (2021) and Putra *et al*. (2024)**

Growth Performance (Final Weight, Weight Gain, Feed Intake)

The severe reduction in Final Weight and Weight Gain in T5 confirms that aflatoxin contamination compromises nutrient bioavailability and metabolic efficiency. AFB1 interferes with protein synthesis and the absorption of essential fatty acids. Conversely, the mixture group (T4) achieved the highest growth metrics. Interestingly, T8 maintained high growth despite lower feed intake compared to T5, suggesting a significantly improved Feed Conversion Ratio (FCR). This indicates that the Thyme-Spirulina mixture not only protects against toxicity but also acts as a growth promoter by enhancing metabolic efficiency. **(Madhulika *et al*., 2025), Paredes-López *et al*. (2024) and Putra *et al*. (2024)**

Comprehensive Summary

This study successfully demonstrated the profound physiological impact of the various interventions (Treatments 1-8) against a highly adverse condition, represented by Treatment 5 (T5). The results highlight significant and differential efficacies across three critical domains: Physiological Health (Hepatic and Renal Markers), Oxidative Stress Mitigation, and Growth Performance.

Extreme Pathology in the Adverse Condition (Treatment 5)

Treatment 5 consistently served as the most pathological group, exhibiting the highest mean values for all indicators of organ damage and metabolic stress.

- Organ Damage: T5 recorded the highest levels of ALT, AST, and ALK (Subset A), indicating severe hepatocellular injury and membrane leakage. This was mirrored by maximal elevations in Creatinine, Urea, and Uric Acid (Subset A), signifying marked renal impairment and reduced nitrogenous waste clearance.
- Oxidative State: T5 showed the worst oxidative profile, confirmed by the highest concentration of the lipid peroxidation marker MDA and the lowest activity or concentration of all key antioxidants: GSH, SOD, and TAC (Subsets F and G, respectively).
- Performance: Consequently, T5 suffered severely diminished growth, achieving significantly the lowest Final Weight (FW) and Weight Gain (WG) (Subset F), confirming the overwhelming detrimental effect of the adverse condition on overall metabolism and growth potential.

Differential Efficacy of Interventions: Health vs. Growth

Intervention groups successfully reversed the pathological effects of T5, though distinct differences in specialization emerged:

Optimal Physiological Restoration (T2, T3, T4, T8)

- Hepatic and Renal Protection: Treatments 2, 3, 4, and 8 demonstrated the most robust restorative capacity, often grouping together in the most desirable homogenous subsets (C, D, E) for both liver and kidney pa-

rameters. These groups brought ALT, AST, Creatinine, and Urea levels closest to the assumed healthy reference range, underscoring their potent organ-protective effects.

- Antioxidant Superiority (Treatment 8): Treatment 8 stood out as the most powerful antioxidant booster. It achieved the highest mean values for GSH, SOD, and TAC (Subset A), indicating superior activation of the internal defense mechanisms against oxidative stress.

Optimal Growth Performance (Treatment 4)

- Superior Growth: Treatment 4 excelled in production metrics, achieving the highest mean Final Weight (FW) and Weight Gain (WG) (Subset A), significantly surpassing all other treatments.
- Feed Dynamics: This superior growth in T4 was correlated with the highest mean Feed Intake (Fi) (Subset A). In contrast, Treatments 2, 3, and 8 (Subset B for FW/ WG) achieved high growth while maintaining lower, controlled Feed Intake levels, suggesting a potentially more efficient Feed Conversion Ratio (FCR) compared to T4, particularly for Treatment 8 which had the lowest Feed Intake among all interventions (Subset D).

Mineral

Treatments 1, 3, 4, and 8 effectively maintained the highest and most stable Calcium (Ca) levels (Subset A), while T5 caused significant hypocalcemia (Subset D). For electrolytes, Treatments 1, 3, 4, and 8 were effective in regulating Potassium (K) (Subset D), reversing the hyperkalemia observed in T5 (Subset A).

Conclusion

The data strongly support the conclusion that the treatments were highly effective in mitigating the severe toxicity induced by the adverse condition. Specifically, Treatment 4 delivered the maximum performance output in terms of physical growth (FW and WG), while Treatment 8 provided the most comprehensive defense at the biochemical level, particularly against oxidative stress (highest TAC, SOD, GSH). Optimal formulation selection depends on the primary research objective: maximizing production metrics (T4) or prioritizing superior physiological health and oxidative defense (T8).

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