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Scientific field evaluation report

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Abstract

This field investigation evaluated mycotoxin exposure in poultry breeder flocks receiving **B.I.O.Tox®**, a mycotoxin-binding feed additive manufactured by Biochem, Germany.

Two feed samples showed substantial T-2 toxin contamination (601 and 620 µg/kg) and lower-level fumonisin contamination (7.65 and 4.94 mg/kg). In the corresponding blood analyses of breeder flocks at 34 and 56 weeks of age, neither toxin was detected.

Despite the absence of detectable circulating toxin, abnormalities were present in selected serum biochemical indicators. Direct bilirubin was elevated in both age groups, ALT was elevated particularly at 56 weeks, and creatinine and blood urea nitrogen (BUN) were markedly higher in the older birds.

Collectively, these observations are compatible with reduced systemic mycotoxin exposure alongside continued organ-level biochemical stress. Because this was a field diagnostic assessment without a contemporaneous untreated contaminated-feed control group, the results should be interpreted as *supportive field evidence* rather than a controlled quantitative determination of B.I.O.Tox® binding efficacy

Keywords: B.I.O.Tox; Mycotoxin binder; Poultry breeders; T-2 toxin; Fumonisin; Mycotoxin exposure; Blood mycotoxin; Serum biochemistry; Hepatic stress; Renal and metabolic indicators

Introduction

Mycotoxin contamination remains an important challenge in poultry production because multiple toxins may occur simultaneously and negatively influence liver function, kidney function, intestinal integrity, immunity, feed efficiency, and reproduction and production performance. A toxin binder is intended primarily to interact with susceptible mycotoxins within the gastrointestinal tract, thereby reducing their bioavailability and subsequent systemic exposure. An appropriate field evaluation should not rely solely on the concentration of toxin present in feed. Ideally, an assessment combines feed mycotoxin concentration, validated biomarkers of systemic exposure, serum biochemical indicators of organ response, pathological or histopathological findings where available, and productive performance.

Objective

The objective of this field investigation was to assess whether observations obtained from feed and blood analysis were consistent with a protective effect during the use of B.I.O.Tox® under naturally contaminated commercial poultry conditions. A secondary objective was to determine whether serum biochemical indicators suggested residual hepatic or renal/metabolic stress despite the absence of detectable circulating toxin in the submitted blood samples.

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Analytical Results

Parameter	Feed Report 1	Feed Report 2	Blood 34 wk	Blood 56 wk	Scientific interpretation
T-2 toxin	601 µg/kg	620 µg/kg	Not detected	Not detected	High dietary exposure; no detectable circulating T-2 in submitted samples
Fumonisin	7.65 mg/kg	4.94 mg/kg	Not detected	Not detected	Dietary exposure confirmed; no detectable circulating fumonisin in submitted samples
Direct Bilirubin	-	-	0.56 mg/dL	0.32 mg/dL	Elevated per reference range used in original diagnostic report
ALT (SGPT)	-	-	37 IU/L	55 IU/L	Greater biochemical alteration in older birds
Creatinine	-	-	0.16 mg/dL	0.37 mg/dL	Higher value in 56-week birds
BUN	-	-	4.69 mg/dL	13.24 mg/dL	Marked increase in 56-week birds

Feed Contamination in Regulatory Context

Comparing the measured feed concentrations against European Union guidance levels for poultry feedingstuffs

adds an important layer of interpretation that concentration values alone do not convey.

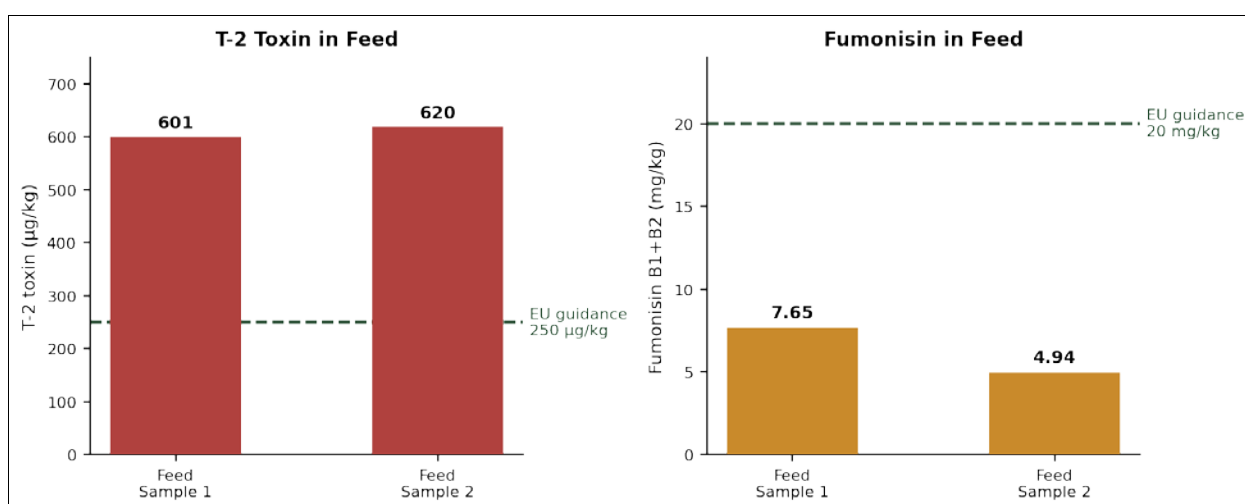


Fig 1: Feed T-2 toxin and fumonisin concentrations relative to EU guidance levels for poultry (Commission Recommendations 2013/165/EU and 2006/576/EC).

Toxin	Measured range	EU guidance level poultry	Assessment
T-2 + HT-2 toxin	601–620 µg/kg	250 µg/kg (complete feed)	~2.4–2.5× guidance level genuinely high exposure
Fumonisin B1+B2	4.94–7.65 mg/kg	20 mg/kg (complete feed)	Within guidance level, though still a measurable co-exposure

This distinction matters: the feed challenge in this field case was driven predominantly by T-2 toxin, which substantially exceeded EU guidance, while fumonisin though present remained within the guidance range. The exposure is best characterised as a T-2–dominant, multi-mycotoxin challenge rather than a case of severe combined overload on both toxins.

Interpretation of Feed Mycotoxin Challenge

T-2 Toxin

The two feed samples contained 601 µg/kg and 620 µg/kg T-2 toxin, roughly 2.4–2.5 times the EU guidance level of 250 µg/kg for poultry complete feed. These concentrations demonstrate a substantial dietary T-2 challenge in the sampled feeds. T-2 toxin is a type-A trichothecene associated with oxidative stress, gastrointestinal epithelial injury, impaired protein synthesis, immunological effects and reduced productive performance.

Fumonisin

Fumonisin concentrations were 7.65 mg/kg and 4.94 mg/kg below the EU guidance level of 20 mg/kg for poultry, meaning fumonisin exposure alone would not typically be flagged as a primary concern under current guidance. The first sample nonetheless represented a higher fumonisin exposure than the second, and its co-occurrence with high T-2 toxin

indicates the birds were exposed to a multi-mycotoxin challenge rather than an isolated single-toxin challenge. Combined low-level fumonisin exposure alongside a dominant T-2 challenge can still contribute to cumulative gastrointestinal and hepatic stress even when each toxin is considered individually within guidance.

Blood Mycotoxin Findings

An important observation was that T-2 toxin and fumonisin were reported as not detected in blood samples from both the 34-week and 56-week breeder groups, despite their presence in feed. This feed-to-blood contrast is biologically relevant and is consistent with reduced systemic bioavailability during toxin-binder supplementation. Scientifically, however, 'not detected' means the analyte concentration was below the analytical method's detection capability in that particular sample it should not automatically be interpreted as complete absence of systemic exposure. For stronger confirmation in future work, the analytical method, Limit of Detection (LOD), Limit of Quantification (LOQ), sample timing relative to feeding, and toxin-specific metabolites should be documented.

Serum Biochemistry: Evidence of Biological Response

While direct toxin measurement investigates systemic

exposure, biochemical markers investigate the physiological

consequences associated with exposure and recovery.

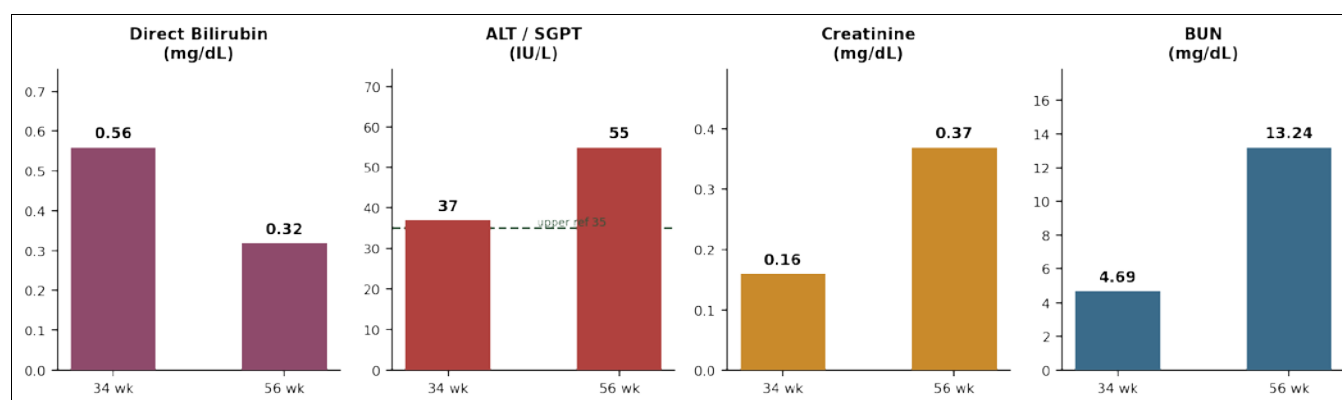


Fig 2: Serum biochemical indicators at 34 and 56 weeks of age.

Direct Bilirubin

Direct bilirubin was 0.56 mg/dL at 34 weeks and 0.32 mg/dL at 56 weeks. Both were classified as high according to the reference range applied in the original diagnostic report. The elevated values are compatible with altered hepatobiliary handling and warrant interpretation together with other hepatic indicators; bilirubin alone is not specific for mycotoxicosis.

ALT / SGPT

ALT values were 37 IU/L at 34 weeks and 55 IU/L at 56 weeks, against a stated reference range of 5–35 IU/L in the original report. The greater elevation in the older flock suggests a greater degree of biochemical disturbance. In birds, ALT is best interpreted alongside additional hepatic markers and pathology rather than as a stand-alone quantitative marker of toxin-binder efficacy.

Renal / Metabolic Indicators

Creatinine increased from 0.16 mg/dL at 34 weeks to 0.37 mg/dL at 56 weeks more than double. BUN increased from 4.69 mg/dL to 13.24 mg/dL nearly a threefold rise. The original diagnostic assessment identified greater renal/metabolic stress in the older birds on this basis. In poultry, uric acid is generally a more physiologically relevant indicator of renal function and nitrogen metabolism than BUN or creatinine, both of which are more established in mammalian clinical pathology. Future studies should therefore preferably include uric acid, phosphorus, electrolytes and kidney gross pathology/histopathology alongside these markers.

Integrated Interpretation of B.I.O.Tox® Performance

Level 1: Exposure

Feed analysis confirmed a T-2–dominant, multi-mycotoxin challenge: T-2 601–620 µg/kg (~2.4–2.5× EU guidance) and fumonisin 4.94–7.65 mg/kg (within EU guidance).

Level 2: Systemic Bioavailability

Despite dietary contamination, the tested toxins were reported as not detected in blood at both breeder ages compatible with reduced systemic exposure during B.I.O.Tox® supplementation.

Level 3: Biological Consequences

Elevated bilirubin and ALT, and particularly higher creatinine/BUN in older birds, indicate that physiological

stress was still present. Reduced absorption does not imply instantaneous organ recovery

Scientific Assessment of B.I.O.Tox® Efficacy

Scientifically defensible interpretation: The concurrent finding of substantial T-2 toxin contamination in feed alongside non-detectable concentrations in the submitted blood samples is consistent with reduced systemic mycotoxin exposure during B.I.O.Tox® supplementation. Serum biochemical abnormalities indicate that hepatic and metabolic/renal stress remained detectable, particularly in older breeder birds.

The findings provide supportive field evidence of a protective effect, but they should not be interpreted as proof of a specific percentage adsorption or protection. An efficacy statement such as "85–95% binding efficacy" cannot be calculated from the present dataset without an appropriately controlled trial.

Value of Serum Biochemistry in Toxin-Binder Evaluation

- **Feed toxin analysis** → establishes dietary challenge.
- **Blood toxin/metabolite analysis** → estimates systemic exposure.
- **Serum biochemistry** → assesses physiological response.
- **Histopathology / organ scoring** → confirms tissue-level injury.
- **Performance parameters** → measure commercial consequences.

Serum biochemistry should therefore be regarded as an important component of a multi-level toxin-binder efficacy assessment, rather than as a stand-alone measurement of adsorption percentage.

Recommended Biomarker Panel for Future B.I.O.Tox® Field Studies

Mycotoxin Exposure

- Feed multi-mycotoxin LC-MS/MS
- T-2 + HT-2
- Fumonisin B1 + B2
- DON
- ZEA
- OTA
- AFB1

Liver Panel

- AST
- GLDH

- GGT
- Total / direct bilirubin, where validated
- Total protein
- Albumin
- Globulin
- Cholesterol / triglycerides
- Validated oxidative-stress biomarkers

Kidney / Metabolic Panel

- Uric acid
- Phosphorus
- Electrolytes
- Creatinine (supplementary)
- BUN (supplementary)

Tissue Assessment

- Liver score
- Liver histopathology
- Kidney histopathology
- Intestinal morphology
- Bursa of Fabricius evaluation

Performance

- Mortality
- Feed intake
- Body weight
- FCR
- Egg production
- Egg weight
- Fertility
- Hatchability

Recommended Controlled Trial Design

Group	Feed condition
Group 1 Negative Control	Low-mycotoxin feed without B.I.O.Tox®
Group 2 Positive Control	Mycotoxin-contaminated feed without B.I.O.Tox®
Group 3 Treatment	Same contaminated feed + B.I.O.Tox®
Group 4 (optional)	Higher / alternative B.I.O.Tox® dose

Samples should be collected at baseline and at predetermined intervals. The strongest evidence would compare a contaminated control showing increased systemic biomarkers and altered biochemical/histological parameters against a contaminated + B.I.O.Tox® group showing significantly reduced systemic biomarkers and protection or normalisation of biochemical, pathological and productive endpoints.

Overall Conclusion

The present commercial breeder observations demonstrate a clear feed–blood–organ response pattern. Feed samples contained substantial T-2 toxin (601–620 µg/kg, well above EU guidance) together with a lower-level fumonisin co-exposure (4.94–7.65 mg/kg, within EU guidance), confirming a genuine T-2–dominant dietary mycotoxin challenge. Nevertheless, T-2 toxin and fumonisin were reported as not detected in the corresponding blood samples.

This observation is consistent with reduced gastrointestinal bioavailability and systemic exposure during the use of B.I.O.Tox®. At the same time, elevated bilirubin and ALT, and increased renal/metabolic indicators particularly in 56-week breeders show that absence of detectable circulating

toxin should not automatically be equated with complete physiological recovery.

Scientific Conclusion

- Under the conditions of this field investigation, B.I.O.Tox® supplementation was associated with non-detectable blood concentrations of the investigated mycotoxins despite measurable dietary contamination.
- The findings are compatible with a reduction in systemic mycotoxin exposure.
- Residual alterations in serum biochemical parameters indicate ongoing or recovering hepatic and metabolic stress, and emphasize the value of combining feed analysis, validated exposure biomarkers, serum biochemistry, pathology and productive performance when evaluating mycotoxin-management strategies.
- Because an untreated contaminated-feed control was not included, these observations should be classified as supportive field evidence rather than a controlled quantitative efficacy trial.

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