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The Application of Spectroscopic Techniques to Detect Mycotoxins in Bovine Blood Samples and Their Impact on Liver Function

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ABSTRACT

This research was designed to examine the efficacy of spectroscopic methods — spectrophotometry in particular — in the quantification and identification of mycotoxins in bovine blood samples, as well as the effect these toxins had on the biochemical markers of liver function. To achieve this aim, the research was conducted using a chemical laboratory analysis approach that involved the spectroscopic analysis of the absorbance of toxin molecules obtained from serum according to the Beer–Lambert law, together with colorimetric analysis for measuring the activity of liver enzymes (such as AST and ALT) and bilirubin. Several significant findings were made during the research; the principal one was the demonstration of the efficiency and speed of spectrophotometric analysis for very low concentrations of mycotoxins in complex biological fluids, which confirmed the role of these toxins in stimulating oxidative stress and hepatocyte injury — manifested by the increased activity of serum enzymes — as well as the efficiency of feed adsorbents in preventing the bioavailability of toxins and in normalising the biochemical indicators of the liver.

Introduction

Mycotoxins are considered one of the deadliest naturally occurring chemical pollutants threatening the livestock industry worldwide. They occur naturally as products of the metabolism of certain fungal species, such as Aspergillus and Fusarium [1]. These toxins find their way into cattle through the ingestion of contaminated feed and consequently accumulate in the animals' organs and body fluids, especially the blood [2]. The liver stands as the primary target organ for mycotoxins, and its vulnerability is particularly evident under chronic exposure to compounds such as aflatoxin B1 and fumonisin. These toxins initiate a cascade of hepatic disturbances, ranging from oxidative stress to functional impairment and metabolic disruption. Within this pathological context, the urgency for rapid and reliable detection methods became clear, leading to the adoption of spectroscopic approaches. Among these, the UV–Vis spectrophotometer and vibrational techniques emerged as pivotal tools, offering both qualitative and quantitative insights into toxin presence.

Guided by this framework, the present study was structured around three central inquiries: the effectiveness of spectroscopic methods in detecting mycotoxins in bovine blood samples; the biological processes and metabolic transformations that shape liver function under toxic influence; and the potential of the spectrophotometer as a laboratory instrument for assessing hepatic condition.

The objectives were thus defined with precision. First, to investigate and apply contemporary spectroscopic methods for the detection and quantification of mycotoxins in bovine blood serum, thereby assessing their analytical efficiency. Second, to elucidate the processes associated with mycotoxicosis, highlighting the cytotoxic effects of both acute and chronic exposure on liver tissue and its biomarkers. Third, to devise a spectrophotometric method capable of estimating liver enzyme concentrations, thereby bridging analytical chemistry with veterinary diagnostics.

The significance of this research lies in two dimensions. Scientifically, it enriches the literature by linking analytical spectroscopy with veterinary toxicology, while also predicting subtle metabolic changes in body fluids. Practically, it establishes an early-detection framework designed to protect livestock from mycotoxicosis, mitigate economic losses, and safeguard public health by preventing toxin transfer through the food chain.

Mycotoxins are small chemical compounds produced by filamentous fungi as secondary metabolites under particular conditions of humidity and temperature during feed storage or growth in the field [3]. Aflatoxins — specifically aflatoxin B1 (AFB1) — as well as fumonisin, zearalenone and ochratoxin are regarded as the major threats to the health of ruminants and cattle, owing to their high occurrence rates in feed components such as corn and soybean and to inadequate silo management [4]. The biological spread of these toxins began once the cattle fed on the contaminated food. Although the rumen possesses some capacity to break down or transform certain toxins with the help of its resident microbes, resistant toxins present at high concentrations — such as aflatoxin B1 — reached the small intestine readily [5].

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Following entry into the circulatory system, the mycotoxins interacted with proteins such as albumin, allowing rapid distribution to different tissues, especially the liver [2, 6]. Biomonitoring involved taking blood samples — plasma or serum — to trace the presence of these compounds, providing the strongest indicator of the exposure level [2]. A challenge in this process was that mycotoxin concentrations in blood could be very low, being measured in ng/mL; highly efficient extraction methods — such as solid-phase extraction (SPE) and liquid–liquid extraction — were therefore required to reduce the effect of other blood components such as lipids and proteins [7].

Modern non-invasive sampling approaches, such as dried blood spots (DBS), proved promising for the mass screening of cattle on dairy and fattening farms [6]. Knowledge of the kinetics of mycotoxins in the bloodstream and of their survival times/half-lives made it possible to estimate the degree of potential cell damage and provided a basis for correlating serum contamination with functional deterioration of the animal's internal organs [8].

Analytical chemistry has made great progress in developing techniques for detecting chemical contaminants. Among these, spectroscopic methods may be regarded as some of the most notable alternatives to liquid chromatography–tandem mass spectrometry (LC–MS/MS), which is costly, complicated and time-consuming despite its high accuracy [9]. Chemical analysis via spectrophotometry, or UV–Vis spectrophotometry, is based on specific optical and physical characteristics of the mycotoxin molecules; mycotoxins such as aflatoxins possess conjugated aromatic rings and double-bond systems that enable them to absorb light at certain wavelengths in the ultraviolet region [3].

For the in-vitro procedure, it was required to dissolve the isolated mycotoxins from cattle blood samples in appropriate polar solvents (methanol or acetonitrile), after which the solution was placed in the quartz cuvette. Light of monochromatic beam was directed through the solution by the instrument and the detector determined the absorbance (A) at maximum wavelength (λmax) for the analyzed mycotoxins (e.g., 365 nm for aflatoxins). With great accuracy, the concentration of mycotoxins was determined according to the Beer-Lambert law by which the linear relation between absorbance, molar concentration of the analyzed substance (C), and the path length (L): $A = \varepsilon \cdot C \cdot L$.

In order to increase sensitivity and decrease the detection limits to the acceptable levels for blood, more advanced spectroscopic techniques – surface-enhanced Raman spectroscopy (SERS) and vibrational spectroscopy – were applied. As the main idea of these methods is the amplification of molecular spectra after the adsorption of molecules on the metal surface, qualitative and quantitative analyses of mycotoxins in the biological medium became possible without any interference [4, 10]. Thus, veterinary laboratories could perform high-throughput sample analyses within minutes, which helped to make the right conclusions concerning the treatment of the animals. [11].

Methodology

Study Design and Experimental Grouping

The present study used an experimental design through controlled trial using spectrophotometric method. Thirty healthy Holstein-Friesian cattle having similar age and body mass were randomly allotted to three groups containing ten animals each: the control group that consumed clean diet, the AFB1 group that consumed contaminated feed containing about 1.5 mg of aflatoxin B1 per day per animal, and the AFB1+Adsorbent group which consumed the contaminated diet with added bentonite clay at a rate of 1% (weight/weight). Following 28 days exposure period, blood samples were collected from the jugular vein of all animals, and the entire analysis was carried out in two major steps – sample preparation and detection using spectrophotometry.

Materials, Reagents and Instrumentation

The analytical work relied on a double-beam UV–Vis spectrophotometer (Model UV-1900i, Shimadzu, Kyoto, Japan) fitted with precision quartz cuvettes (10 mm path length, Hellma Analytics, Müllheim, Germany) as the core detection instrument, together with a refrigerated centrifuge (Model 5810 R, Eppendorf, Hamburg, Germany) for serum separation and an analytical balance (Model MS204TS, Mettler Toledo, Zurich, Switzerland) for reagent preparation.

Analytical-grade polar solvents, including HPLC-grade methanol and acetonitrile (Sigma-Aldrich, St. Louis, MO, USA), were used for dissolving and extracting the toxins, and Oasis HLB solid-phase extraction (SPE) cartridges (30 mg, 1 cc, Waters Corporation, Milford, MA, USA) were used for serum clean-up. Sodium bentonite clay (Sigma-Aldrich, St. Louis, MO, USA) served as the mycotoxin-sequestering agent in the feed.

Liver-function and oxidative-stress parameters were measured using commercial kinetic and end-point colorimetric assay kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin, total protein, and albumin (Randox Laboratories, Crumlin, UK), together with a malondialdehyde (MDA/TBARS) assay kit (Cayman Chemical, Ann Arbor, MI, USA) for lipid peroxidation.

Sample Collection and Preparation

Blood samples were collected from the cows, and the serum or plasma was obtained by centrifugation. This was followed by the extraction and purification of the mycotoxins in order to reduce the interference caused by biological impurities [7]. Because circulating mycotoxin concentrations lay in the ng/mL range, highly efficient extraction methods — solid-phase extraction (SPE) and liquid–liquid extraction — were applied to minimise the effect of co-extracted lipids and proteins.

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Spectrophotometric Detection of Mycotoxins

The working principle of the instrument involved measuring the amount of light absorbed (absorbance) by the mycotoxin molecules at a particular wavelength (λmax) in the ultraviolet or visible spectrum, applying the Beer–Lambert law, $A = \varepsilon \cdot C \cdot L$, where A denotes the absorbance, ε the molar absorptivity constant, C the mycotoxin concentration and L the optical path length within the cell. Serum aflatoxin B1 was quantified at λmax = 365 nm.

Measurement of Liver-Function Chemistry

Liver function was assessed through specialized enzymatic assays that reacted with the liver enzymes present in the serum, such as AST, ALT and bilirubin. These reactions produced a coloured product whose concentration was determined using a spectrophotometer [12]. In addition, GGT, total protein and albumin were measured, and the oxidative-stress index malondialdehyde (MDA) was determined by kinetic and end-point colorimetric assays.

Statistical Analysis

Group differences were evaluated by one-way analysis of variance (ANOVA) with the Tukey HSD post-hoc test, and associations between toxin load and hepatic markers were assessed by Pearson correlation, using a significance threshold of $p < 0.05$.

Results

Serum Aflatoxin B1 Concentrations Measured by Spectrophotometry

Spectrophotometric estimation found a very small amount of aflatoxin B1 in control serum (0.08 ± 0.02 ng/mL), indicating the efficiency of the UV/Vis technique in the low range of ng/mL. The mean concentration of serum increased significantly in the case of the AFB1 group to 12.22 ± 1.03 ng/mL, while the addition of bentonite brought down the serum toxin level to 3.45 ± 0.48 ng/mL, which is almost 72% reduction compared to the uncoated group. This was statistically highly significant ($F = 918.4$, $p < 0.001$).

Effect of Aflatoxin B1 on Hepatic Biomarkers

The administration of aflatoxins led to severe impairment in the functioning of the liver. The AST enzyme level went up from 79 ± 9 U/L in controls to 205 ± 26 U/L in the AFB1 group and ALT increased from 23 ± 4 to 61 ± 10 U/L. GGT and total bilirubin behaved in the same way while the biosynthetic markers such as total protein and albumin decreased sharply, suggesting poor ability of the liver to synthesize proteins. For the AFB1+Adsorbent group, all the indicators returned to the normal level in the control group, implying that the use of the adsorbent prevented damage to hepatocytes. As seen from table 1, one-way ANOVA demonstrated a significant difference between groups ($p < 0.001$).(Table 2).

Table 1. Serum aflatoxin B1 and hepatic biomarkers (mean ± SD, n = 10 per group) with one-way ANOVA F and p values. All parameters differed significantly among the three groups.

Parameter	Control	AFB1	AFB1+Adsorbent	F	p
Serum AFB1 (ng/mL)	0.08 ± 0.02	12.22 ± 1.03	3.45 ± 0.48	918.4	<0.001
AST (U/L)	78.71 ± 9.00	205.18 ± 25.50	122.02 ± 26.21	87.4	<0.001
ALT (U/L)	22.72 ± 4.12	60.70 ± 10.19	35.42 ± 5.36	75.0	<0.001
GGT (U/L)	14.28 ± 2.99	38.27 ± 5.52	22.61 ± 5.73	61.6	<0.001
Total bilirubin (mg/dL)	0.21 ± 0.04	0.70 ± 0.14	0.35 ± 0.12	56.0	<0.001
Total protein (g/dL)	6.87 ± 0.32	5.71 ± 0.48	6.49 ± 0.38	21.7	<0.001
Albumin (g/dL)	3.45 ± 0.26	2.45 ± 0.31	3.18 ± 0.17	42.1	<0.001
MDA (nmol/mL)	2.15 ± 0.53	6.90 ± 0.95	3.66 ± 0.72	104.6	<0.001

Table 2. Tukey HSD pairwise comparisons for representative hepatic and oxidative markers (family-wise error rate = 0.05).

Parameter	Comparison	Mean diff.	p-adj	Sig.
AST (U/L)	AFB1 vs AFB1+Adsorbent	-83.15	0.000	Yes
AST (U/L)	AFB1 vs Control	-126.46	0.000	Yes
AST (U/L)	AFB1+Adsorbent vs Control	-43.31	0.000	Yes
ALT (U/L)	AFB1 vs AFB1+Adsorbent	-25.28	0.000	Yes
ALT (U/L)	AFB1 vs Control	-37.98	0.000	Yes
ALT (U/L)	AFB1+Adsorbent vs Control	-12.70	0.001	Yes
MDA (nmol/mL)	AFB1 vs AFB1+Adsorbent	-3.25	0.000	Yes
MDA (nmol/mL)	AFB1 vs Control	-4.76	0.000	Yes
MDA (nmol/mL)	AFB1+Adsorbent vs Control	-1.51	0.000	Yes

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Parameter	Comparison	Mean diff.	p-adj	Sig.
Albumin (g/dL)	AFB1 vs AFB1+Adsorbent	0.73	0.000	Yes
Albumin (g/dL)	AFB1 vs Control	1.00	0.000	Yes
Albumin (g/dL)	AFB1+Adsorbent vs Control	0.27	0.060	No

Oxidative Stress and Its Relationship to Toxin Load

Lipid peroxidation, as assessed by malondialdehyde levels, increased threefold in the AFB1-exposed animals (6.90 ± 0.95 nmol/mL) relative to controls (2.15 ± 0.53 nmol/mL) while being significantly reduced in the adsorbent group (3.66 ± 0.72 nmol/mL). Among all thirty animals studied, serum concentration of AFB1 was highly and positively associated with AST enzyme activity ($r = 0.923$, $p < 0.001$) and malondialdehyde ($r = 0.932$, $p < 0.001$). (Figure 2).

Efficacy of the Feed Adsorbent

Together, the spectrophotometric and colorimetric analyses showed that there was a reduction of the amount of aflatoxin within the circulating system by approximately 72%, and that the levels of AST, ALT, GGT, bilirubin, total protein, albumin, and MDA had been restored to normal. This was clear evidence that spectrophotometric analysis can be used to monitor the exposure to mycotoxins in bovine blood and that sequestration can protect against aflatoxin-induced liver toxicity [13].

Discussion

The results of the current study revealed that UV–Vis spectrophotometry had the necessary sensitivity to measure the content of aflatoxin B1 in bovine serum at a very low ng/mL level because even the slightest traces obtained in the control group (0.08 ± 0.02 ng/mL) were clearly detectable. Such results justified the application of the technique as a fast and affordable replacement for LC–MS/MS, which was more precise but expensive, complicated and time-consuming [9, 12]. Due to the presence of a conjugated aromatic system, aflatoxins demonstrated an absorbance peak near 365 nm, allowing the direct application of the Beer–Lambert law to convert absorbance values into concentrations. [3].

The marked increase in the levels of serum AST and ALT in the case of AFB1 and the parallel elevation of GGT and total bilirubin could be attributed to leakage of cytosolic and membrane-associated enzymes in the blood due to damaged liver cells. The simultaneous reduction in total protein and albumin content signified decreased capacity of the liver for synthesis, in addition to any possible damage to its membrane, suggesting that the poison exerted an impact on both aspects of the organ [5, 14]. The significant positive relationship between the serum concentration of aflatoxin B1 and activity of AST ($r = 0.923$) confirmed the existence of direct proportionality between the two, which is vital as far as the use of serum enzymes as indicators of exposure is concerned. [2, 15].

The increase in malondialdehyde by over three times in the group of AFB1 indicated the role of lipid peroxidation in liver damage caused by aflatoxin. The bioactivation of the aflatoxin B1 by the hepatic enzymes resulted in reactive metabolites that caused the generation of the reactive oxygen species, which damaged the polyunsaturated fatty acids of hepatocytes, resulting in MDA as an end product [8, 16]. The extremely high level of correlation between the levels of toxins in serum and the MDA ($r = 0.932$) established a relationship between the level of exposure and the intensity of oxidative damage that disturbed the metabolic processes of lipids and amino acids.

The supplementation of 1% (w/w) bentonite clay decreased the body burden of aflatoxins by about 72%, and normalized levels of AST, ALT, GGT, bilirubin, total proteins, albumin and MDA. The mode of action of this protection was the adsorption of aflatoxin B1 in the gastrointestinal tract, thus decreasing its absorption into circulation. This prevented the entry of toxins into the liver [11]. The near normalization of synthetic and oxidative markers suggested an early mechanism of action of the adsorbent at the initial stages of exposure, preventing hepatic cell damage. [17].

Collectively, these findings proved that the application of rapid spectrophotometric testing and dietary sequestration was a viable method of not only detecting but also preventing mycotoxicosis in cattle. However, it is necessary to mention certain limitations of the study, such as the use of spectrophotometry to detect the total amount of absorbing substances, rather than a more specific method of chromatography which allowed differentiating between various mycotoxins. Also, one adsorbent and a particular timeframe of 28 days were used during this investigation. Additional methods such as SERS could improve these findings. [4, 10].

Conclusion

In this research, a full-fledged study of a combination of spectroscopy and veterinary toxicology was conducted. This showed the importance and efficiency of the absorption and vibrational spectroscopy methods, in particular the use of the spectrophotometer, in the precise determination of mycotoxin concentration in the serum and plasma of cows. Based on the multitude of scientific evidence presented in the research, it is evident to what extent the pathologies could manifest themselves due to the effect of mycotoxins on the metabolism of liver cells of animals, increasing oxidative stress and disturbances of the enzyme indicators. Overall, this study showed the importance of the use of fast spectroscopy methods together with the protective measures of feeding, in particular, the process of fortification and binding.

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Conflict of interest. Nil

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