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Research Article

Ameliorative Potential of Afrocarpus falcatus Leaf Powder on Growth Performance and Hematological Indices of Broiler Chicks Exposed to Aflatoxin B1

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Abstract

A 42-day feeding trial was conducted to evaluate the ameliorative potential of Afrocarpus falcatus leaf powder on the growth performance and hematological indices of broiler chicks exposed to Aflatoxin B1 (AFB1). A total of 200 one-day-old broiler chicks were randomly assigned to 4 dietary treatments in a Completely Randomized Design (CRD). Each treatment was replicated 5 times with 10 birds per replicate, housed in a sanitized battery cage system. Treatment 1 (T1) served as the absolute control, receiving a standard basal diet without toxin or additives. Treatment 2 (T2) was fed a basal diet contaminated with 0.5 g AFB1/kg feed and supplemented with 250 mg synthetic ascorbic acid/kg. Treatments 3 (T3) and 4 (T4) were fed the 0.5 g AFB1 /kg contaminated diet supplemented with Afrocarpus falcatus leaf powder at 150 g/kg and 200 g/kg of feed, respectively. At the end of the 42-day experimental period, final body weight (FBW) was significantly lower ($P<0.05$) in T2 compared to T1, T3, and T4, which maintained statistically similar weights. Feed intake (FI) followed a distinct trend, being highest in T1, intermediate in T3 and T4, and lowest in T2 ($P<0.05$). The feed conversion ratio (FCR) was significantly higher ($P<0.05$) in the unprotected T2 group, whereas T3 and T4 effectively restored FCR values to par with the T1 control. Hematological analysis revealed that Red Blood Cell (RBC) counts, Packed Cell Volume (PCV), and Hemoglobin (Hb) concentrations remained within the normal physiological reference range for broilers across all groups; however, values were significantly lower ($P<0.05$) in the T2 group than in T1, T3, and T4. Total White Blood Cell (WBC) counts were not significantly altered ($P>0.05$) by any of the dietary interventions. In conclusion, dietary exposure to 0.5 g AFB1/kg causes severe growth depression and sub-clinical microcytic anemia in broilers, which cannot be fully mitigated by 250 mg of ascorbic acid. However, dietary inclusion of Afrocarpus falcatus leaf powder at both 150 g/kg and 200 g/kg effectively neutralizes AFB1 toxicity, protecting erythrocytic indices and restoring growth performance parameters to control levels. Afrocarpus falcatus shows strong potential as a functional phytogenic feed additive for organic mycotoxin management in commercial poultry production.

Keywords: Afrocarpus falcatus, Aflatoxin B1 , Broilers, Growth performance, Hematology, Phytogenics

Introduction

The global poultry industry plays a critical role in meeting human demand for high-quality animal protein, with broiler production serving as one of its most dynamic and fast-growing sectors [1]. To keep pace with this demand, commercial producers rely heavily on intensive feeding practices and standardized management protocols [2]. However, the econom-

ic sustainability of intensive broiler production is frequently threatened by the contamination of feed ingredients with mycotoxins [3, 4]. Among these, Aflatoxin B 1 (AFB1) a highly toxic secondary metabolite primarily synthesized by the ubiquitous fungal species Aspergillus flavus and Aspergillus parasiticus is considered the most prevalent and economically devastating threat in tropical and subtropical regions [4]. In commercial broiler facilities, the co-exposure of intensive production systems and compromised feed hygiene creates a high-risk environment for systemic mycotoxicity [5, 6]. When broilers ingest AFB1-contaminated rations, it triggers severe economic and biological failures, commonly characterized by structural tissue damage, reduced growth, and suppressed physiological function [7]. Intensive broiler production systems require optimal health and defense against feed-borne toxins [7].

The core problem facing the poultry sector is that conventional post-harvest drying and storage techniques often fail to prevent the proliferation of Aspergillus species in key feed ingredients like corn and soybean meal, especially under hot and humid tropical climates [8]. Once synthesized, AFB1 is exceptionally heat-stable and resistant to typical feed manufacturing processes, making its elimination from finished rations a major technical challenge [9]. When consumed by broiler chicks, AFB1 undergo rapid hepatic biotransformation cytochrome P450 enzymes [10]. This pathway generates highly reactive intermediate metabolites, most notably aflatoxin-B1 -8, 9-epoxide. This electrophilic intermediate binds covalently to cellular DNA, forming toxic adducts that disrupt protein translation and transcription [10]. At the systemic level, this biochemical disruption manifests as severe hepatotoxicity, lipid peroxidation of intestinal and erythrocytic biomembranes, and suppressed nutrient assimilation [11]. Consequently, affected flocks experience a drop in feed intake, stunted body weight gain, and high feed conversion ratios (FCR) [11]. Furthermore, the induced oxidative stress destabilizes mature red blood cell membranes, leading to accelerated hemolysis, anemia, and altered hematological profiles [12]. This reduces production margins and causes significant economic losses worldwide [13].

To mitigate the destructive impacts of aflatoxicosis, historical research has evaluated various therapeutic interventions, including synthetic chemical binders, inorganic clays, and single-source vitamins [14]. For example, supplementing diets with synthetic antioxidants like ascorbic acid (Vitamin C) has been a common mitigation strategy due to its role as an electron donor that neutralizes free radicals and supports tissue regeneration [15]. However, previous studies have demonstrated that relying on synthetic ascorbic acid alone offers limited, narrow-spectrum protection when animals face a high-dose mycotoxin challenge [16]. While synthetic vitamins can partially offset systemic oxidative stress, they fail to provide a physical barrier against toxin absorption in the intestine, nor do they possess the structural complexity needed to fully protect hepatic or hematopoietic tissues from severe AFB1 exposure [15]. As a result, recent livestock research has shifted toward identifying complex, multi-component phytogenic feed additives [17]. These plant-derived alternatives offer broad-spectrum protection by combining direct radical scavenging with physical bio-sorption properties [17].

Given the limitations of single-molecule synthetic alternatives, there is a clear need to evaluate underexplored, bioactive tropical plants as sustainable, low-cost solutions for managing mycotoxin risks. Afrocarpus falcatus (Outeniqua yellowwood) is a promising candidate for this purpose due to its dense profile of secondary metabolites, including a natural blend of polyphenols, complex tannins, and unique biflavonoids such as podocarpusflavone and sciadopitysin [18]. The foliage of Afrocarpus falcatus contains a rich profile of bioactive compounds. Using the whole crude leaf powder of Afrocarpus falcatus provides a dual-action defense against AFB1 toxicity that synthetic alternatives cannot match [18]. The rich concentrations of flavonoids and polyphenols acts as a potent antioxidant network, scavenging reactive oxygen species (ROS) and preventing the lipid peroxidation of erythrocyte and hepatic membranes. The structural, condensed tannins within the leaf powder can form stable complexes with AFB1 within the gastrointestinal tract, reducing its bioavailability and preventing its absorption into the portal circulation [17].

By investigating the inclusion of Afrocarpus falcatus leaf powder at graded levels in comparison to a standard synthetic ascorbic acid treatment, this study provides valuable data on organic mycotoxin management. The findings establish a practical, plant-based strategy to protect the health and performance of broiler chickens, offering a reliable alternative to safeguard poultry production from the economic impact of aflatoxin contamination.

Materials and Methods

Experimental Site, Environmental Conditions, and Ethical Approval

The 42-day feeding trial was executed at the Poultry Section of the Gandhi College of Agriculture, Rajasthan, India. The institutional study site is geographically positioned between latitude 23^0 03 ′N to 30^0 12 ′ N and longitude 69^0 30 ′ E to $78^0$17 ′E. Environmental monitoring throughout the experimental window recorded a mean annual temperature range fluctuating between 25.8^0 C and 35.1^0 C, alongside a mean annual rainfall ranging from 1100 mm to 1650 mm. All standard management, handling, and experimental interventions involving live birds were stringently subjected to review and secured formal clearance from the Institutional Animal Care and Ethics Committee under the specific authorization protocol code (AGN/30221/2026189).

Preparation and Processing of Afrocarpus falcatus Leaf Powder

Fresh leaves of Afrocarpus falcatus (Outeniqua yellowwood) were gathered during early morning hours from designated botanical repositories in the region. The harvested leaves were immediately transported to the Botany Department laboratory where their taxonomy was verified and recorded under voucher reference number AF/10002/202F deposited for future verification. The raw leaves were meticulously sorted manually to strip away extraneous organic matter and debris, followed by washing in clean distilled water. The cleaned leaves were evenly distributed across plastic trays and subjected to unheated shade-drying at room temperature for a duration of 12 consecutive days until a constant weight was recorded was achieved. This deliberate thermal preservation strategy prevents the degradation of heat-sensitive phytogenic metabolites. The dried leaves were then crushed using a heavy-duty mechanical mill to achieve a homogeneous coarse leaf powder. This mechanical modification maximizes the cellular surface area, optimizing the bioavailability of active polyphenolic matrices, tannins, and complex flavonoids when homogeneously blended into the daily raw feed fractions.

Fungal Inoculation, Extraction, and High-Performance Liquid Chromatography (HPLC) Quantification of Aflatoxin B1

The creation of the target mycotoxin challenge was initiated by using an active, toxigenic strain of Aspergillus flavus obtained from the Microbiology Laboratory, Sumitra Research Institute, Gujarat to inoculate a clean batch of cracked white maize matrix. The moisture content of the maize substrate was adjusted to a constant 22 % by adding sterile distilled water, before it was autoclaved at 121^{0}C for 20 minutes to eliminate competing native microbial flora. The sterile substrate was then inoculated with the A. flavus spore suspension and incubated under controlled environmental dark chambers at a constant temperature of 28^{0}C and 85% relative humidity for a 14-day production window to stimulate secondary fungal metabolism and optimize the biosynthesis of Aflatoxin B1(AFB1).Following the incubation cycle, the fungal activity was halted by steaming the toxic corn matrix at 100^0 C for 15 minutes, followed by complete dehydration in a forced-air oven at 60^0 C until a stable dry mass was achieved. The toxic corn was ground into a uniform meal. The quantification of AFB1 concentrations within the generated substrate meal was determined using High-Performance Liquid Chromatography (HPLC) equipped with post-column photochemical derivatization. A 25 g representative sample of the toxic corn meal was extracted using a 100 mL mixture of methanol and water (80:20 v/v) by shaking vigorously on an orbital shaker for 45 minutes. The crude extract slurry was filtered through Whatman No. 4 filter paper, and a 10 mL aliquot of the filtrate was diluted with 40 mL of phosphate-buffered saline (PBS, pH 7.4). The diluted extract was then passed through an AFB -specific immunoaffinity clean-up column at a flow rate of 1 drop per second to isolate the mycotoxin molecules. The column was washed with 10 mL of pure distilled water, and the bound AFB1molecules were eluted by passing 1.5 mL of HPLC-grade methanol through the matrix.The purified eluate was directly injected into an Agilent 1260 Infinity II HPLC System (Agilent Technologies, United States). The operational configuration utilized a reversed-phase C 18 analytical column (4.6 mm×250 mm, 5 µm particle size) maintained at an internal oven temperature of 35^{0}C. The mobile phase consisted of an isocratic blend of water, methanol, and acetonitrile (60:20:20 v/v/v) driven at a constant flow rate of 1.0 mL/min. Detection was accomplished using a high-sensitivity fluorescence detector with excitation and emission wavelengths configured precisely at 365 nm and 435 nm, respectively. Using the determined potency of the stock corn meal, precise portions were calculated and incorporated into the basal broiler rations to establish an exact, uniform contamination exposure level of 0.5 g (500 mg) of pure AFB1per kilogram of finished experimental diet across treatments T2, T3, and T4.

Experimental Design, Housing Management

A total of 200 one-day-old broiler chicks (Cobb 500) was purchased from a reputable source in Rajathan, India. Chicks were unboxed and their average weight was recorded using a digital sensitive scale before it was randomly distributed split into 4 distinct dietary treatments, with each individual treatment comprised of 5 independent replicates containing 10 birds each using Completely Randomized Design (CRD). The birds were housed within a strictly sanitized and disinfected multi-tier battery cage system located inside an environmentally regulated poultry house. Prior to the arrival of the chicks, all pre-experimental hygiene procedures were carried out, including thorough pressure washing, chemical disinfection of the floors, cages, and boundaries, and setting up clean feeding and watering troughs.

The baseline environmental temperature within the brooding zone was initialized at 33^0 C using automated gas heaters, then gradually scaled down by 2.5^0 C every week until a steady ambient comfort threshold of 24^0 C was reached. Clean water and the standard experimental diets which was formulated according to the standard nutritional requirement for broilers [18] was provided on an ad libitum basis throughout the 42-day production cycle. Proximate composition of experimental diet was carried out according to the method outlined by [19].

The experimental feeding groups were formulated as follows:

Treatment 1 (T1): Control group receiving only the standard

basal diet without any AFB1 contamination or antioxidant additives.

Treatment 2 (T2): Basal diet contaminated with 0.5 g AFB1/ kg diet, supplemented with 250 mg of synthetic ascorbic acid per kilogram of feed.

Treatment 3 (T3): Basal diet contaminated with 0.5 g AFB1/ kg diet, supplemented with 150 g of processed Afrocarpus falcatus leaf powder per kilogram of feed.

Treatment 4 (T4): Basal diet contaminated with 0.5 g AFB1/ kg diet, supplemented with 200 g of processed Afrocarpus falcatus leaf powder per kilogram of feed.

Growth Performance Parameters

Final Body Weight (FBW) and Feed Intake (FI) were measured at the end of the 42-day trial using a high-precision electronic digital platform scale. The Feed Conversion Ratio (FCR) for each replicate was calculated mathematically by dividing the total feed consumed within that replicate by the corresponding total live weight gain recorded over the 42-day timeline.

Blood Collection and Analysis

At the exact termination of the 42-day experiment, 5 birds were randomly chosen from each treatment group for blood profiling. Blood samples (3 mL) were collected via branchial vein puncture using sterile needles and deposited into vacuum tubes containing ethylene diamine tetra acetic acid (EDTA) anticoagulant to preserve cellular integrity. The complete blood count focused on Red Blood Cell (RBC) count, Packed Cell Volume (PCV), Hemoglobin (Hb) concentration, and total White Blood Cell (WBC) count—was performed using a Mindray BC-2800 Vet Automated Hematology Analyzer (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). The analyzer was configured using verified, animal-specific software parameters calibrated for avian blood morphology to account for the nucleated nature of avian erythrocytes and thrombocytes. The operational specifications of the kit has a sample aspiration volume of 20 µL (whole blood mode); 20 µL (pre-diluted mode), aperture diameter (80 µm) and the measurement principle is via electronic impedance method for cell counting and cyanide-free colorimetric method for hemoglobin quantification.

Statistical Analysis

All data collected on growth performance parameters (Final Body Weight, Feed Intake, and Feed Conversion Ratio) and hematological indices (RBC, PCV, Hb, and WBC) were subjected to a one-way Analysis of Variance (ANOVA) for a Completely Randomized Design (CRD) using the General Linear Model (GLM) procedure of SAS (Statistical Analysis System, version 9.4, SAS Institute Inc., Cary, NC, USA).The underlying statistical model used for the analysis is formulated as follows:

$Y_{ij} = \mu + T_i + \epsilon_{ij}$

Where:

Y_{ij} represents the individual observation of the dependent variable from the j-th replicate within the i-th dietary treatment.

μ is the overall population mean for the parameter measured.

T_i represents the fixed effect of the i-th dietary treatment (i=1, 2, 3, 4).

ϵ_{ij} is the random residual error associated with the j-th replicate in the i-th treatment, assumed to be independently and identically distributed following a normal distribution: ϵ_{ij}

Results

Phyto-components in Afrocarpus falcatus leaf powder is presented in Table 2. The plant contained tannins (10.24 mg/g), saponins (35.62 mg/g), phenols (276.33 mg/g), alkaloids (27.11 mg/g), terpenoids (131.4 mg/g), steroids (65.72 mg/g) and flavonoids (290.2 mg/g). In ranking order: phenols > flavonoids > terpenoids > steroids > saponins > alkaloids > tannins.

Growth performance of broiler chickens exposed to Afrocarpus falcatus leaf powder and a diet contaminated with Aflatoxin B1 (AFB1) is presented in Table 3. Body weight gain was lower in T2 (1763.94 g) than T1 (2406.23 g), T3 (2443.46 g) and T4 (2450.98 g) ($p<0.05$). Cummulative feed intake was more in T1 compared to the other groups ($p<0.05$). Feed conversion ratio varied from 2.00 – 2.54, value obtained was higher in T2 relative to the other treatments ($p<0.05$).

Haematological indices of broiler chickens exposed to Afrocarpus falcatus leaf powder and a diet contaminated with Aflatoxin B1 (AFB1) is presented in Table 4. Whereas pack cell volume, haemoglobin and red blood cell were lower ($p<0.05$) for T2 than for T1, T3 and T4. White blood cells were similar ($p >0.05$) among the treatments.

	Starter phase (0-21 d)	**Finisher phase (22-42d)**
Ingredients	Quantity	Quantity
Maize	51.00	55.00
Wheat bran	2.00	4.39
Soyabean meal	35.05	29.05
Fish meal	4.89	3.00
Limestone	2.00	2.50
Dicalcium Phosphate	4.00	5.00
DL-Methionine	0.25	0.25

L-Lysine HCl	0.25	0.25
Min-Vit Premix	0.25	0.25
Salt	0.20	0.20
Toxin binder	0.11	0.11
Total	100	100
Determined analysis (%)		
Crude protein	23.12	21.06
Crude fibre	3.88	4.01
Ether extract	4.08	4.21
Calcium	1.17	1.19
Phosphorus	0.56	0.58
ME (kcal/kg)	2908.2	3016.5

2.5 kg of vitamin-mineral premix contains: Vitamin D3 - 2,000,000 IU; Vitamin K - 2,250 mg; Vitamin A 10,000,000 IU; Vitamin E - 20,000 IU; Thiamine B1 - 1,750 mg; Niacin - 27,500 mg; Pantothenic acid - 7,500 mg; Biotin - 50 mg; Choline chloride - 400 g; Riboflavin B2 - 5,000 mg; Pyridoxine B6 - 2,750 mg; Antioxidant - 125 g; Magnesium - 80 g; Iodine - 1.2 g; Selenium - 200 mg; Cobalt - 200 mg; Zinc - 50 mg; Iron - 20 g; Copper - 5 g

Table 1: Ingredient and chemical composition of experimental (basal) diet

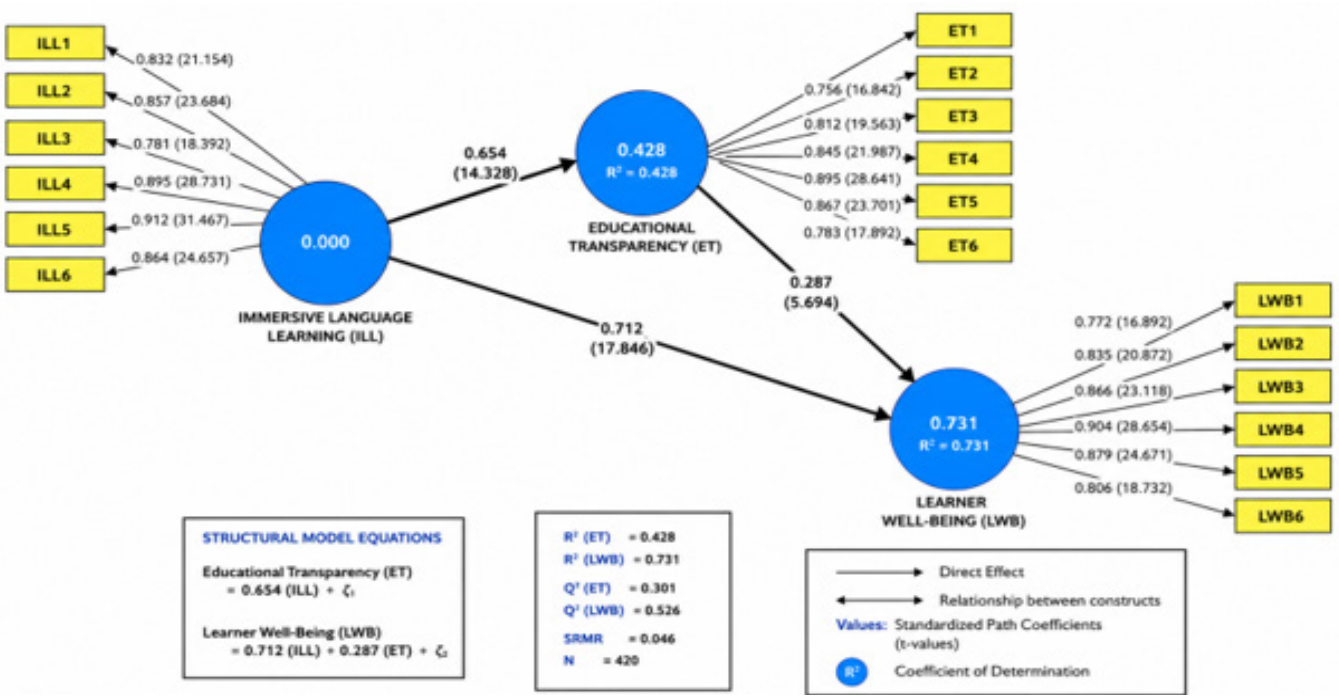


Table 2: Phyto-components in Afrocarpus falcatus leaf powder

Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**
Initial live weight (g)	45.67	45.26	45.14	45.22	0.02
Final Live Weight (g)	2451.9a	1809.2b	2488.6a	2496.2a	98.91
Body Weight Gain (g)	2406.23a	1763.94b	2443.46a	2450.98a	87.40
Daily Weight Gain (g)	57.29a	41.99b	58.18a	58.36a	0.03
Cummulative Feed Intake (g)	5106.5a	4490.6b	4900.6b	4900.9b	164.2
Daily Feed Intake (g)	121.6a	106.9b	116.7b	116.6b	0.07
Feed Conversion Ratio	2.01b	2.54a	2.00b	2.00b	0.01

Note on Superscripts: [a, b, c] Means along the same row with different superscripts are significantly different ($p<0.05$)
Treatment 1 (T1): Control group receiving only the standard basal diet without any AFB1 contamination or antioxidant additives; Treatment 2 (T2): Basal diet contaminated with 0.5 g AFB1/kg diet, supplemented with 250 mg of synthetic ascorbic acid per kilogram of

feed; Treatment 3 (T3): Basal diet contaminated with 0.5 g AFB1/kg diet, supplemented with 150 g of processed Afrocarpus falcatus leaf powder per kilogram of feed; Treatment 4 (T4): Basal diet contaminated with 0.5 g AFB1/kg diet, supplemented with 200 g of processed Afrocarpus falcatus leaf powder per kilogram of feed.

Table 3: Growth performance of broiler chickens exposed to Afrocarpus falcatus leaf powder and a diet contaminated with Aflatoxin B1 (AFB1)

Constituents	T1	T2	T3	T4	SEM
Packed cell volume (%)	31.72a	28.65b	32.91a	32.82a	0.01
Haemoglobin (g/dL)	10.12a	8.97b	10.42a	10.83a	0.02
Red blood cells (1012/L)	2.97a	2.00b	2.98a	3.00a	0.01
White blood cells (109/L)	21.56	20.88	22.87	22.96	0.02

Note on Superscripts: [a, b] Means along the same row with different superscripts are significantly different ($p<0.05$)
Treatment 1 (T1): Control group receiving only the standard basal diet without any AFB1 contamination or antioxidant additives; Treatment 2 (T2): Basal diet contaminated with 0.5 g AFB1/kg diet, supplemented with 250 mg of synthetic ascorbic acid per kilogram of feed; Treatment 3 (T3): Basal diet contaminated with 0.5 g AFB1/kg diet, supplemented with 150 g of processed Afrocarpus falcatus leaf powder per kilogram of feed; Treatment 4 (T4): Basal diet contaminated with 0.5 g AFB1/kg diet, supplemented with 200 g of processed Afrocarpus falcatus leaf powder per kilogram of feed.

Table 4: Haematological indices of broiler chickens exposed to Afrocarpus falcatus leaf powder and a diet contaminated with Aflatoxin B1 (AFB1)

Discussion

The inclusion of Afrocarpus falcatus leaf powder at graded levels (150 g/kg and 200 g/kg) successfully countered the severe systemic damage caused by Aflatoxin B1(AFB1) over the 42-day broiler trial. Ingestion of 0.5 g AFB1/kg without sufficient protection as seen in the T2 group supplemented with only 250 mg of ascorbic acid triggered classic symptoms of aflatoxicosis [20]. At the cellular level, AFB1 is converted by hepatic cytochrome P450 enzymes into the highly reactive intermediate aflatoxin-B1-8, 9-epoxide [21]. This electrophilic compound binds to cellular macromolecules to form toxic DNA adducts, which block transcription and translation [21]. This broad disruption of protein synthesis directly explains the significantly lower final body weights observed in T2 [22]. Furthermore, the unmitigated oxidative stress caused by AFB1generates a surge of reactive oxygen species (ROS) that attack the lipid membranes of the intestinal mucosa [23]. This lipid peroxidation causes the breakdown of intestinal villi, reducing their height and lowering the gut's overall capacity to absorb nutrients [23, 24]. This structural damage, paired with toxin-induced appetite suppression, led to the lower feed intake and elevated feed conversion ratio (FCR) in T2. This demonstrates that the birds consumed feed but could not convert it efficiently into muscle mass because their metabolic resources were redirected toward hepatic detoxification [24]. This result is in agreement with the report of [6] when ginger extract was supplemented in the diet of broiler chicken.

The higher final body weight and feed conversion efficiency in the T3 and T4 groups proves that Afrocarpus falcatus leaf powder provides superior protection against aflatoxicosis compared to synthetic ascorbic acid alone. This impressive therapeutic effect is directly tied to the rich and diverse profile of phytochemicals within the plant's leaves [2]. The leaf powder contains exceptionally high levels of flavonoids (290.2 mg/g) and phenols (276.3 mg/g). These polyphenolic compounds function as a powerful antioxidants [15, 33]. They easily donate hydrogen atoms or electrons to neutralize ROS and lipid peroxyl radicals, halting the oxidative destruction of hepatic and intestinal cell membranes [16]. Additionally, the terpenoids (131.4 mg/g) and steroids (65.72 mg/g) in the leaf powder likely worked alongside these antioxidants to reduce inflammation and stabilize cell walls, protecting the structural integrity of the gut lining [34]. By preserving the intestinal villi, the leaf powder maintained normal nutrient transport and assimilation [35]. This allowed the birds in T3 and T4 to utilize their feed efficiently, resulting in an optimal feed conversion ratio matching the toxin-free control (T1), despite showing an intermediate feed intake. The performance of the leaf powder is further explained by its content of tannins (10.24 mg/g) and alkaloids (27.11 mg/g), which provide an elegant physical defense mechanism within the digestive tract [33]. Tannins are high-molecular-weight polyphenols known for their ability to bind to various chemical complexes [15]. In the small intestine of birds, these tannins can form stable, insoluble hydrophobic complexes with the hydrophobic phenanthrene rings of AFB1. This physical binding effectively traps a

portion of the mycotoxin inside the digestive tract, preventing it from crossing the intestinal wall into the portal bloodstream [16].

By acting as a natural bio-sorbent, the leaf powder reduced the total amount of AFB1 absorbed by the birds. This significantly eased the toxic burden on the liver and bone marrow. This dual action where tannins physically block toxin absorption while high concentrations of flavonoids and phenols chemically neutralize systemic oxidative stress explains why Afrocarpus falcatus leaf powder protected the birds far more effectively than the single-molecule, water-soluble ascorbic acid treatment used in T2.

The hematological results provide clear evidence of this systemic protection. The lower Red Blood Cell (RBC) counts, Packed Cell Volume (PCV), and Hemoglobin (Hb) levels in the T2 group indicate a state of sub-clinical microcytic anemia caused by unmitigated aflatoxicosis [25]. This decline happens because AFB1 directly suppresses the bone marrow, slowing down the production of new red blood cells (erythropoiesis) [26]. At the same time, circulating free radicals weaken mature red blood cell walls, causing them to rupture prematurely (hemolysis) [26]. In contrast, the birds in T3 and T4 maintained significantly higher RBC, PCV, and Hb values than those in T2, keeping them well within normal reference ranges [27]. The abundant flavonoids (290.2 mg/g) and saponins (35.62 mg/g) in the leaf powder helped stabilize erythrocyte membranes, protecting them from oxidative damage and hemolysis [27]. Moreover, by protecting liver tissue from damage, the leaf powder ensured the continuous synthesis of key proteins like transferrin, which are essential for normal iron transport and hemoglobin production [28]. Finally, the fact that White Blood Cell (WBC) counts remained statistically unaffected across all groups indicates that the dietary challenge of 0.5 g AFB1/kg did not cause acute immune cell depletion or severe systemic inflammation [29]. This suggests that the primary damage caused by this level of aflatoxin over 42 days is metabolic rather than destructive to white blood cell lines [28]. This result is in agreement with the report of [30].

Conclusion

In conclusion, the outcome of this experiment demonstrate that Afrocarpus falcatus leaf powder contains bioactive compounds with various therapeutic properties. Its inclusion at both 150 g and 200 g per kg of diet successfully ameliorates the destructive effects of Aflatoxin B1 in broiler chickens and protects hematopoietic system from oxidative damage and preserves gut and liver health, allowing for optimal feed conversion, growth performance on par with a toxin-free diet. It represents a highly promising, organic phytogenic feed additive for managing mycotoxin risks in poultry production.

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