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Research Article

Efficacy of Phytogenic Additive (*Spondia mombin*) to Mitigate the Toxic Effects of Aflatoxicosis in Cockrel Chickens at Grower Phase

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About Article

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ABSTRACT

The presence of mycotoxins in poultry feed can cause growth retardation, immune suppression, organ damage, and intestinal dysfunction, with the most prevalent being aflatoxin B1 (AFB1). In this study, the ameliorative effects of ethanol extract of *Spondias mombin* (EEHP) leaves were compared against cockrel chickens fed diets containing 35 µg/kg feed of AFB1 on growth performance, carcass characteristics, lymphoid organs, and intestinal morphology. The ethanol extract was made with ethanol as the extraction solvent. A total of 150 cockrel chickens were randomly distributed into five treatment groups (AFB1, EEHP, AFB1 + EEHP, positive control, and negative control) in two experimental periods (6–9 weeks and 9–12 weeks). Growth performance, organ weights, carcass traits, and intestinal morphometry data were analyzed by analysis of variance. In the first phase (6–9 weeks), significant differences ($p < 0.05$) were found with the body weight and the weight gain, where EEHP supplemented birds exhibited higher values at the start of the exposure (960.00 g and 260.00 g, respectively), while aflatoxin exposed birds showed lower values at the end of the phase (1150.00 g and 413.64 g, respectively). Feed intake also varied significantly ($p < 0.05$) with control group having the highest feed intake (1250.78 g). During this period, there were no significant differences ($p > 0.05$) in carcass characteristics, but the weights of liver (30.00 g vs. 18.50 g), kidney (7.50 g vs. 5.00 g), and heart (7.33 g vs. 4.17 g) were significantly lower ($p < 0.05$) in the birds exposed to aflatoxin. During the second phase (9–12 weeks), the birds receiving EEHP had significantly higher final body weight (1500.00 g) and dressed weight (1256.00 g) than did the birds receiving only aflatoxin. There was also improved intestinal development with enhanced jejunal weight (27.50 g vs. 14.03 g) and ileal length (56.37 cm vs. 45.10 cm). In summary, overall growth performance, organ integrity, and intestinal morphology were improved with EEHP supplementation in both phases, suggesting the protective effect on AFB1 induced toxicity. The result indicated EEHP could be used as a natural feed supplement to control the aflatoxicosis in poultry production.

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1. INTRODUCTION

Secondary metabolites, which are fungal in origin, are referred to as mycotoxins. They are a global risk to food security and safety, especially in poultry farming. Mycotoxins are secondary metabolites of moulds that grow on animal feeds, especially in favourable climatic conditions characterized by high humidity and temperature (Adepoju & Oyewole, 2008). Over 200 species of fungi are capable of producing these toxins, with the majority of them, however, being from three different genera: *Fusarium*, *Penicillium*, and *Aspergillus*, which produce 500 different mycotoxins, a small fraction of which are acutely toxic (Adepoju & Oyewole, 2008; Cloft *et al.*, 2023). Among these, aflatoxins have been identified as the most common and potent, with AFB1 identified as the most toxic, which affects animal health (Cloft *et al.*, 2023).

Grains and feed materials can be contaminated by mycotoxins during the growth, harvesting, and storage phases. The storage of grains and feed materials under anaerobic and moisture-rich conditions is a major contributing factor to mycotoxin contamination (Gómez-Osorio *et al.*, 2024). In the case of *Aspergillus* species, such as *Aspergillus flavus* and *Aspergillus parasiticus*, the major mycotoxins produced are aflatoxins (Gómez-Osorio *et al.*, 2024). These species of molds are widespread in the soil and are responsible for the contamination of major poultry feed materials such as maize, sorghum, soybeans, rice, wheat, and groundnuts (Cloft *et al.*, 2023; Graham *et al.*, 2023). Under moisture-rich and storage conditions, the rate of aflatoxin production is high, resulting in a high level.

Aflatoxicosis in poultry is of significant interest because of the significant relationship established between aflatoxin exposure and unfavourable physiological outcomes, including decreased feed efficiency, growth, feed conversion, and increased mortality (Cloft *et al.*, 2023; Graham *et al.*, 2023). AFB1 is known for its hepatotoxic, carcinogenic, and immunosuppressive properties, mediated through reactive oxygen species and macromolecular interactions, which affect protein synthesis and lead to oxidative stress (Kumar *et al.*, 2022; Cloft *et al.*, 2023). Aflatoxin contamination is not an issue of developing countries only, but it is a global problem, and it is a threat to both animal and human health, as it is detected in poultry meat for human consumption (Cloft *et al.*, 2023). In poultry, for instance, exposure to AFB1 has been demonstrated to result in a complex of clinical manifestations referred to as aflatoxicosis. The various effects include reduced appetite, lethargy, reduced growth rate, reduced egg production and quality, fatty liver, altered organ weights, reduced levels of serum protein, and reduced immunity (Cloft *et al.*, 2023; Graham *et al.*, 2023). The histopathological impacts encompass apoptosis of the immune-related organs like the bursa of Fabricius and spleen, thereby affecting immunity (Nabi *et al.*, 2024). In addition, aflatoxins have been demonstrated to cause damage to the epithelium in the gastrointestinal tract, thereby affecting villus height and nutrient absorption (Nwidu *et al.*, 2017; Okechukwu *et al.*, 2024). Meanwhile, conventional methods of mitigation, such as physical decontamination, use of chemical binders, and feed additives, have shown varied success and can result in nutrient interactions and costs (Cloft *et al.*, 2023). Conversely, there has

been an interest in phytogetic additives, which are of plant origin and have been used as medicine due to their antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties (Graham *et al.*, 2023). Flavonoids and phenolic acids are examples of phytochemicals that have the potential to upregulate endogenous antioxidant pathways, preserve cell membrane integrity, and activate immune responses, thereby classifying these as a multifaceted method of alleviating the effects of mycotoxins.

Spondia mombin, also called hog plum, is a deciduous tree that grows in tropical regions of the world. It has yellow-coloured plum-like fruits, which are traditionally used for nutritional and medicinal purposes (Olariu *et al.*, 2025). The tree has leaves, bark, and fruits that are abundant in bioactive compounds, such as flavonoids, phenolic acids, tannins, saponins, carotenoids, and vitamin C (Cloft *et al.*, 2023; Omotayo *et al.*, 2019). The antioxidant properties of these bioactive compounds scavenge the free radicals and activate the antioxidant defence mechanism of the body. The antioxidant activities of the compounds include the enhancement of collagen synthesis, the stimulation of the immune system, and the protection of cellular structures from oxidative damage (Patathananone *et al.*, 2019). The high amount of phenolic compounds and flavonoids in the hog plum tree indicates that the tree might provide protective effects against oxidative and inflammatory damage caused by aflatoxins.

Medicinal plants with high levels of phytochemicals have shown the potential to reduce oxidative stress, inflammation, and microbial infections, which are major pathological changes associated with aflatoxicosis (Rajput *et al.*, 2019; Graham *et al.*, 2023). Medical plant extracts have recorded encouraging outcomes in alleviating the extent of organ injury, as well as enhancing the functionality of animals exposed to toxins (Lee & Ryu, 2017). But few studies have compared the effectiveness of *S. mombin* in alleviating the toxicity of aflatoxin in poultry, particularly with regard to its effects on the growth performance, carcass traits, and the weight of organs and gut integrity of the animals.

Accordingly, the present study aimed at assessing the ameliorative effect of the ethanol extract of the leaves of *Spondias mombin* on growth performance, carcass traits, lymphoid organ development, and intestinal morphology of cockerel chickens fed an AFB1-contaminated diet. Based on these observations, we hypothesized that dietary supplementation with ethanol extract of *S. mombin* would reduce the toxicity caused by AFB1 as reflected by improved growth, organ integrity, and intestinal morphology in challenged cockerels during the experimental period.

2. LITERATURE REVIEW

Aflatoxin B1 (AFB1) has been shown to have negative impacts on poultry productivity and health in previous studies. Yunus *et al.* (2011) reported that broiler chickens fed with AFB1 had decreased feed intake, decreased body weight gain, poor feed conversion ratio, and immunosuppression. Likewise, Denli *et al.* (2009) reported that aflatoxin in the feed had a significant negative effect on the growth performance and induced pathological changes in the liver and kidney of broiler chickens. Adverse effects associated with these have been attributed to



oxidative stress, decreased protein synthesis, and effects on hepatic metabolism due to the reactive metabolites of AFB1 (Rawal *et al.*, 2010).

The negative impact of exposure to aflatoxin is not restricted to growth depression, but also affects carcass and organ characteristics. Nabi *et al.* (2024) showed that exposure to aflatoxin caused a reduction in the relative weight of lymphoid organs (spleen and bursa of Fabricius), which may impair immune function. Aflatoxins also affect the structures of the intestinal epithelium of poultry birds, decreasing the height of the villi and affecting the absorption of nutrients. These changes in the intestine are a major factor in poor growth performance and decreased feed efficiency.

Several methods of detoxification of aflatoxins have been studied, such as the use of adsorbents, probiotics, antioxidants, and phytogetic feed additives. Rajput *et al.* (2019) reported that phytogetic compounds with flavonoids and phenolics had a beneficial role on antioxidant status and decreasing tissue damage in toxin-challenged poultry. Similarly, Lee and Ryu (2017) reported that the extracts of medicinal plants had a beneficial effect on the liver function and growth performance of the birds fed with toxic diets. The efficacy of phytogetic additives, however, depends on plant source and extraction process as well as bioactive compound concentrations.

Due to its rich phytochemical content and medicinal properties, *Spondias mombin* has been brought into focus. Some of the major constituents of the plant, identified by Omotayo *et al.* (2019), include flavonoids, tannins, saponins, and phenolic compounds. They have antioxidant and anti-inflammatory properties that protect tissues from oxidative damage and remove free radicals. Ajolo *et al.* (2023) also showed that the leaf meal of *S. mombin* protected broiler chickens from organ damage caused by AFB1, which indicated its protective effect against aflatoxicosis.

Although these results were obtained, little information is available on the effect of the ethanol extract of *S. mombin* on growth performance, carcass traits, development of lymphoid organs, and intestinal morphology of cockerel chickens fed AFB1-contaminated diets. The available literature has concentrated primarily on broilers and organ pathology, and very few have paid attention to intestinal morphometry and growth responses at various growth stages. Thus, more research is needed to elucidate the ameliorative activity of *S. mombin* ethanol extract in aflatoxin-challenged cockerels.

3. METHODOLOGY

3.1. Experimental location

The study was conducted at the Training and Research Unit, The Federal Polytechnic, Ilaro, Animal Health and Production Technology Department. It is in the Yewa South Local Government in Ogun State, Nigeria. Its coordinates are 6 3746oN and 6 5542oN and 2 4724oE and 3 648oE (14).

3.2. Aflatoxin production & controlled contamination

Aspergillus flavus isolated on DG-18 and OMA, cultivated in acidified PDB for 7 days;

Stock quantified by TLC/UV; working solution prepared to contaminate feed at 35 mg AFB1/kg.

Aflatoxin B1 Concentration Quantification and Confirmation
Before the experimental use, the concentration of aflatoxin B₁ in the stock suspension was determined. The quantification was done by applying thin-layer chromatography (TLC), in accordance with the official methods of the AOAC for aflatoxin determination. The aliquots of the aflatoxin extract were processed simultaneously with certified AFB1 standards for chromatographic analysis. For TLC, detection and quantification were done by measuring fluorescence intensity under ultraviolet light (365 nm). Only those extracts having confirmed and measurable AFB1 concentrations were used to contaminate the experimental setup.

3.3. Preparation of ethanolic extract of hog plum (*Spondias mombin*)

The ethanolic extraction of hog plum (*Spondias mombin*) was done according to the standard maceration method mentioned earlier by Hassan and Jaffer (2019). In summary, 100 g of hog plum material that had been finely ground was weighed and then put into a sterile and air-tight container. 1000 mL of ethanol (absolute) was added to the plant material, covering the plant material completely. The mixture was left for 24 hours in the laboratory at a normal temperature, shaken occasionally to ensure better penetration of the solvent and better extraction. After this time, the mixture was filtered through Whatman filter papers to remove the undissolved plant debris. The filtered liquid was a crude ethanolic extract, which was stored in labelled containers in a refrigerator until use. 1 ml of the extract was mixed with 1 litre of water before administering to the birds.

3.4. Experimental animal and management

One hundred and fifty (150) day-old cockerel chicks were bought in a well-established commercial hatchery, and were maintained in the brooding pen during a period of two weeks. Then, the birds were reared for another 4 weeks to get 6 weeks of the grower phase. The chicks were further allocated into five (5) treatments. The treatments were repeated three times, consisting of thirty (10) birds each. Routine and periodic (vaccinations (Newcastle and Gumboro), management of chickens had been adhered to. During the experiment, *ad libitum* commercial feed and water were given. The experiment was for 6 weeks.

3.5. Experimental design

The experiment used a Completely Randomized Design (CRD). The experiment had two (2) Phases. In the first phase, each treatment was given the test ingredients as follows:

Treatment 1 (T1): 1 ml of EEHP + 0 µl of Aflatoxins

Treatment 2: 1 ml EEHP per litre of water + 35 µl of Aflatoxins per kg of feed

Treatment 3: 35 µl of Aflatoxins + 0 ml of EEHP per litre of water

Treatment 4: 0 Aflatoxins per kg of feed + 0 of EEHP per litre of water

Treatment 5: 35 µl Aflatoxins per kg of feed + 0 ml of EEHP per litre of water

For the last three weeks of the grower phase, the treatments were given the following amounts of the test ingredient:



Treatment 1: 0 ml of EEHP + 35 µl of Aflatoxins
 Treatment 2: 0 ml of EEHP per litre of water + 0 µl of Aflatoxins per kg of feed
 Treatment 3: 0 µl of Aflatoxins per kg of feed + 1 ml of EEHP per litre of water
 Treatment 4: 0 µl of Aflatoxins per kg of feed + 0 ml of EEHP per litre of water
 Treatment 5: 0 µl of Aflatoxins per kg of feed + 0 ml of EEHP per litre of water.

3.6. Data collection

3.6.1. Feed intake

This was recorded on a weekly basis for each replicate. Average feed consumed by a bird was calculated with the formula;
 Feed Intake = Total Feed Offered – Total Feed Leftover

$$\text{Average Feed Intake (g/bird)} = \frac{\text{Feed Intake}}{\text{No of birds per replicate}}$$

3.6.2. Average body weight gain

Average Body Weight Gain (g/bird) = [(Final Weight (g) – Initial Weight (g)) / (No of Birds Per replicate)]

3.6.3. Feed conversion ratio

The feed conversion ratio was calculated by dividing the feed intake by the weight gain.

$$\text{FCR} = \frac{\text{Total Feed Intake (g)}}{\text{Weight Gain}}$$

3.7. Carcass characteristics and weight of lymphoid organs

Two (2) birds were picked randomly at the end of the

experiment in each replicate and examined with regard to their carcass and body organs. The feed was not eaten 6 hours prior to slaughtering to cleanse the digestive system. The measurements of carcass and body organs were examined in terms of dressing weight, breast, thigh, abdominal fat, gizzard, heart, liver, and lung weight. The weight of the dresses is the weight without visceral and the weights of other body organs were computed.

3.8. Statistical analysis

The data collected was analysed for analysis of variance (ANOVA), and Duncan's Multiple Range Test was used to compare the treatment means. Statistical significance was considered to be $P < 0.05$ (IBM SPSS 26, 2022).

3.9. Ethical considerations

The University of Ibadan Animal Care and Use Research Committee gave approval to the use of animals as per the international and institutional guidelines. UI ACUREC NO: 017-0124/29 Ibadan, Oyo state Nigeria.

4. RESULTS AND DISCUSSION

4.1. Growth performance (6–9 weeks)

As presented in Table 1, feed intake, final weight, and weight gain were significantly ($p < 0.05$) affected by treatments. Birds in T3 showed the best weight gain and final weight, while T5 showed relatively poor results. The feed conversion ratio (FCR) was not significantly different among treatments ($p > 0.05$), but improved values were observed for birds that received EEHP. The initial weight showed no significant difference, indicating uniformity among birds at the start of the experiment.

Table 1. Initial growth performance of cockerel fed aflatoxin b1 contaminated feed and ethanol extract hog plum at grower stage (6-9 weeks).

Parameters	T1 (EPH before afl)	T2(EEHP + afl)	T3 (Afl before EPH)	T4(+control)	T5 (-ve control)	P-value
Feed Intake (g/bird)	1167.14 ^{ab}	1128.57 ^a	1185.00 ^b	1250.78 ^c	1126.23 ^a	0.002
Initial Weight (g)	700.00 ^a	730.43 ^a	736.36 ^a	699.77 ^a	702.17 ^a	0.129
Final Weight (g)	960.00 ^a	1028.57 ^b	1150.00 ^c	1033.92 ^b	1009.52 ^b	0.001
Weight Gain (g)	260.00 ^a	298.14 ^{ab}	413.64 ^c	334.15 ^b	307.35 ^b	0.001
Feed Conversion Ratio	4.49 ^a	3.79 ^a	2.86 ^a	3.74 ^a	3.69 ^a	0.256

^{a,b,c} Means in the same column by factor with different superscripts are significantly ($p > 0.05$) different.

4.2. Growth performance (9–12 weeks)

From Table 2, it is evident that there were differences in feed intake, weight gain, and FCR, which were all significant ($p < 0.05$). Birds in treatment 1 had the highest feed intake but the poorest efficiency in feed utilization, whereas the birds

in treatment 3 had improved growth performance due to the supplementation of EEHP. The final weight of the birds was not significantly different ($p > 0.05$), although the values were higher in the treatment with EEHP.

Table 2. Final growth performance of cockerel fed aflatoxin B1 contaminated feed and ethanol extract Hog plum at grower stage (9-12 weeks).

Parameters	T1 (Afl + 0 EEHP)	T2(water)	T3 (EPH alone)	T4(+control)	T5 (-ve control)	P-value	SEM
Feed Intake (g)	1699.46 ^c	1325.76 ^a	1378.59 ^{ab}	1480.97 ^b	1295.63 ^a	0.001	19.02



Initial weight (g)	960.00 ^a	1028.57 ^b	1150.00 ^c	1033.92 ^b	1009.52 ^b	0.001	15.01
Final weight (g)	1733.33 ^b	1647.06 ^{ab}	1803.92 ^b	1576.47 ^a	1645.03 ^{ab}	0.001	18.12
Weight Gain (g)	773.33 ^c	618.49 ^{ab}	653.92 ^b	542.55 ^a	635.51 ^{ab}	0.001	8.95
FCR	2.20 ^a	2.14 ^a	2.11 ^a	2.74 ^a	2.04 ^a	0.015	0.45

a, b, c Means in the same column by factor with different superscripts are significantly ($p > 0.05$) different.

4.3. Carcass and organ characteristics (6–9 weeks)

As presented in Table 3, carcass characteristics such as final weight, dressed weight, and eviscerated weight were not significantly ($p > 0.05$) affected by the treatments. However, some carcass components, such as head, neck, and shank, showed significant differences. The weights of internal organs such as liver, kidney, heart, and proventriculus were

significantly reduced in birds exposed to aflatoxin, while birds treated with EEHP showed improved results. Lymphoid organs such as the bursa and spleen were also significantly affected. Intestinal weights and lengths, including duodenum, jejunum, and ileum, were significantly affected, and birds treated with EEHP showed improved results.

Table 3. Carcass and weight of lymphoid organs at first phase of the growers phase (6-9 weeks).

Parameters	T1(EPH before afl)	T2(EEHP + afl)	T3 (Afl before EPH)	T4(+control)	T5 (-ve control)	P-Value
Final weight	1079	1005	1007	1014	913.2	0.358
Dressed weight	901.0	814.0	815.0	843.5	758.0	0.281
Eviscerated weight	653.37	627.67	630.00	687.3	596.67	0.532
Head	21.45 ^a	9.20 ^c	9.00 ^c	21.25 ^a	19.21 ^b	0.00
Neck	38.00 ^b	57.00 ^a	57.13 ^a	39.67 ^b	38.10 ^b	0.00
Breast Muscle	131.53	134.00	135.33	131.7	122.20	0.818
Thigh	47.46	47.47	47.50	47.67	43.07	0.777
Drum stick	54.00	48.17	47.00	49.33	44.67	0.193
Back	103.47	115.53	115.67	114.80	96.17	0.399
Wings	41.00	40.50	40.43	45.00	41.00	0.738
Shank	32.38 ^a	28.40 ^{ab}	28.63 ^{ab}	30.33 ^{ab}	27.00 ^b	0.035
Weight of Organs						
Proventriculus	8.00 ^a	3.50 ^{bc}	8.00 ^{bc}	4.50 ^b	3.00 ^c	0.000
Gizzard	35.50	30.50	30.60	30.83	29.73	0.178
Lungs	4.50	5.00	5.20	5.67	4.70	0.094
Liver	30.00 ^a	18.50 ^b	18.50 ^b	20.67 ^b	19.03 ^b	0.000
Kidney	7.50 ^a	6.00 ^{ab}	6.00 ^{ab}	5.00 ^b	4.40 ^b	0.001
Heart	7.33 ^a	5.33 ^{bc}	5.50 ^b	4.17 ^{bc}	3.90 ^c	0.00
Bursa	1.00 ^{abc}	0.00 ^c	0.33 ^{bc}	1.67 ^{ab}	2.20 ^a	0.002
Thymus	3.00	3.167	3.23	2.83	2.33	0.740
Spleen	2.00 ^a	1.50 ^{abc}	1.00 ^c	1.17 ^{bc}	1.83 ^{ab}	0.008
Duodenum weight (g)	16.83 ^a	15.50 ^a	15.27 ^a	13.50 ^b	11.33 ^c	0.000
Duodenum length(cm)	28.57	25.13	24.67	27.17	23.13	0.103
Jejunum weight (g)	27.50 ^a	14.03 ^c	14.03 ^c	18.83 ^b	14.93 ^c	0.00
Jejunum length (cm)	60.43 ^a	48.47 ^b	48.50 ^b	58.33 ^a	50.37 ^b	0.00
Ileum weight (g)	22.30 ^a	11.00 ^c	10.00 ^d	13.83 ^b	11.17 ^c	0.00
Ileum length (cm)	56.37 ^a	51.00 ^b	45.10 ^c	50.83 ^b	51.23 ^b	0.00



4.4. Carcass and organ characteristics (9–12 weeks)

Table 4 indicates that carcass characteristics differed significantly ($p < 0.05$). Birds in T3 (EEHP alone) and T4 (+control) had higher final, dressed, and eviscerated weights than those in other treatments. However, the birds infected with aflatoxin alone (T1) had lower carcass yield. The organ weights, including liver, kidney, heart, and gizzard, were significantly

affected ($p < 0.05$). EEHP supplementation improved organ weight development. The lymphoid organs, including the bursa, thymus, and spleen, were suppressed in the aflatoxin-infected birds but improved in the EEHP-treated birds. The intestinal parameters were also significantly affected, and the EEHP-treated birds had better organ development than the other birds.

Table 4. Carcass and weight of lymphoid organs at second phase of the growers phase (9-12 weeks).

Parameters	T1 (Afl + 0 EEHP)	T2(water)	T3 (EPH alone)	T4(+control)	T5 (-ve control)	P-Value
Final weight	1397.00 ^a	558.00 ^c	1500 ^a	1352.50 ^a	867.00 ^b	0.000
Dressed weight	1142.00 ^a	463.00 ^b	1256.00 ^a	1196.00 ^a	679.00 ^b	0.000
Eviscerated weight	863.00	323.00	1037.00	879.00	516.00	0.000
Head	49.00 ^{ab}	30.00 ^{cd}	66.00 ^a	46.50 ^{bc}	15.00 ^d	0.000
Neck	59.00 ^a	24.00 ^b	25.00 ^b	49.50 ^a	34.50 ^b	0.000
Breast Muscle	197.00 ^a	37.00 ^c	217.00 ^a	182.50 ^a	98.50 ^b	0.000
Thigh	71.00 ^a	21.00 ^c	65.00 ^a	65.00 ^a	40.00 ^b	0.000
Drum stick	66.00 ^b	26.00 ^d	78.00 ^a	65.50 ^b	43.50 ^c	0.000
Back	156.00 ^a	43.00 ^c	170.00 ^a	153.50 ^a	82.00 ^b	0.000
Wings	58.00 ^a	26.00 ^b	63.00 ^a	55.50 ^a	36.00 ^b	0.000
Shank	35.00 ^b	23.00 ^c	47.00 ^a	39.50 ^b	26.50 ^c	0.000
Weight of Organs						
Proventriculus	6.00 ^{ab}	2.00 ^c	5.00 ^{ab}	7.50 ^a	3.50 ^b	0.000
Gizzard	37.00 ^a	15.00 ^c	36.00 ^a	40.00 ^a	26.50 ^b	0.000
Lungs	7.00 ^{ab}	3.00 ^c	7.00 ^{ab}	9.00 ^a	5.00 ^b	0.000
Liver	32.00 ^a	13.00 ^c	26.00 ^{ab}	28.50 ^{ab}	19.50 ^{bc}	0.000
Kidney	9.00 ^a	4.00 ^b	8.00 ^a	7.00 ^a	7.00 ^a	0.001
Heart	8.00 ^b	3.00 ^a	5.00 ^b	8.50 ^a	3.50 ^b	0.000
Bursa	1.00 ^{ab}	0.33 ^{ab}	1.67 ^a	0.50 ^a	0.00 ^b	0.057
Thymus	5.00 ^b	0.00 ^a	4.00 ^c	4.00 ^c	0.00 ^a	0.000
Spleen	4.00 ^a	1.00 ^d	2.00 ^{bc}	2.50 ^b	1.50 ^{cd}	0.000
Duodenum weight (g)	17.00 ^a	13.00 ^b	16.00 ^a	16.50 ^a	13.00 ^b	0.000
Duodenum length(cm)	28.00 ^b	33.00 ^a	29.00 ^b	29.00 ^b	28.50 ^b	0.008
Jejunum weight (g)	20.83 ^{bc}	27.00 ^a	24.00 ^{ab}	24.50 ^a	19.50 ^c	0.000
Jejunum length (cm)	61.00 ^{ab}	64.00 ^a	53.00 ^c	57.50 ^{abc}	55.50 ^{bc}	0.003
Ileum weight (g)	15.00 ^{ab}	17.00 ^{ab}	18.00 ^{ab}	19.50 ^a	13.50 ^b	0.035
Ileum length (cm)	65.67 ^a	57.00 ^b	56.00 ^b	58.50 ^b	59.00 ^b	0.001

4.5. Discussion

In the present study, the growth performance of cockerel chickens was investigated when they were fed AFB1-contaminated diets and then supplemented with ethanol extract of *Spondias mombin* (EEHP). The results showed that the AFB1 exposure and EEHP supplementation affected growth performance, with more pronounced effects during the later stage of growth. There were significant differences in feed intake, body weight, and

weight gain between the treatment groups in weeks 6–9, but no significant differences in feed conversion ratio. There were no significant differences in feed conversion ratios in the early growth phase, which implies that if the toxic effect of AFB1 on nutrient utilisation and feed efficiency were sufficiently great at this growth stage to significantly affect the efficiency of conversion, it has not been. The same was observed by Yunus *et al.* (2011), who reported that aflatoxin in poultry causes



depression in growth performance primarily due to disruption of nutrient metabolism and organ dysfunction, but not through changes in feed conversion efficiency.

In the later growth period, birds exposed to AFB1 and then fed the EEHP supplementation gained more weight, numerically, than the birds not fed EEHP. The present study, however, did not measure nutrient digestibility, compensatory feed intake, or nutrient retention parameters to definitively conclude compensatory growth, though this observation may suggest it. So, caution should be used when interpreting the higher weight gain as evidence of compensatory growth. The compensatory growth of broilers following nutritional or toxic stress has frequently been linked to an increase in feed consumption and a greater efficiency in nutrient utilization, which were not specifically measured in the present study (Zubair & Leeson, 1996). Likewise, Rawal *et al.* (2010) noted that antioxidant supplementation had a beneficial effect on the growth performance of broilers challenged with aflatoxin, suggesting that antioxidants help to reduce the effect of oxidative stress and restore normal levels of metabolic activity.

The negative growth performance effects reported in this study are consistent with previous reports that indicated that AFB1 can inhibit protein synthesis, interfere with hepatic metabolism, and that the AFB1 can form reactive metabolites that bind to cellular macromolecules, causing hepatocellular damage and decreased productive performance. According to Sameh *et al.* (2018), AFB1 is toxic because it forms reactive epoxide metabolites, which disrupt nucleic acid and protein synthesis. Similarly, Rawal *et al.* (2010) found the liver to be the key target organ for aflatoxin toxicity due to its role in bioactivating and detoxifying aflatoxin. Therefore, AFB1 damages the liver and reduces the growth performance of the poultry. Hence, the relatively good performance of EEHP-supplemented birds may be linked with the antioxidant and hepatoprotecting effects previously reported for phytogetic extracts. Manafi *et al.* (2017) have shown that antioxidants containing phytogetic feed additives could decrease the oxidative damage and enhance growth performance in broilers fed with aflatoxin. Furthermore, Sarker *et al.* (2023) found that the juice of *Spondias mombin* leaves had protective effects against organ damage caused by AFB1 in broilers, indicating the presence of bioactive phytochemicals that could aid in enhancing the physiological functions and recovery of growth.

Carcass characteristics included some parameters that numerically differed between treatment groups, but were not statistically significant. As such, the numerical differences should not be interpreted as treatment effects. Non-significant results suggest that although there was a tendency to be affected by the AFB1 exposure and EEHP supplementation, the effects of these on some of the carcass yield parameters were of limited magnitude in the environment of the present study. Denli *et al.* (2009) reported a similar observation that exposure to aflatoxins had more consistent effects on growth performance than on carcass yield traits in broiler chickens.

The better performance of birds that received EEHP treatment after exposure to aflatoxin can be attributed to the bioactive compounds that are present in *Spondia mombin*, such as flavonoids and phenolics. These compounds have antioxidant

properties that scavenge reactive oxygen species that are produced during the metabolism of aflatoxin. This minimizes oxidative stress and protects the hepatic tissue. The role of oxidative stress in aflatoxin-induced toxicity and the role of antioxidant defense in reducing its effects have been well established (Sameh *et al.*, 2018; Souza *et al.*, 2024). In the final phase (9-12 weeks), marked differences were observed in feed intake, final weight, and weight gain, while FCR was statistically similar across treatments. Birds receiving aflatoxin without EEHP supplementation showed significantly higher feed intake but not proportionate weight gain. This is a manifestation of poor nutrient utilization efficiency. This is characteristic of aflatoxicosis, in which nutrient utilization is compromised by reduced enzyme activity and nutrient absorption in the gut, causing increased feed intake without proportionate weight gain (Souza *et al.*, 2024).

On the other hand, birds that were administered EEHP only showed the best final weight, indicating that the compound may have a positive effect on growth performance even without toxin exposure. This could be attributed to improved digestive efficiency and nutrient uptake due to improved gut integrity and metabolic function. Phytogetic compounds have been known to promote gut integrity and influence various physiological activities that can boost growth performance in birds (Sameh *et al.* 2018). The consistency in FCR among all birds in both phases may indicate that birds may have adapted to the dietary treatments over time, even in the presence of aflatoxin. However, the numerically lower FCR among untreated birds in the final phase may indicate that aflatoxin effects on metabolism were not completely resolved, even though they were not statistically different.

The increased weight gain of aflatoxin-exposed birds in the later phase might also be due to compensatory growth following earlier physiological stress. This is because compensatory growth is often seen in birds reared in unfavorable conditions; in such cases, there is an increase in growth rate after the reduction of the unfavorable factor or after physiological adaptation occurs in birds (Sameh *et al.*, 2018). However, it is also clear that such compensatory growth is not an indicator of optimal metabolic efficiency. Overall, it is clear that the results of this study confirm that aflatoxin B1 affects growth performance in cockerel chickens. However, it is also clear that there is a potential ameliorating effect of the ethanol extract of *Spondia mombin* in alleviating the effect of aflatoxin B1. These study results emphasize the potential of using phytogetic compounds as an alternative strategy in controlling aflatoxicosis in birds.

Table 3 and Table 4 show the effects of carcass and weight of lymphoid organs at different phases of the grower's phase. At the first phase (6-9 weeks), there was no significant difference in final weight, dressed weight, and weight of eviscerated carcass ($p > 0.05$). This implies that early exposure to AFB1 has a subclinical effect on the growth of animals. However, other components, such as the weight of the head, neck, and shank, were significantly affected ($p < 0.05$). This implies that early exposure to AFB1 causes different effects on the development of different tissues due to early disruption of physiological activities. This has been observed in broilers that are exposed to mycotoxins (Cloft *et al.*, 2023; Graham *et al.*, 2023).



The weights of the organs, especially the liver, kidney, and heart, were also significantly reduced in AFB1-treated birds during this period (weeks 6-9). The reduction in liver weight is associated with hepatocellular stress and damage due to the action of reactive epoxides of AFB1, which affect protein synthesis and cause oxidative stress (Lee & Ryu, 2017). The reduction in kidney and heart weight also indicates the toxicity of AFB1, which has also been supported by previous studies on the effects of mycotoxins on the health of birds (Cloft *et al.*, 2023). Lymphoid organs like the bursa of Fabricius and the spleen also showed reduced weight in the AFB1-treated birds, indicating immunosuppressive effects of AFB1. The reduction in the weight of the bursa of Fabricius in the AFB1-treated birds also indicates the apoptosis of the lymphoid organs due to AFB1, which has also been supported by previous studies on the effects of AFB1 on the health of birds (Nabi *et al.*, 2024; Lee & Ryu, 2017). In the birds supplemented with EEHP, the lymphoid organs maintained their weight, indicating the immune-protective and antioxidant effects of phytogetic extracts.

Moreover, the effect on intestinal morphology in the first phase showed that aflatoxin-exposed birds had reduced weights and lengths of the duodenum, jejunum, and ileum. Consequently, aflatoxin-induced epithelial damage may result in reduced villus height and, therefore, reduced nutrient uptake (Nwidu *et al.*, 2017; Okechukwu *et al.*, 2024). However, birds that were administered EEHP showed improved intestinal measurements, suggesting that bioactive compounds in EEHP may help to mitigate oxidative stress and thus promote nutrient uptake.

On the other hand, during the second phase (9–12 weeks), the prolonged exposure of birds to AFB1 resulted in more profound effects on carcass characteristics. Birds that were exposed to AFB1 but were not supplemented with EEHP experienced a reduction in final weight, dressed weight, and eviscerated weight at the conclusion of the experiment, as indicated by the results ($p < 0.05$). Conversely, EEHP-supplemented birds had a higher carcass yield, which can be attributed to the protective and growth-promoting effects of EEHP, possibly by improving nutrient assimilation and antioxidant activities (Lee & Ryu, 2017). The weights of the organs in the second phase further established the progressive nature of aflatoxin toxicity. For instance, liver, kidney, and heart weights were significantly reduced in birds that only received aflatoxin, while EEHP supplementation reduced this effect, thus confirming the hepato-protective and systemic antioxidant properties of phytogetic extracts (Cloft *et al.*, 2023; Lee & Ryu, 2017). The weights of the lymphoid organs, including the bursa, thymus, and spleen, were reduced in birds that were exposed to AFB1, thus confirming the immunosuppressive effects of aflatoxin and its ability to influence vaccine efficacy and pathogen resistance (Nabi *et al.*, 2024; Graham *et al.*, 2023).

The intestinal morphological features of the second phase showed that there were significant reductions in the weights and lengths of the duodenum, jejunum, and ileum of the AFB1-exposed birds. This shows that there was a chronic effect on gut integrity. The efficacy of EEHP in maintaining the weights and lengths of the intestine can be explained by the fact that phytogetic extracts have been shown to increase the regeneration of epithelial cells, reduce oxidative stress,

and maintain gut microflora balance, thereby maintaining gut integrity (Nwidu *et al.*, 2017).

Overall, the results show that while early exposure to aflatoxin has a small impact on the physiological status of the birds, prolonged exposure to the toxin results in a marked decline in the performance of the carcass, organs, immunity, and morphology of the intestines of the birds. However, the supplementation of the ethanol extract of *Spondia mombin* was seen to reduce the adverse effects of the aflatoxin, which might have been mediated by antioxidant, hepatoprotective, immunomodulatory, and gut-protective effects of the EEHP, thereby confirming its potential as a natural feed additive for improving the resistance of the birds to aflatoxin toxicity.

5. CONCLUSION

The present research has discovered that the AFB1 exposure had major effects on growth performance, carcass traits, lymphoid organ development, and intestinal morphology in cockerel chickens. The adverse outcomes of AFB1 are augmented with an increase in the exposure time. The performance of birds exposed to AFB1 was identified by decreased increase in weight, low feed conversion ratio, and a large decrease in liver weight, kidney weight, heart weight, bursa weight, and spleen weight. There was a significant effect of intestinal features in the AFB1-exposed birds on duodenum weight, jejunum weight, ileum weight, duodenum length, jejunum length, and ileum length.

The supplementation of EEHP alleviated the adverse effects of AFB1, as indicated by the improved growth performance, carcass characteristics, lymphoid organ weights, and the protective effects on the intestinal morphology of the challenged birds. The results of the study indicated that EEHP supplementation to the diet of broiler chickens challenged with AFB1 has protective effects, which might be mediated by antioxidant, hepatoprotective, immunomodulatory, and gut-protective activities.

The results of the study demonstrated the efficacy of EEHP as a natural feed supplement to counteract the adverse effects of aflatoxicosis, which warrants its potential use in the control of the syndrome in poultry, thereby improving the productivity and health of the birds, as well as protecting the immune system of the birds from the toxic effects of AFB1.

RECOMMENDATION

Further studies are recommended to understand the molecular mechanisms of the protective effects of EEHP against the toxicity of AFB1 to the birds.

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