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Investigating the effects of dietary supplementation with Moringa leaf powder and vitamin C in aflatoxin B1-exposed broilers.

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Abstract

This study investigates the use of Moringa Leaf Powder (MLP) and Vitamin C in the diets of broiler chickens exposed to Aflatoxin B1 (AFB1) to enhance performance and health. Two hundred one-day old Cobb 500 broiler breed chicks were divided into four diet groups: CON (no AFB1, no MLP), AFB (0.2 mg AFB1 per kg of feed), AFV (0.2 mg AFB1 with 200 mg vitamin C), and AFM (0.2 mg AFB1 with 500 mg MLP). Supplementation with MLP and vitamin C led to improved broiler performance, with AFM and AFV groups exhibiting higher body weight gain, similar or lower feed intake, and better feed conversion ratios compared to AFB. Mortality rates were lower in AFM and AFV, and dressing percentages and liver weights were higher. Haematological parameters showed significant improvements in AFM and AFV compared to AFB. MLP and vitamin C reduced serum cholesterol levels and normalised liver enzymes. MLP improved kidney function. Using 200 mg kg^{-1} vitamin C or 500 mg kg^{-1} Moringa oleifera powder as dietary supplements for broiler chickens exposed to aflatoxin B1 is recommended for improved productivity and health.

Keywords: ascorbic acid, poultry, supplements, toxin

1 Introduction

Aflatoxin B1 (AFB1) contamination in poultry feed remains a persistent concern in the poultry industry due to its detrimental effects on broiler chickens' health, performance, and the quality of their meat products. AFB1 is a naturally occurring mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* molds, commonly found in feed ingredients such as corn and peanuts (Kang'ethe *et al.*, 2009). The consumption of AFB1-contaminated feed by broiler chickens can lead to a range of adverse effects, including reduced growth performance, compromised immune function, and increased susceptibility to various diseases (Liao *et al.*, 2014; Kolawole *et al.*, 2022).

In response to the ongoing challenges posed by AFB1 contamination, researchers have explored various strategies to mitigate its negative impacts on poultry. One such approach involves the use of natural feed additives with proven antioxidant and detoxifying properties. *Moringa oleifera*, commonly referred to as the "drumstick tree" or "horseradish tree", has garnered significant attention in recent years for its potential as a dietary supplement in animal nutrition. Moringa leaves, in particular, are rich in bioactive compounds, including vitamins, minerals, polyphenols, and flavonoids, which are known to possess antioxidant and anti-inflammatory properties (Mahfuz & Piao, 2019). The utilisation of Moringa leaf powder (MLP) as a dietary supplement for broiler chickens exposed to AFB1-contaminated diets holds promise as a potential solution to mitigate the

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negative effects of mycotoxin exposure. Existing literature suggests that MLP supplementation may improve growth performance, enhance carcass traits, and modulate serum enzymes and metabolites in broiler chickens (Hosseini-Vashan *et al*., 2016; Mahfuz and Piao, 2019). Additionally, MLP's antioxidant properties may contribute to the maintenance of meat quality and the protection of haematological indices in AFB1-exposed birds (Peñalver *et al*., 2022).

The utilisation of vitamin C as an antioxidant against aflatoxin contamination in poultry has garnered significant attention due to its potential to mitigate the adverse effects of mycotoxin exposure. Aflatoxins, notably aflatoxin B1 (AFB1), are known to induce oxidative stress in birds, leading to cellular damage, compromised immune responses, and reduced overall health and performance (Hieu *et al*., 2022). Vitamin C, also known as ascorbic acid, is a powerful water-soluble antioxidant that plays a crucial role in scavenging free radicals and preventing oxidative damage (Traber & Stevens, 2011). When incorporated into the diet of poultry exposed to AFB1, vitamin C can bolster the birds' antioxidant defense mechanisms. It helps neutralize harmful reactive oxygen species generated by aflatoxins, thereby reducing oxidative stress and minimizing cellular damage. Additionally, vitamin C may enhance the birds' immune function, which is often impaired by mycotoxin exposure (Fouad *et al*., 2019).

Supplementing broiler chicken diets with Moringa leaf powder (MLP) and vitamin C represents a multifaceted approach to mitigating the adverse effects of AFB1 exposure. Literature suggests that MLP and vitamin C supplementation may improve growth performance, enhance carcass traits, modulate serum enzymes and metabolites, bolster meat antioxidant enzymes, and positively influence haematological indices in broiler chickens (Hosseini-Vashan *et al*., 2016; Attia *et al*., 2017; Peñalver *et al*., 2022).

In view of these promising findings, this study aims to comprehensively investigate the impact of incorporating Moringa leaf powder and vitamin C into the diets of broiler chickens exposed to AFB1. This work aims at evaluating the performance parameters, carcass traits, serum enzymes, metabolites, meat antioxidant enzymes, and haematological indices to gain a comprehensive understanding of the potential benefits of MLP and vitamin C supplementation in the context of AFB1 challenge conditions.

2 Materials and methods

2.1 Ethical approval; Phytogen collection, processing, and analysis

The regulations for the animals and the animal method were approved by the Research and Ethics Committee of the Department of Animal Health and Production, The Federal College of Agriculture, Akure, Nigeria. Fresh leaves of *Moringa oleifera* were collected within the premises of the Federal College of Agriculture, Akure, Nigeria and allowed to dry in the shade for 14 days before being ground into "Moringa leaf meal" (MLP). The MLP was analysed for ferric acid reducing power (Benzie & Strain, 1996), alkaloid (Adeniyi *et al*., 2009), saponins (He *et al*., 2014), flavonoids (Surana *et al*., 2016), tannins (Biswas *et al*., 2020), phenols (Otles & Yalcin (2012) and lipid peroxidation activities (Bajpai *et al*., 2015).

2.2 Experimental diet and Aflatoxin B1

To adhere to the recommended dietary standards for broiler chickens during both the starter and finisher phases, we formulated a foundational/basal diet as outlined in Table 1. Aflatoxin production originated from a pure culture of *Aspergillus flavus* (NRRL 3251). Aflatoxin B1 (AFB1) was generated through solid fermentation using grit maize as a substrate, following the method detailed in Gbore *et al*.'s (2016) study. After the cultivation process, the grit maize underwent a drying procedure at 50 °C for 20 hours, after which it was finely pulverised using an electric blender. To assess the AFB1 content, we conducted a triplicate analysis using thin-layer chromatography, following the AOAC (2010) method. The quantification of AFB1 in the ground maize was carried out at the Animal Care Disease Diagnosis/Control and Feed Analysis Laboratory in Ibadan, Nigeria, employing the thin-layer chromatography technique.

To achieve the desired concentration of approximately 0.2 mg AFB1 kg^{-1} in the chicken feed during both the starter and finisher phases, a precise procedure was employed. Initially, 1 gram of AFB1-infused maize powder was meticulously blended with 1 kilogram of uncontaminated milled maize. This blending process resulted in a detected AFB1 concentration of 0.00403 mg AFB1 kg^{-1} in the maize mixture. Subsequently, the known quantity of AFB1 from the combination of 1 g AFB1 with uncontaminated milled maize was used to calculate the amount of AFB1-infused maize powder required to be mixed with 1 kg of uncontaminated maize to achieve an AFB1 contamination level of approximately 0.4 mg kg^{-1}. Consequently, AFB1-infused maize was blended with uncontaminated maize at a ratio of 99.39 g kg^{-1} to attain the desired 0.4 mg AFB1 kg^{-1} contamination, resulting in what we will refer to as AFB1-contaminated maize. Ultimately, to achieve an approximate AFB1 contamination level of 0.2 mg AFB1 kg^{-1} in treatment groups 2, 3, and 4, the feed ingredients were supplemented with 50.36 % and 58.36 % of AFB1-contaminated maize to make up the total feed composition for both the starter and

Table 1: *Composition of the experimental diets*

	Broiler starter experimental diets				*Broiler finisher experimental diets*			
Ingredients	*CON*	*AFB*	*AFV*	*AFM*	*CON*	*AFB*	*AFV*	*AFM*
Maize	50.36	50.36	50.36	50.36	58.36	58.36	58.36	58.36
Maize bran	3.00	3.00	3.00	3.00	0	0	0	0
Rice bran	0	0	0	0	3.02	3.02	3.02	3.02
Fish meal	3	3	3	3	3	3	3	3
Soybean meal	38	38	38	38	30	30	30	30
Bone meal	3	3	3	3	3	3	3	3
Premix	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Limestone	0.49	0.49	0.49	0.49	0.47	0.47	0.47	0.47
Salt	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Lysine	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Methionine	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Soy oil	1	1	1	1	1	1	1	1
Composition (%)								
Metabolisable energy (kcal kg^{-1})	3018.1	3018.1	3018.1	3018.1	3108.2	3108.2	3108.2	3108.2
Available phosphorus	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.43
Calcium	1.03	1.03	1.03	1.03	1.04	1.04	1.04	1.04
Crude fibre*	3.52	3.52	3.52	3.52	3.58	3.58	3.58	3.58
Crude fat*	4.23	4.23	4.23	4.23	2.38	2.38	2.38	2.38
Crude protein*	22.17	22.17	22.17	22.17	20.04	20.04	20.04	20.04
Metabolisable energy (kcal kg^{-1})	3018.1	3018.1	3018.1	3018.1	3108.2	3108.2	3108.2	3108.2
Aflatoxin B1* (mg kg-$^{-1}$)	NN	0.19	0.19	0.19	NN	0.18	0.18	0.18

* Analysed composition; NN: Not negligible; CON: No AFB1 contamination; no MLP supplementation; AFB: 0.2 mg of AFB1 per kg of feed; AFV: 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin per kg of feed; AFM: 0.2 mg of AFB1 per kg of feed, along with 500 mg of MLP per kg of feed.

finisher feeds, respectively. Following this adjustment, the experimental diets or treatments (1, 2, 3, and 4) were analysed for AFB1 quantification (Table 1). For each phase of the broiler chicken production, the diets were formulated as follows:

CON: No AFB1 contamination; no MLP supplementation.

AFB: 0.2 mg of AFB1 per kg of feed

AFV: 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin C per kg of feed

AFM: 0.2 mg of AFB1 per kg of feed, along with 500 mg of MLP per kg of feed

The nutritional composition of these diets was assessed in accordance with AOAC (2010).

The concentration of approximately 0.2 mg AFB1 kg^{-1} in the chicken diet represented a substantial elevation, being 10 times higher than the permissible limit of 0.02 mg kg^{-1} as specified by NAFDAC, the EU, the USFDA, and the CFIA (Boudergue *et al*., 2009). Subsequently, the four distinct dietary treatments underwent AFB1 quantification analysis to accurately determine the AFB1 levels within each treatment.

2.3 Experimental site and experimental birds

A feeding study was conducted at the Teaching and Research Farm of the Federal College of Agriculture in Akure, Nigeria. The study involved 200 one-day-old chicks of the Cob 500 broiler breed. These chicks were randomly assigned to four distinct experimental diets, with each diet group comprising 50 birds, organised into 5 replications of 10 birds each.

To house the birds, experimental pens measuring 2 m × 2 m were used, and they were filled with dry wood shavings to a depth of 3 cm. Ensuring an appropriate environmental temperature was crucial for the birds' well-being. Therefore, the temperature in the experimental room was meticulously controlled. In the initial week of the study, the room temperature was maintained at a constant 31±2 °C. Over the subsequent two weeks, the temperature was gradually reduced by 2 °C per week. During weeks 4 to 6 of the

rearing period, the birds were exposed to the natural ambient temperature of the environment.

Lighting conditions were also managed systematically to optimize growth conditions. On the first day of the experiment, the lights were continuously on for 24 hours. From day 2 through 7, lighting was provided for 23 hours each day. For the remainder of the rearing period (weeks 4 to 6), the birds received 18 hours of illumination daily. Throughout the entire six-week duration of the experiment, the birds had uninterrupted access to their respective diets as well as to drinking water.

2.4 Performance characteristics

At regular intervals of seven days, critical growth parameters, including feed intake, body weight gain, and total body weight, were carefully recorded. These data were essential for assessing the birds' growth performance. The feed conversion ratio (FCR), representing the proportion of feed consumed to the increase in body weight, was calculated as an indicator of feed efficiency.

2.5 Slaughter and carcass evaluation

On the 42nd day of the experiment, we randomly selected 12 birds from each treatment group, with two birds chosen from each replication. The slaughtering process was conducted by following the guidelines established by the European Union for the humane treatment of animals during slaughter and killing, as detailed by Uijttenboogaart (1999). The carcasses underwent a series of procedures, including spray-washing and chilling at 2 °C for 30 minutes. To assess meat yield, the dressing percentage, which signifies the ratio of carcass weight to the final body weight was calculated. Additionally, the relative weights of specific organs (liver, heart, lung, proventriculus, gizzard, spleen, and pancreas) as a percentage of the slaughter weight were determined.

2.6 Blood collection and analysis

Blood samples were collected from the chickens and divided into two types of sample bottles: one without any anticoagulant (referred to as plain bottles) and the other containing ethylenediaminetetraacetic acid (EDTA). The samples in the plain bottles were subjected to centrifugation to separate the serum, which was then transferred to another set of plain bottles and stored at −20 °C for subsequent analysis. The assessments of various haematological parameters, including red blood cell count (RBC), haemoglobin concentration (HbC), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and white blood cell count (WBC), were determined by following the methodology outlined by Cheesbrough (2000). Furthermore, the serum markers such as cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine with a Reflectron® Plus 8C79 (Roche Diagnostic, GmbH Mannheim, Germany), were determined using commercial kits (Oloruntola *et al*., 2018).

2.6.1 Statistical analysis

The data collected for this study underwent statistical analysis using SPSS software, version 20. A one-way analysis of variance (ANOVA) was employed to examine all the collected data. The ANOVA model was structured as follows: $\mathrm{Adj} = \bar{u} + \mathrm{ad} + \mathrm{edj}$

In this equation, "Adj" represents the response variables, encompassing the various measurements and parameters gathered during the experiment. "$\bar{U}$" denotes the overall mean, signifying the average value of the response variables across all treatment groups. "ad" signifies the effect of the dth treatment, where 'd' corresponds to the four distinct dietary treatments (diets 1, 2, 3, and 4). "edj" takes into account the random error inherent in the experimental process. Subsequent to the ANOVA, the Duncan multiple range test, a post-hoc test, was utilized to differentiate significant differences among the means of the different treatment groups. The level of statistical significance was set at $p < 0.05$.

3 Results

MLP exhibits several noteworthy characteristics: it possesses a ferric reducing antioxidant power of 56.78 mg g^{-1}, contains 2.91 % alkaloids, has a substantial saponin content at 90 mg g^{-1}, boasts a significant flavonoid content of 55.21 mg g^{-1}, contains 60.64 mg g^{-1} of tannins, demonstrates a phenolic compound content of 56.76 mg g^{-1}, and showcases significant lipid peroxidation activity at 35.43 % (as presented in Fig. 1).

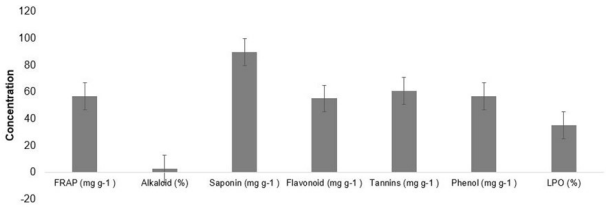

Fig. 1: *The Chemical composition and properties of Moringa oleifera leaf powder FRAP: Ferric Reducing Antioxidant Power; LOP: Lipid Peroxidation*

Table 2 depicts the influence of dietary supplementation with *Moringa oleifera* leaf powder (MLP) and vitamin C on

Table 2: *Effects of* Moringa oleifera *leaf powder (MLP) and vitamin C supplementations on the performance of broiler chickens fed aflatoxin B1 contaminated diets.*

Parameters	*CON*	*AFB*	*AFV*	*AFM*	*SEM*	*P value*
Initial weight (g bird^{-1})	44.91	44.42	44.99	44.75	0.14	0.53
Final body weight (g bird^{-1})	2655.88^{a}	2420.52^{b}	2633.19^{a}	2568.08^{a}	31.60	0.01
Body weight gain (g bird^{-1})	2799.79^{a}	2464.94^{b}	2578.18^{a}	2612.83^{a}	31.66	0.01
Feed intake	4094.97^{a}	3849.25^{c}	3952.64^{bc}	3988.26^{b}	29.84	0.01
Feed conversion ratio	1.52	1.56	1.48	1.53	0.01	0.09
Mortality	0.00^{b}	7.00^{a}	0.33^{b}	0.67^{b}	0.95	0.01

Means in the same row with distinct letters indicate statistically significant differences (P < 0.05); SEM: Standard error of mean; CON: No AFB1 contamination; no MLP supplementation; AFB: 0.2 mg of AFB1 per kg of feed; AFV: 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin per kg of feed; AFM: 0.2 mg of AFB1 per kg of feed, along with 500 mg of MLP per kg of feed.

the performance of broiler chickens exposed to aflatoxin B1-contaminated diets. Birds in the AFM and AFV groups exhibited comparable body weight gain (BWG) ($p > 0.05$) to the CON group, which represents chickens without AFB1 contamination and without MLP supplementation. However, their BWG was significantly higher ($p < 0.05$) than that of the AFB group. Feed intake (FI) in the AFM group was comparable ($p > 0.05$) to that in the AFV group but significantly lower ($p < 0.05$) than that in the CON group. However, FI in the AFB group, although similar ($p > 0.05$) to the AFV group, was lower ($p < 0.05$) than in the CON and AFM groups. The Feed Conversion Ratio (FCR) displayed a tendency ($p = 0.09$) to be influenced by dietary treatments. Mortality rates in the AFM and AFV groups were similar ($p > 0.05$) to the CON group but significantly lower ($p < 0.05$) than in the AFB group.

Table 3: *Effects of* Moringa oleifera *leaf powder (MLP) and vitamin C supplementations on the performance of broiler chickens fed aflatoxin B1 contaminated diets.*

Parameters	*CON*	*AFB*	*AFV*	*AFM*	*SEM*	*P value*
Dressing	73.14^{a}	65.80^{b}	72.86^{a}	70.80^{a}	1.03	0.01
Liver	1.65^{b}	1.85^{a}	1.60^{b}	1.68^{b}	0.03	0.00
Heart	0.42	0.48	0.40	0.40	0.01	0.14
Lung	0.38	0.35	0.30	0.45	0.02	0.06
Proventr.	0.30	0.33	0.37	0.34	0.01	0.10
Gizzard	1.62	1.51	1.71	1.55	0.03	0.17
Spleen	0.08^{b}	0.12^{a}	0.08^{b}	0.07^{b}	0.01	0.00
Pancreas	0.15	0.16	0.17	0.14	0.00	0.16

Proventr. = Proventriculus; Means in the same row with distinct letters indicate statistically significant differences (P < 0.05); SEM: Standard error of mean; CON: No AFB1 contamination; no MLP supplementation; AFB: 0.2 mg of AFB1 per kg of feed; AFV: 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin per kg of feed; AFM: 0.2 mg of AFB1 per kg of feed, along with 500 mg of MLP per kg of feed.

Table 3 presents the impact of dietary supplementation with *Moringa oleifera* leaf powder (MLP) and vitamin C on the dressing percentage and relative organ weights in broiler chickens exposed to aflatoxin B1 (AFB1) contaminated diets. Dressing percentages of broiler chickens in the AFM and AFV groups were not significantly different ($p > 0.05$) from those in the CON group (no AFB1 contamination and no MLP supplementation). However, both AFM and AFV groups had significantly higher dressing percentages ($p < 0.05$) compared to the AFB group (0.2 mg of AFB1 per kg of feed). The relative weight of the liver was significantly higher ($p < 0.05$) in all treatment groups (CON, AFV, and AFM) compared to the AFB group. Although not statistically significant ($p = 0.06$), there is a tendency for the lung's relative weight to be influenced by the supplementation with MLP and vitamin C. The relative weights of the spleens in the AFM and AFV groups were similar ($p > 0.05$) to those in the CON group but significantly lower ($p < 0.05$) than those in the AFB group.

Table 4 presents the impact of dietary supplementation with *Moringa oleifera* leaf powder (MLP) and vitamin C on the haematological parameters of broiler chickens exposed to aflatoxin B1 (AFB1) contaminated diets. Red blood cell count (RBC), haemoglobin concentration (HbC), and packed cell volume (PCV) in the AFV and AFM groups showed no significant differences ($p > 0.05$ when compared to the CON group, representing chickens without AFB1 contamination and without MLP supplementation. However, these parameters were notably higher ($p < 0.05$) in both the AFV and AFM groups in comparison to the AFB group, which was subjected to 0.2 mg of AFB1 per kg of feed. Moreover, various other haematological indices, (mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, white blood cell count, granulocytes, lymphocytes, and monocytes), remained unaffected by the dietary interventions and exhibited similar values across all treatment groups.

Table 5 illustrates the impact of dietary supplementation with *Moringa oleifera* leaf powder (MLP) and vitamin C on

Table 4: *Effects of* Moringa oleifera *leaf powder (MLP) and vitamin C supplementations on the performance of broiler chickens fed aflatoxin B1 contaminated diets.*

Parameters	*CON*	*AFB*	*AFV*	*AFM*	*SEM*	*P value*
Red blood cells (x10^6 l^{-1})	2.70^a	2.30^b	2.80^a	2.85^a	0.08	0.04
Haemoglobin conc. (g dl^{-1})	11.50^a	10.44^b	11.83^a	11.37^a	0.19	0.03
Packed cell volume (%)	34.50^a	31.33^b	35.50^a	34.12^a	0.58	0.03
Mean cell volume (fl)	128.20	136.49	126.79	120.93	3.01	0.37
Mean cell haemoglobin (pg)	42.73	45.50	42.26	40.31	1.00	0.37
Mean cell haemoglobin conc. (g dl^{-1})	33.33	33.34	33.34	33.34	0.00	0.49
White blood cells (x10^9 l^{-1})	3.70	5.60	2.90	3.30	0.62	0.48
Granulocytes (x10^9 l^{-1})	1.38	2.90	0.58	1.00	0.43	0.26
Lymphocytes (x10^9 l^{-1})	2.26	2.30	2.32	2.28	0.19	1.00
Monocytes (x10^9 l^{-1})	0.08	0.40	0.00	0.03	0.07	0.10

Means in the same row with distinct letters indicate statistically significant differences ($P < 0.05$); SEM: Standard error of mean; CON: No AFB1 contamination; no MLP supplementation; AFB: 0.2 mg of AFB1 per kg of feed; AFV: 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin per kg of feed; AFM: 0.2 mg of AFB1 per kg of feed, along with 500 mg of MLP per kg of feed.

Table 5: *Effects of* Moringa oleifera *leaf powder (MLP) and vitamin C supplementations on the performance of broiler chickens fed aflatoxin B1 contaminated diets.*

Parameters	*CON*	*AFB*	*AFV*	*AFM*	*SEM*	*P value*
Cholesterol (mmol l^{-1})	2.70^a	1.83^c	2.23^b	2.23^b	0.11	0.00
Alanine aminotransferase (U I^{-1})	36.60^b	48.62^a	39.90^b	41.07^b	1.65	0.03
Aspartate aminotransferase (U I^{-1})	69.73^b	94.92^a	77.58^b	79.12^b	3.21	0.01
Creatinine (mmol l^{-1})	77.05^{bc}	141.50^a	84.70^{ab}	87.85^b	8.50	0.00

Means in the same row with distinct letters indicate statistically significant differences ($p < 0.05$); SEM: Standard error of mean; CON: No AFB1 contamination; no MLP supplementation; AFB: 0.2 mg of AFB1 per kg of feed; AFV: 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin per kg of feed; AFM: 0.2 mg of AFB1 per kg of feed, along with 500 mg of MLP per kg of feed.

the serum chemistry indices of broiler chickens exposed to aflatoxin B1 (AFB1) contaminated diets. Notably, the serum cholesterol level in the AFB group was significantly lower ($p < 0.05$) compared to the CON, AFV, and AFM groups. Additionally, the serum cholesterol levels in both the AFV and AFM groups were significantly lower ($p < 0.05$) than the CON group. In the AFV and AFM groups, the levels of serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were comparable ($p > 0.05$) to those in the CON group. However, these levels were significantly higher ($p < 0.05$) in the AFB group. The serum creatinine concentration, which reflects kidney function. In the AFM group, the serum creatinine concentration was similar ($p > 0.05$) to that in the CON and AFV groups but significantly lower ($p < 0.05$) than in the AFB group.

4 Discussion

Antioxidants present in Moringa play a crucial role in mitigating oxidative stress and safeguarding against cellular damage (Kou *et al*., 2018). The existence of alkaloids within *Moringa oleifera* implies the presence of potential bioactive compounds. Alkaloids can exert various physiological effects and may contribute to the plant's therapeutic attributes (Pop *et al*., 2022). Notably, Moringa boasts a substantial saponin content of 90 mg g^{-1}. Saponins have gained recognition for their diverse biological functions, including antioxidant and anti-inflammatory properties (Vergara-Jimenez *et al*., 2017). The significant presence of flavonoids, quantified at 55.21 mg g^{-1}, in *Moringa oleifera* aligns with its reputation as a rich source of these compounds.

Flavonoids are renowned for their antioxidative, anti-inflammatory, and potential health-enhancing effects (Ullah *et al*., 2020). The elevated tannin content, measuring 60.64 mg g^{-1}, in *Moringa oleifera* suggests possible astringent properties. Tannins may also contribute to its antioxidant activity (Peñalver *et al*., 2022). *Moringa oleifera* showcases a notable phenolic content of 56.76 mg g^{-1}. Phenolic compounds are celebrated for their antioxidant attributes and potential health advantages (Tungmunnithum *et al*.,

2018). The substantial lipid peroxidation activity, recorded at 35.43 % , hints at *Moringa oleifera*'s potential to protect lipids, possibly due to its abundance of antioxidant constituents (Vergara-Jimenez *et al*., 2017).

The BWG in broiler chickens is a vital indicator of their growth performance and overall health. In this study, it is evident that the inclusion of either MLP or vitamin C in the diet of birds exposed to AFB1 contamination had a beneficial effect on BWG compared to the AFB group. Both AFM (0.2 mg of AFB1 per kg of feed with 500 mg of MLP per kg of feed) and AFV (0.2 mg of AFB1 per kg of feed with 200 mg of vitamin C per kg of feed) groups exhibited BWG similar to the CON group, which had no AFB1 contamination and no MLP supplementation. This finding suggests that both MLP and vitamin C may mitigate the negative impact of AFB1 on growth performance. The improvement in BWG can be attributed to the antioxidant properties of MLP and vitamin C, which may have counteracted the oxidative stress induced by AFB1 (Alpsoy *et al*., 2009; Jobe *et al*., 2023). Feed intake (FI) is another critical parameter reflecting the dietary preferences and overall health of broiler chickens (Classen, 2017). The results indicate that birds in the AFM group had FI comparable to those in the AFV group. However, both groups showed significantly lower FI compared to the CON group. The reduced FI observed in the AFB group, is consistent with previous studies (Mesgar *et al*., 2023). It is noteworthy that the inclusion of MLP or vitamin C did not completely restore FI to the levels seen in the uncontaminated CON group. This may be due to the persistent effects of AFB1 on feed intake, despite the supplementation. The ability of AFB1 to reduce FI is a well-documented phenomenon (Saleemi *et al*., 2020). Although the FCR showed a tendency to be affected by the dietary treatments, the differences did not reach statistical significance in this study. FCR is a key indicator of feed efficiency, and while the numerical trends suggest some variations. Mortality rates provide crucial insights into the overall health and well-being of broiler chickens. The significantly lower mortality rates in the AFM and AFV groups compared to the AFB group indicate that the supplementation of MLP and vitamin C had a protective effect against the adverse consequences of AFB1. This aligns with the known antioxidant and health-promoting properties of these dietary additives (Huang 2018; Peñalver *et al*., 2022).

The dressing percentage of broiler chickens is a crucial parameter in the meat industry, as it reflects the yield of edible meat after processing (Sin-Young *et al*., 2021). In this study, the dressing percentages of birds in the AFM and AFV groups were not different from those in the CON group, suggesting that the supplementation of either MLP or vitamin C helped maintain dressing percentages similar to those in uncontaminated diets (CON). Importantly, both the AFM and AFV groups exhibited significantly higher dressing percentages compared to the non-supplemented AFB group,. This finding is notable as it indicates that AFB1 contamination had a negative impact on dressing percentage, possibly due to its adverse effects on broiler chicken health and growth (Chen *et al*., 2022). However, the inclusion of MLP or vitamin C in the diet appears to have mitigated this negative influence, resulting in higher dressing percentages (Mahfuz & Piao, 2019). This aligns with previous research suggesting that MLP and vitamin C possess antioxidant properties that may counteract the adverse effects of AFB1 (Huang 2018; Peñalver *et al*., 2022). The significant increase in the relative weight of the liver in all treatment groups (CON, AFV, and AFM) compared to the AFB group indicates that AFB1 contamination likely led to changes in the liver, such as enlargement or congestion. AFB1 is known to exert hepatotoxic effects on poultry (Xi *et al*., 2022). The higher liver relative weight in the supplemented groups (AFM and AFV) suggests that MLP and vitamin C may have helped mitigate the AFB1-induced liver alterations. These findings align with the hepatoprotective properties of MLP and vitamin C (Su *et al*., 2019; Vergara-Jimenez *et al*., 2019). The significant decrease in the relative weights of the spleens in the AFM and AFV groups compared to the AFB group suggests that AFB1 contamination may have led to an increase in spleen weight. This is consistent with the immunotoxic effects of AFB1, which can stimulate the immune system (Meissonnier *et al*., 2008). The supplementation with MLP or vitamin C appears to have partially mitigated this effect by reducing spleen size. This is in line with the immunomodulatory properties reported for MLP and vitamin C (Nfambi *et al*., 2015; Carr & Maggini, 2017).

Haematological parameters provide valuable insights into the physiological status of animals and can serve as indicators of overall health (Pessini *et al*., 2020). The RBC, HbC, and PCV are essential components of the complete blood count and play a crucial role in oxygen transport and overall blood health. The results demonstrated that RBC, HbC, and PCV levels in the AFV and AFM groups were not significantly different from those in the CON group, which indicates that supplementation with either MLP or vitamin C effectively maintained these parameters at levels comparable to those in uncontaminated diets (CON). However, it's important to note that both the AFV and AFM groups exhibited significantly higher levels of RBC, HbC, and PCV compared to the AFB group, which experienced AFB1 contamination. This is a significant finding as it suggests that MLP and vitamin C supplementation may counteract the negative impact

of AFB1 on these crucial haematological parameters. The improvement in RBC, HbC, and PCV can be attributed to the potential antioxidant properties of MLP and vitamin C, which may have mitigated the oxidative stress induced by AFB1 (Al Balushi *et al*., 2019).

Serum cholesterol is a critical parameter that reflects lipid metabolism and overall health in animals (Stellaard, 2022). The results indicate that chickens exposed to AFB1 contamination (AFB group) exhibited significantly lower serum cholesterol levels compared to the control group (CON), which represents chickens with no AFB1 contamination and no MLP supplementation. This finding aligns with previous research suggesting that AFB1 exposure can lead to disruptions in lipid metabolism and result in reduced serum cholesterol levels (Rotimi *et al*., 2017).

Remarkably, the supplementation of either MLP or vitamin C in the AFV and AFM groups resulted in even lower serum cholesterol levels compared to the CON group. This is a noteworthy observation, as it suggests that these supplements had a lipid-lowering effect, potentially contributing to improved overall health. The cholesterol-lowering properties of *Moringa oleifera* have been reported in previous studies (Kou *et al*., 2018). This may be attributed to the presence of bioactive compounds, including flavonoids and saponins, in Moringa leaves that are known for their lipid-lowering effects (Vergara-Jimenez *et al*., 2017).

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) are enzymes produced by the liver and are used as indicators of liver function (Huang *et al*., 2006). In the AFV and AFM groups, the levels of ALT and AST were like those in the CON group, indicating that MLP and vitamin C supplementation effectively maintained normal liver enzyme levels. However, these levels were significantly higher in the AFB group, suggesting impaired liver function due to AFB1 contamination. This is consistent with previous findings that AFB1 exposure can lead to liver damage and elevated liver enzymes (Mekuria *et al*., 2023). The ability of MLP and vitamin C to normalize ALT and AST levels is significant and suggests potential hepatoprotective properties. *Moringa oleifera* is known for its hepatoprotective effects, which may be attributed to its antioxidant compounds, including phenolics and flavonoids (Asgari-Kafrani *et al*., 2020). Similarly, vitamin C is recognized for its role in protecting the liver from oxidative stress and maintaining liver health (He *et al*., 2021).

Serum creatinine concentration is a crucial parameter for assessing kidney function. In the AFM group, the serum creatinine concentration was similar to that in the CON and AFV groups but lower than in the AFB group. Elevated serum creatinine levels in the AFB group suggest impaired kidney function, a known consequence of aflatoxicosis (Yilmaz *et al*., 2018). However, the normalization of serum creatinine levels in the AFM group indicates that MLP supplementation may have a protective effect on kidney function.

5 Conclusion

Moringa oleifera powder and vitamin C supplementation improved the performance characteristics and positively influence essential haematological parameters, while other indices remain stable. In addition, these supplements also lead to lower serum cholesterol levels, normalized liver enzymes, and improved kidney function. The use of 200 mg kg^{-1} vitamin C or 500 mg kg^{-1} Moringa oleifera powder as dietary supplements for broiler chickens exposed to aflatoxin B1 is recommended to enhance productivity and sustain health status.

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Ethical standards

The regulations for the animals and the animal method were approved by the Research and Ethics Committee of the Department of Animal Health and Production, The Federal College of Agriculture, Akure, Nigeria.

Conflict of interest

The authors declare no conflict of interest.

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