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Original Paper

Evaluating the Effects of Including Vitamin C and *Parquetina nigrescens* Leaf Powder in the Diets of Broiler Chicks Exposed to Aflatoxin B1

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In this study, we evaluated the effects of dietary vitamin C and *Parquetina nigrescens* leaf powder (PLP) in broiler chicks exposed to aflatoxin B1 (AFB1). The treatments consisted of CONT (receiving a diet without AFB1 contamination and without PLP supplementation); AFLB (fed a diet containing 0.2 mg of AFB1 per kg of feed); AFVC (fed a diet containing 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin C per kg of feed); and AFLP (fed a diet containing 0.2 mg of AFB1 per kg of feed along with 500 mg of PLP per kg of feed). Our findings demonstrate the rich bioactive profile of PLP, including significant antioxidant capacity and various beneficial compounds. When it comes to broiler chicken performance, we observed that the groups supplemented with vitamin C and PLP (AFVC and AFLP) outperformed those with AFB1 alone (AFLB). These supplemented groups exhibited improved body weight gain, feed intake, and reduced mortality rates, suggesting the potential of these natural additives to counteract mycotoxin challenges in poultry production, especially in tropical conditions. Moreover, our results indicated that vitamin C and PLP supplementation ameliorated the adverse effects of AFB1 contamination on dressing percentage and liver weight. Notably, Haematological parameters were significantly impacted by AFB1 exposure, with lower values in the AFLB group, while serum chemistry indices reflected liver and kidney dysfunction. In contrast, supplemented groups showed improved profiles. Overall, our study highlights the potential of PLP, and vitamin C dietary supplementation in enhancing poultry health and production under aflatoxin-B1 exposure, contributing to sustainable poultry farming strategies.

Keywords: ascorbic acid, botanicals, feed supplements, mycotoxins, poultry

1 Introduction

The poultry industry plays a pivotal role in meeting global protein demands, with broiler chickens representing a significant component of this industry (Oloruntola et al., 2022). However, the productivity and health of broiler chickens are frequently challenged by various factors, including mycotoxin contamination of feed ingredients (Murugesan et al., 2022). Mycotoxins are toxic secondary metabolites produced by various species of fungi, particularly under warm and humid conditions, and are known to contaminate a wide range of agricultural commodities (Olarotimi et al., 2023). Among these mycotoxins, Aflatoxin B_1 (AFB1), produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*, poses a substantial threat to both animal and human health (Alameri et al., 2023).

In tropical regions with elevated humidity and temperatures, conditions conducive to fungal growth, the risk of AFB1 contamination in poultry feed is particularly pronounced (Mutuli et al., 2022). Aflatoxin B_1 is known to impair growth performance, compromise immune function, and lead to economic losses in poultry production (Monson et al., 2015). Aflatoxin B_1 is also known to be a highly toxic mycotoxin that increases the production of reactive oxygen species (ROS), which

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harms both humans and animals through oxidative stress (Ma et al., 2021). Moreover, the residual presence of AFB1 in poultry products raises concerns about food safety and public health (Alameri et al., 2023).

To mitigate the adverse effects of AFB1 on broiler chickens, various strategies have been explored, including the use of mycotoxin-binding agents and the development of feed management protocols (Elwan et al., 2021). However, recent research has increasingly focused on the potential of dietary phytogenic antioxidant supplements to ameliorate the harmful effects of AFB1 exposure in broiler chickens (Sarker et al., 2021).

Among these dietary supplements, vitamin C (ascorbic acid) and *Parquetina nigrescens* leaf powder have garnered attention for their potential ameliorative properties (Alabi et al., 2021; Chambial et al., 2021). Vitamin C is recognized for its antioxidant and immune-boosting properties (Chambial et al., 2013), while PLP, derived from the leaves of *Parquetina nigrescens*, is rich in bioactive compounds with reported antioxidant and hepatoprotective effects (Alabi et al., 2021). *Parquetina nigrescens*, a medicinal plant commonly found in tropical Africa, has been traditionally used for the treatment of anemia, inflammation, and liver disorders. Its leaves contain a variety of phytochemicals, including flavonoids, alkaloids, tannins, saponins, phenolic compounds, and glycosides, which contribute to its pharmacological properties (Adase et al., 2022; Daramola et al., 2022). As a result of its antioxidant, antibacterial, anti-inflammatory, analgesic, and aphrodisiac characteristics, *Parquetina nigrescens* has been utilised in ethnomedicine for decades in various regions of West and Central Africa, including Nigeria, Ghana, Benin, and Cameroon (Alabi et al., 2021; Adase et al., 2022).

In tropical conditions, where AFB1 contamination is prevalent, the combination of vitamin C and PLP supplementation may offer a synergistic approach to mitigating the adverse effects of mycotoxin exposure in broiler chickens (Stefanović et al., 2023). This study investigates the potential ameliorative effects of dietary vitamin C and PLP supplementation on broiler chickens raised under tropical conditions and exposed to AFB1-contaminated diets. By evaluating growth performance, haematological parameters, organ relative weight, and serum markers, this research aims to provide valuable insights into the efficacy of these dietary supplements in promoting the health and productivity of broiler chickens in challenging tropical environments.

2 Material and Methods

2.1 Ethical Approval

The regulations pertaining to animal care and the use of animals in this study were formally approved by the Research and Ethics Committee within the Department of Animal Health and Production at The Federal College of Agriculture in Akure, Nigeria.

2.2 Parquetina Nigrescens Leaf Collection, Processing, and Analysis

To prepare the *Parquetina nigrescens* leaves for analysis, fresh leaves were harvested from their parent plants and allowed to undergo a 14-day drying process in the shade. Subsequently, they were finely ground to create what we refer to as "*Parquetina nigrescens* leaf powder" or PLP.

The PLP underwent a comprehensive analysis, including assessments for its ferric acid reducing power following the method outlined by Benzie and Strain (1996). Additionally, evaluations were conducted for alkaloid content based on the procedure established by Adeniyi et al. (2009), saponin levels in accordance with He et al. (2014), flavonoid content following the approach detailed by Surana et al. (2016), tannin content as per the method proposed by Biswas et al. (2020), phenolic compounds in line with Otles and Yalcin (2012), and finally, an assessment of lipid peroxidation activities, as guided by Bajpai et al. (2015).

2.3 Diet and Aflatoxin B_1

To ensure compliance with recommended dietary standards as stated in Cobb500 Broiler Management Guide for broiler chickens during both the starter and finisher phases, we developed a standard/basal diet, detailed in Table 1. Aflatoxin production was initiated from a pure culture of *Aspergillus flavus* (NRRL 3251). Aflatoxin B_1 was generated through solid fermentation using grit maize as a substrate, following the method outlined in the study by Gbore et al. (2016). After the cultivation process, the grit maize underwent a drying procedure at 50 °C for 20 hours, followed by fine pulverization using an electric blender. To evaluate the AFB1 content, a triplicate analysis was conducted using thin-layer chromatography, in accordance with the AOAC (2010) method. Quantification of AFB1 in the ground maize was carried out at the Animal Care Disease Diagnosis/Control and Feed Analysis Laboratory in Ibadan, Nigeria, using the thin-layer chromatography technique.

To attain the target AFB1 concentration of approximately 0.2 mg AFB1·kg^{-1} in the chicken feed for both the starter and finisher phases, a precise procedure

was meticulously followed. Initially, 1 gram of AFB1-infused·cultured^{-1} maize powder was carefully blended with 1 kilogram of uncontaminated milled maize. As a result of this blending process, a measured AFB1 concentration of AFB1·kg^{-1} was detected in the maize mixture. Subsequently, we used the known quantity of AFB1 obtained from the combination of 1g AFB1 with uncontaminated milled maize to calculate the amount of AFB1-infused/cultured maize powder needed to be mixed with 1 kg of uncontaminated maize. This was done to achieve an AFB1 contamination level of approximately 0.4 mg·kg^{-1}. Consequently, we blended AFB1-infused·cultured^{-1} maize with uncontaminated maize at a precise ratio of 99.38 g·kg^{-1}, resulting in the desired 0.4 mg AFB1·kg^{-1} contamination. This mixture is referred 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, we blended the other feed ingredients with 50.50% and 58.34% of AFB1-contaminated maize. This carefully calculated addition constituted the total feed composition for both the starter and finisher feeds, respectively. Following this adjustment, the experimental diets or treatments (1, 2, 3, and 4) were analyzed for AFB1 quantification (Table 1). For each phase of broiler chicken production, the diets were supplemented as follows:

- CONT: No AFB1 contamination; no PLP supplementation.
- AFLB: 0.2 mg of AFB1 per kg of feed.
- AFVC: 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin per kg of feed.
- AFLP: 0.2 mg of AFB1 per kg of feed, along with 500 mg of PLP per kg of feed.

The nutritional composition of these foundational diets was assessed in accordance with the AOAC (2010) guidelines. It is worth noting that the concentration of approximately 0.2 mg AFB1·kg^{-1} in the chicken diet represents a substantial elevation, exceeding the permissible limit of 0.02 mg·kg^{-1} as specified by the National Agency for Food and Drug Administration and Control (NAFDAC), the European Union (EU), the United States Food and Drug Administration (USFDA), and the Canadian Food Inspection Agency (CFIA) (Boudergue et al., 2009).

Table 1 Composition of the experimental diets

Ingredients (%)	Broiler starter				Broiler finisher			
	CONT	AFLB	AFVC	AFLP	CONT	AFLB	AFVC	AFLP
Maize	50.50	50.50	50.50	50.50	58.34	58.34	58.34	58.34
Maize bran	3.20	3.20	3.20	3.20	0.00	0.00	0.00	0.00
Rice bran	0.00	0.00	0.00	0.00	3.02	3.02	3.02	3.02
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soybean meal	37.56	37.56	37.56	37.56	30.02	30.02	30.02	30.02
Bone meal	3.10	3.10	3.10	3.10	3.00	3.00	3.00	3.00
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	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Composition (%)								
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	3.54	3.52	3.54	3.53	3.57	3.58	3.55	3.57
*Crude fat	4.25	4.23	4.24	4.23	2.38	2.36	2.35	2.38
*Crude protein	22.18	22.19	22.17	22.179	20.06	20.05	20.04	20.05
Metabolizable energy (Kcal·kg^{-1})	3,018.11	3,018.11	3,018.11	3,018.11	3,108.24	3,108.24	3,108.24	3,108.24
*Aflatoxin B1 (mg·kg^{-1})	NN	0.187	0.185	0.192	NN	0.176	0.179	0.175

*Analyzed composition; NN – not negligible; CON – no AFB1 contamination; no PLP 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 PLP per kg of feed

2.4 Experimental Site and Birds

A feeding study was conducted at the Avian Experimental Pen, located at the Teaching and Research Farm located at the Federal College of Agriculture in Akure, Nigeria. This study involved a total of 200 day-old chicks of both sexes belonging to the Cobb 500 breed. These chicks were allocated randomly into four distinct experimental diet groups, each comprising 50 birds. To ensure robust statistical analysis, each diet group was further divided into 5 replications, each containing 10 birds.

To provide suitable living conditions for the birds, we utilized floor pens measuring 2 meters by 2 meters, each constructed with concrete flooring. These pens were filled with dry wood shavings to a depth of 3 centimeters, offering a comfortable and hygienic environment for the chicks. Maintaining an appropriate environmental temperature was of paramount importance for the well-being of the birds. To achieve this, we exercised meticulous control over the temperature in the experimental room. During the first week of the experiment, the room temperature was maintained at 31 ±2 °C to support optimal brooding conditions for the chicks. Thereafter, the temperature was gradually decreased by approximately 2 °C per week during the second and third weeks to facilitate thermoregulatory adaptation. From the fourth week onward, no artificial heating was provided, and the birds were exposed to natural ambient environmental conditions, with an average temperature of 29 ±2 °C and relative humidity of 69.5 ±4%. This temperature management approach ensured a gradual and physiologically appropriate transition from brooding to ambient conditions over the 6-week rearing period. Lighting conditions were also managed systematically to optimize growth conditions throughout the study. On the first day of the experiment, the lights were kept on continuously for 24 hours to provide consistent illumination. From days 2 through 7, lighting was provided for 23 hours each day, promoting a conducive environment for the chicks' development. For the remaining period of the rearing phase (weeks 2 to 6), the birds received 18 hours of illumination daily, aligning with their growth requirements. Throughout the entire six-week duration of the experiment, the birds had uninterrupted access to their respective diets, ensuring that they received consistent nutrition throughout the study period.

2.5 Performance Characteristics

At the beginning of the experiment (day 0), the initial body weight of all birds was recorded to establish a baseline. Subsequently, at consistent seven-day intervals, critical growth parameters – including feed intake, weekly body weight, and body weight gain – were meticulously recorded. These measurements were essential for evaluating the overall growth performance of the birds throughout the experimental period. Additionally, we calculated the feed conversion ratio (FCR), which signifies the ratio of feed consumed to the increase in body weight. Feed conversion ratio is an important indicator of feed efficiency, helping us assess the effectiveness of the feeding regimen.

2.6 Slaughter and Carcass Evaluation

On the 42nd day of the experiment, we implemented a random selection process to choose 10 birds from each treatment group. This selection included two birds from each replication, ensuring a representative sample. The slaughtering procedure was carried out meticulously, adhering to the established guidelines for the humane treatment of animals during slaughter and killing, as detailed by Oloruntola et al. (2025). Euthanasia was performed through electrical stunning in a water bath, in accordance with recognised animal welfare protocols. The equipment was adjusted to deliver a minimum of 100 milliamperes per bird, as recommended by European Union (EU) standards, ensuring immediate loss of consciousness. To achieve consistent stunning, the voltage was regulated to supply adequate current, and the shackles were moistened to minimise electrical resistance. After stunning, humane slaughter was carried out by severing at least one major neck artery using a sharp, dry, and sanitized stainless steel knife to allow thorough exsanguination

Following slaughter, the carcasses were subjected to standard post-slaughter handling procedures, including thorough spray-washing to remove visible contaminants and chilling at 2 °C for 30 minutes. This chilling step is critical as it rapidly reduces the carcass temperature, thereby inhibiting bacterial proliferation, minimizing the risk of microbial spoilage, and ensuring that the meat remains hygienic and safe for human consumption (Jainonthee et al., 2024). To evaluate the meat yield, we calculated the dressing percentage, which represents the ratio of the carcass weight to the final body weight of the birds. Additionally, we determined the relative weights of specific organs, including the liver, heart, lung, proventriculus, gizzard, spleen, and pancreas. These organ weights were expressed as a percentage of the slaughter weight, allowing for a comprehensive assessment of organ development and health.

2.7 Blood Collection and Analysis

Blood samples were collected from the chickens and divided into two types of sample bottles: one set without any anticoagulant (plain bottles) for serum separation, used in the analysis of serum biochemical parameters,

and another set containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The EDTA-treated samples were gently mixed and processed immediately for haematological analysis, including measurements of 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). These assessments were carried out following the methodology outlined by Cheesbrough (2000). The samples in the plain bottles were then subjected to centrifugation to separate the serum component. This serum was carefully transferred to a separate set of plain bottles and stored at a temperature of -20 °C for subsequent analysis. Furthermore, we analyzed serum markers such as cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine using a Reflectron® Plus 8C79 instrument from Roche Diagnostic, GmbH Mannheim, Germany. Commercial kits, as detailed in the study by Oloruntola et al. (2018), were employed for these serum marker determinations.

2.8 Statistical Analysis

The data collected in this study underwent thorough statistical analysis using SPSS software, specifically version 20. To assess the differences among the collected data, we employed a one-way analysis of variance (ANOVA). The ANOVA model was structured as follows:

$$Adj = m + \alpha t + edj$$

where: *Adj* – represents the response variables, encompassing all the measurements and parameters gathered during the experiment; *m* – denotes the overall mean, which signifies the average value of the response variables across all treatment groups; αt – represents the effect of the d^{th} treatment, where *t* corresponds to the four distinct dietary treatments (diets 1, 2, 3, and 4); *edj* – takes into account the random error that is inherent in the experimental process

Following the ANOVA analysis, we utilized the Duncan multiple range test, which is a post-hoc test, to discern significant differences among the means of the different treatment groups. This test allows for a detailed comparison of treatment effects. We established the level of statistical significance at $P < 0.05$, ensuring that any observed differences were statistically meaningful.

3 Results and Discussion

The examination of *Parquetina nigrescens* leaf powder (PLP) demonstrates the presence of 132.28 mg·g^{-1} of Ferric-reducing antioxidant power (FRAP), 3.92% alkaloids, 112.78 mg·g^{-1} of saponins, 204.11 mg·g^{-1} of flavonoids, 77.81 mg·g^{-1} of tannins, 182.07 mg·g^{-1} of phenol, and 33.72% lipid peroxidation activities (Figure 1). The analysis of PLP has revealed a spectrum of bioactive compounds with potential health benefits and therapeutic applications. These findings highlight the significance of PLP as a valuable natural resource for various biomedical and nutritional purposes. The remarkable FRAP value of 132.28 mg·g^{-1} indicates the potent antioxidant capacity of PLP. Antioxidants play a crucial role in neutralising harmful free radicals and reactive oxygen species (ROS) in biological systems, which can cause oxidative stress and damage to cellular

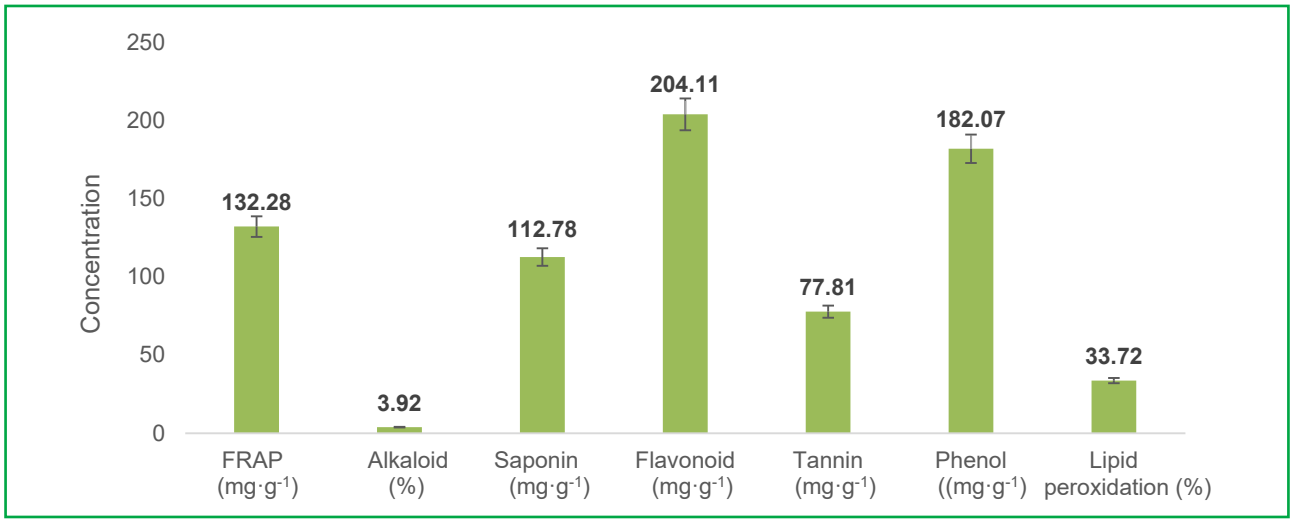

Figure 1 The chemical composition of *Parquetina nigrescens* leaf powder (PLP)
FRAP – ferric reducing antioxidant power

components (Lobo et al., 2010). *Parquetina nigrescens* leaf powder's high FRAP value suggests its potential in mitigating oxidative stress-related disorders and protecting cellular health. The presence of alkaloids in PLP (3.92%) is of interest due to their diverse pharmacological activities. Alkaloids have been associated with various biological effects, including analgesic, anti-inflammatory, and antimicrobial properties (Thawabteh et al., 2019). Further isolation and characterization of these alkaloids could provide insights into their specific bioactivities and potential medicinal applications. The significant content of saponins (112.78 mg·g^{-1}) in PLP is noteworthy. Saponins are known for their ability to lower cholesterol levels, enhance immune function, and exhibit antifungal and antitumor activities (Shi et al., 2004). The presence of saponins in PLP suggests its potential as a functional food ingredient or dietary supplement. Flavonoids, with a content of 204.11 mg·g^{-1} in PLP, are renowned for their antioxidant, anti-inflammatory, and anticancer properties (Ullah et al., 2020). These compounds may contribute to PLP's protective effects against oxidative stress and inflammatory conditions, making it a promising candidate for dietary interventions. Tannins, present at 77.81 mg·g^{-1} in PLP, are known for their astringent properties and ability to bind to proteins, making them useful in various applications, including the food and pharmaceutical industries (Soares et al., 2020). The phenol content of 182.07 mg·g^{-1} in PLP is significant, as phenolic compounds have been associated with antioxidant, anti-inflammatory, and anticancer activities (Xi et al., 2017). These compounds may contribute to the overall bioactivity of PLP. The observation of lipid peroxidation activities at 33.72% indicates that PLP may possess protective effects against lipid oxidation. Lipid peroxidation is a key factor in various chronic diseases, and compounds that inhibit this process are of interest in the context of health promotion (Ayala et al., 2014).

The impact of dietary supplementation of PLP and vitamin C on the performance of broiler chickens fed AFB1-contaminated diets is presented in Table 2. The results reveal that the body weight gain (BWG) of broiler chickens in the AFVC and AFLP groups is comparable ($P = 0.01$) to that of the CONT group but significantly ($P < 0.05$) higher than that of the AFLB group. Furthermore, the feed intake (FI) of birds fed AFLB is similar ($P > 0.05$) to that of those fed AFVC and AFLP but significantly ($P < 0.05$) lower than that of those fed CONT. However, the FI of CONT and AFLP does not exhibit a significant difference ($P > 0.05$). Notably, the mortality rate is significantly ($P < 0.05$) higher in the AFLB group when compared to the CONT, AFVC, and AFLP groups. The performance of broiler chickens exposed to aflatoxin B_1 (AFB1) contamination and dietary supplementation with PLP and vitamin C (ascorbic acid) offers valuable insights into the ameliorative effects of these interventions under challenging conditions. The body weight gain is a crucial indicator of broiler chicken health and productivity. In this study, the BWG of broiler chickens in the AFVC and AFLP groups was comparable to that of the CONT group, demonstrating that these supplementation strategies effectively counteracted the growth-inhibiting effects of AFB1 contamination. Notably, the BWG in the AFLB group was significantly lower than in the other groups. This outcome aligns with previous research that has reported the growth-reducing impact of AFB1 on broiler chickens (Hou et al., 2022). Aflatoxin B_1 (AFB1) impairs liver function through hepatocellular necrosis and bile duct hyperplasia, disrupting key metabolic processes. This hepatic damage compromises protein synthesis, lipid metabolism, and bile production, leading to poor nutrient utilization and feed efficiency (Cheng et al., 2023). Consequently, broiler chickens exposed to AFB1 exhibit reduced body weight gain. Feed intake in broiler chickens is influenced by a combination of appetite and the palatability of the diet, which together reflect both internal physiological needs and sensory preferences. In this study, the FI of birds in the AFLB group was similar to that of those in the AFVC and AFLP groups, indicating that AFB1 contamination did not deter feed consumption in these groups. However, the FI in the AFLB group was

Table 2 Effects of *Parquetina nigrescens* leaf powder (PLP) and vitamin c supplementation on the performance (age 42 day) of broiler chickens fed aflatoxin B_1 contaminated diets

Parameters	CONT	AFLB	AFVC	AFLP	SEM	*P*-value
Initial weight (g/b)	44.83	44.75	44.81	44.74	0.15	0.65
Body weight gain (g/b)	2,621.74^{a}	2,380.09^{b}	2,590.23^{a}	2,591.76^{a}	33.84	0.01
Feed intake	4,078.29^{a}	3,772.55^{c}	3,889.29^{bc}	3,936.48^{ab}	38.71	0.02
Feed conversion ratio	1.57	1.59	1.50	1.52	0.01	0.14
Mortality	0.00^{b}	5.00^{a}	1.00^{b}	1.33^{b}	0.63	0.02

Means in the same row with distinct letters indicate statistically significant differences ($P < 0.05$); SEM – standard error of mean; CONT – no AFB1 contamination; no PLP supplementation; AFLB – 0.2 mg of AFB1 per kg of feed; AFVC – 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin C per kg of feed; AFLP – 0.2 mg of AFB1 per kg of feed, along with 500 mg of MLM per kg of feed

lower than in the CONT group and AFLP. Interestingly, the FI in the CONT and AFLP groups did not differ significantly. This suggests that PLP supplementation may have mitigated the impact of AFB1 on feed intake, as supported by previous findings on the role of natural additives in enhancing feed consumption (Kolawole et al., 2022). Dietary supplementation with either vitamin C or *Parquetina nigrescens* leaf powder (PLP) has been shown to improve the performance of broiler chickens exposed to aflatoxin B_1 (AFB1). Vitamin C, a potent antioxidant, enhances the scavenging of reactive oxygen species, supports hepatic antioxidant defense, and modulates immune responses, thereby improving feed intake, nutrient utilization, and growth (Oloruntola et al., 2024). Similarly, PLP, rich in phytochemicals such as flavonoids and phenolics, exhibits antioxidant, anti-inflammatory, and hepatoprotective properties, which help preserve liver integrity and support immune function (Daramola et al., 2022). These actions contribute to improved feed efficiency and body weight gain in AFB1-challenged broilers, highlighting the potential of both additives as effective dietary interventions for mitigating aflatoxicosis in poultry. The mortality rate is a critical parameter that reflects the overall health and survival of broiler chickens. The higher mortality rate in the AFLB group compared to the CONT, AFVC, and AFLP groups underscores the detrimental consequences of AFB1 contamination on broiler chicken survival. This aligns with established knowledge of the acute toxicity of AFB1 (Benkerroum, 2020). The absence of a significant difference in mortality between the CONT, AFVC, and AFLP groups suggests that vitamin C and PLP supplementation may have contributed to enhanced resilience against AFB1-induced mortality (Sahoo et al., 2003; Adase et al., 2022).

Table 3 presents the impact of dietary supplementation with PLP and vitamin C on the dressing percentage and the relative weights of internal organs of broiler chickens exposed to AFB1 contaminated diets. The findings reveal that the dressing percentage of birds in the AFVC and AFLP groups is not significantly different ($P > 0.05$) from that of the CONT group, but it is significantly ($P < 0.05$) higher than that of the AFLB group. Conversely, the relative liver weight of broiler chickens in the AFLB group is significantly ($P < 0.05$) greater than that of those in the CONT, AFVC, and AFLP groups. Additionally, there are indications that the relative weights of the proventriculus ($P = 0.08$), gizzard ($P = 0.07$), and pancreas ($P = 0.08$) may be influenced by dietary AFB1 contamination and the inclusion of vitamin C and PLP. Specifically, AFB1 contamination tends to cause hypertrophy or increased relative weights of these organs due to its toxic effects on the digestive system, while supplementation with vitamin C and PLP appears to mitigate these effects, possibly by reducing oxidative stress and improving tissue health. The dressing percentage is an essential parameter in poultry production, reflecting the proportion of the live weight that contributes to the carcass weight. In this study, the dressing percentage of broiler chickens in the AFVC and AFLP groups is similar to that of the CONT group. This observation indicates that the inclusion of vitamin C and PLP in the diet mitigates the negative impact of AFB1 contamination on the dressing percentage, allowing the birds in AFVC and AFLP to achieve a comparable dressing percentage to that in the CONT. Significantly, the dressing percentage in the AFLB group is lower than in the supplemented groups. This finding aligns with earlier research reporting that AFB1 exposure can result in reduced carcass yield (Pu et al., 2022). Dietary supplementation with vitamin C or *Parquetina nigrescens* leaf powder (PLP) alleviates the adverse effects of aflatoxin B_1 (AFB1) on dressing percentage in broiler chickens. AFB1-induced hepatotoxicity and impaired nutrient utilization reduce

Table 3 Effects of *Parquetina nigrescens* leaf powder (PLP) and vitamin c supplementations on the dressing percentage and relative internal organs' weight (% Slaughter weight) of broiler chickens fed aflatoxin B1 contaminated diets

Parameters (%)	CONT	AFLB	AFVC	AFLP	SEM	P value
Dressing	74.00^{a}	66.60^{c}	74.19^{a}	72.79^{ab}	0.98	0.001
Liver	1.65^{b}	1.88^{a}	1.64^{b}	1.69^{b}	0.03	0.01
Proventriculus	0.31	0.33	0.38	0.30	0.01	0.08
Gizzard	1.62	1.54	1.73	1.51	0.03	0.07
Heart	0.42	0.48	0.41	0.42	0.01	0.17
Lung	0.37	0.36	0.28	0.35	0.02	0.16
Spleen	0.08	0.06	0.08	0.07	0.01	0.44
Pancreas	0.16	0.17	0.18	0.16	0.00	0.08

Means in the same row with distinct letters indicate statistically significant differences ($P < 0.05$); SEM – standard error of mean; CONT – no AFB1 contamination; no PLP supplementation; AFLB – 0.2 mg of AFB1 per kg of feed; AFVC – 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin C per kg of feed; AFLP – 0.2 mg of AFB1 per kg of feed, along with 500 mg of PLP per kg of feed

muscle growth and increase the relative weight of non-edible organs, lowering carcass yield (Zou et al., 2023). Vitamin C and PLP mitigate these effects by exerting antioxidant and hepatoprotective actions that preserve organ function and support muscle development, thereby improving dressing percentage in AFB1-exposed birds (Daramola et al., 2022; Oloruntola et al., 2024).The liver is a vital organ responsible for various metabolic processes in chickens. The increase in the relative liver weight of broiler chickens in the AFLB group compared to those in the CONT, AFVC, and AFLP groups underscores the adverse impact of AFB1 contamination on liver health. The liver is a vital organ responsible for various metabolic functions, including the detoxification of harmful substances such as mycotoxins. Elevated liver weights observed in this study are indicative of hepatotoxicity caused by AFB1 exposure. This increase in liver size likely results from cellular hypertrophy and inflammation as the liver enhances its metabolic and detoxification activities to process and eliminate the toxin. These changes reflect the physiological stress imposed on the liver during mycotoxin exposure (Chen et al., 2021). Conversely, the supplementation of vitamin C and PLP appears to have mitigated this effect (Daramola et al., 2022). While not statistically significant, there are trends ($P = 0.08$ for proventriculus, $P = 0.07$ for gizzard, $P = 0.08$ for pancreas) suggesting potential effects of dietary AFB1 contamination and vitamin C and PLP supplementation on the relative weights of these internal organs.

In Table 4, we observe the impacts of introducing PLP and vitamin C supplements into the diets of broiler chickens exposed to aflatoxin B_1 contamination with a focus on haematological indices. It is noteworthy that the packed cell volume (PCV), red blood cell (RBC) count, and hemoglobin concentration of birds in the AFLB group were notably lower ($P < 0.05$) compared to those in the CONT, AFVC, and AFLP groups. In contrast, the mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and white blood cell (WBC) counts did not show any significant changes ($P > 0.05$) due to the dietary treatments. Haematological indices are crucial for assessing the health and physiological status of poultry. The PCV, also known as hematocrit, measures the volume of red blood cells in the blood. The lower PCV in the AFLB group compared to CONT, AFVC, and AFLP groups indicates anemia, which is consistent with the known haematotoxic effects of AFB1 (Oloruntola, 2024). The absence of a significant difference between the supplemented groups (AFVC and AFLP) suggests a potential ameliorative effect of antioxidants: vitamin C and PLP (Cotoraci et al., 2021). The RBC count reflects the number of red blood cells in circulation. Similar to PCV, the lower RBC count in the AFLB group underscores the adverse impact of AFB1 on erythropoiesis (Dönmez et al., 2012). Conversely, the CONT, AFVC, and AFLP groups did not exhibit significant differences in RBC count, indicating potential protective effects of dietary supplementation (Katavetin et al., 2007). Hemoglobin is essential for oxygen transport in erythrocytes. The lower hemoglobin concentration (HbC) levels in the AFLB group align with the observed anemia in broiler chickens (Oloruntola, 2024). The stability of HbC levels in the supplemented groups suggests that vitamin C and PLP may help preserve hemoglobin function under AFB1 exposure (Alayash, 2022). The results suggest that AFB1 contamination adversely affects hematological indices, leading to anemia in broiler chickens. However, dietary inclusion of vitamin C and *Parquetina nigrescens* leaf powder appeared to ameliorate these effects. This protective role is likely due to their antioxidant properties. Vitamin C is a well-established antioxidant that scavenges reactive oxygen species and protects erythrocyte membranes from oxidative damage (Tzounakas et al., 2022). Similarly, *Parquetina nigrescens* has been reported to enhance antioxidant enzyme activities and improve hematological parameters in toxin-challenged animals

Table 4 Effects of *Parquetina nigrescens* leaf powder (PLP) and vitamin c supplementations on the haematological indices of broiler chickens fed aflatoxin B1 contaminated diets.

Parameters	CONT	AFLB	AFVC	AFLP	SEM	P value
Packed cell volume (%)	35.83^a	30.20^b	35.70^a	33.93^a	0.76	0.001
Red blood cells ($\cdot 10^6 \cdot l^{-1}$)	2.80^a	2.31^b	2.72^a	2.74^a	0.06	0.001
Haemoglobin conc. ($g.dl^{-1}$)	11.94^a	10.07^b	11.90^a	11.34^a	0.25	0.001
Mean cell volume (fl)	128.12	131.60	131.53	124.09	2.00	0.58
Mean cell haemoglobin (pg)	42.71	43.87	43.84	41.46	0.67	0.60
Mean cell haemoglobin conc. ($g.dl^{-1}$)	33.33	33.35	33.34	33.34	3.37	0.58
White blood cells ($\cdot 10^9 \cdot l^{-1}$)	3.71	5.28	2.91	2.96	2.47	0.47

Means in the same row with distinct letters indicate statistically significant differences ($P < 0.05$); SEM – standard error of mean; CONT – no AFB1 contamination; no PLP supplementation; AFLB – 0.2 mg of AFB1 per kg of feed; AFVC – 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin C per kg of feed; AFLP – 0.2 mg of AFB1 per kg of feed, along with 500 mg of PLP per kg of feed

Table 5 Effects of *Parquetina nigrescens* leaf powder (PLP) and vitamin c supplementations on the serum chemistry indices of broiler chickens fed aflatoxin B1 contaminated diets

Parameters	CONT	AFLB	AFVC	AFLP	SEM	P value
Alanine aminotransferase ($U \cdot l^{-1}$)	36.60^{b}	47.95^{a}	39.90^{b}	38.55^{b}	2.39	0.02
Creatinine ($mmol \cdot l^{-1}$)	75.46^{b}	118.17^{a}	82.63^{b}	77.47^{b}	6.30	0.02
Aspartate aminotransferase ($U \cdot l^{-1}$)	78.40	88.25	79.85	77.57	0.05	0.49
Cholesterol ($mmol \cdot l^{-1}$)	2.25^{a}	2.02^{b}	2.04^{ab}	1.87^{b}	0.05	0.02

Means in the same row with distinct letters indicate statistically significant differences (P <0.05); SEM – standard error of mean; CONT – no AFB1 contamination; no PLP supplementation; AFLB – 0.2 mg of AFB1 per kg of feed; AFVC – 0.2 mg of AFB1 per kg of feed along with 200 mg of vitamin C per kg of feed; AFLP – 0.2 mg of AFB1 per kg of feed, along with 500 mg of PLP per kg of feed

(Adase et al., 2022). These findings align with the current results, indicating the potential of these agents to mitigate mycotoxin-induced oxidative stress and its hematological consequences (Alayash, 2022; Tzounakas et al., 2022).

In Table 5, the impacts of introducing PLP and vitamin C supplements into the diets of broiler chickens exposed to aflatoxin B_1 contamination were observed, focusing on serum chemistry indices. Notably, the serum levels of alanine aminotransferase (ALT) and creatinine in the AFLB group were notably higher (P <0.05) compared to those in the CONT, AFVC, and AFLP groups. Conversely, the serum cholesterol levels in the AFLB and AFLP groups did not significantly differ (P >0.05) from those in the AFVC group but were significantly lower (P <0.05) compared to the CONT group. Serum chemistry indices are essential markers for assessing the physiological status and potential liver and kidney dysfunction in poultry (Gowda et al., 2010). Alanine Aminotransferase is a liver enzyme that plays a crucial role in protein metabolism (Yang et al., 2009). Higher ALT levels in the serum of birds in the AFLB group indicate liver damage, consistent with previous findings on AFB1-induced hepatotoxicity (Chu et al., 2017). In contrast, the CONT, AFVC, and AFLP groups show lower ALT levels, suggesting potential hepatoprotective effects of vitamin C and PLP (Abdulrazzaq et al., 2019; Adase et al., 2022). Vitamin C and *Parquetina nigrescens* leaf powder (PLP) protect the liver by reducing oxidative stress and inflammation. Vitamin C enhances antioxidant enzyme activity and supports liver repair (He et al., 2021), while PLP's phytochemicals scavenge free radicals and prevent lipid peroxidation (Adase et al., 2022), helping preserve liver function in toxin-exposed animals.Creatinine is a waste product typically excreted by the kidneys (Salazar et al., 2014). Elevated serum creatinine levels in the AFLB group (P <0.05) point to kidney dysfunction, in agreement with studies highlighting AFB1's nephrotoxic potential (Al-Naimi et al., 2019). Conversely, the CONT, AFVC, and AFLP groups exhibit lower creatinine levels, indicating that vitamin C and PLP may help maintain renal function (Dennis and Witting, 2017). Serum cholesterol levels are important indicators of lipid metabolism (Strashok et al., 2020). Both the AFLB and AFLP groups display similar cholesterol levels to the AFVC group, but lower compared to the CONT group. This reduction in serum cholesterol in the AFB1-exposed groups may be attributed to impaired liver function (Rotimi et al., 2019). The results highlight the hepatotoxic and nephrotoxic effects of AFB1 on broiler chickens, as evidenced by elevated ALT and creatinine levels. However, the inclusion of vitamin C and PLP in the diet appears to mitigate these adverse effects, suggesting their potential as hepatoprotective and nephroprotective agents. These findings align with previous studies demonstrating the antioxidant and hepatoprotective properties of vitamin C (Abdulrazzaq et al., 2019) and the potential detoxifying effects of PLP (Akinrinmade et al., 2016).

In conclusion, dietary inclusion of vitamin C (200 $mg \cdot kg^{-1}$ feed) or PLP (500 $mg \cdot kg^{-1}$ feed) effectively mitigated the adverse effects of aflatoxin B_1 on growth performance, organ integrity, hematological parameters, and serum biochemistry in broiler chickens. Both supplements exhibited hepatoprotective and nephroprotective properties. It is therefore recommended to include either vitamin C at 200 $mg \cdot kg^{-1}$ or PLP at 500 $mg \cdot kg^{-1}$ in broiler diets as a practical strategy to reduce the impact of aflatoxicosis and support poultry health under mycotoxin exposure.

4 Conclusions

In conclusion, our study highlights *Parquetina nigrescens* leaf powder (PLP) as a rich source of bioactive compounds with potential health benefits. Furthermore, dietary supplementation with vitamin C and PLP shows promise in mitigating the detrimental effects of AFB1 contamination in broiler chickens. These interventions not only improve growth and reduce mortality but also hold significant potential for enhancing poultry production in regions susceptible to aflatoxin B_1 exposure. This research contributes to the development of sustainable strategies for poultry health and well-being.

Authors contribution

Olugbenga David Oloruntola: Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization; Samuel Adebowale Adeyeye: Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing-Review and Editing; Deborah Adebukola Oloruntola: Validation, Resources, Supervision, Methodology, Formal analysis, Data curation; Emmanuel Oluwafemi Adeyeye: Validation, Resources, Methodology, Data curation, Conceptualization.

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