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Original Article

Effects of aflatoxin-B_1 on performance, blood and antioxidant status of broiler chickens are lessened by Walnut kernel powder and Jacobinia leaf powder dietary supplementation

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Abstract

This study investigated the effects of Walnut kernel powder (WP) and Jacobinia leaf powder (JP) dietary supplementation on the performance, blood, and antioxidant status of broiler chickens administered aflatoxin B_1 (AF) contaminated diets. 200 day-old broiler chicks were randomly assigned to four diets (50 birds/treatment; 10 birds/replicate): CONT: No contamination/ supplementation; AFNS: 0.5 mg/kg AF; AFWP: 0.5 mg/kg AF +350 mg/kg WP; AFJP: 0.5 mg/kg AF+350 mg/kg JP. The final weight and weight gain of the birds in AFNS were significantly ($P<0.05$) lower than those birds fed the rest diets; while those of the birds fed the CONT, AFWP, and AFJP were similar ($P>0.05$). The total feed intake (FI) and feed conversion ratio (FCR) of the broiler birds fed AFNS were significantly ($P<0.05$) lower than those fed CONT, AFWP, and AFJP. The Dressed percentage of the birds fed AFNS reduced significantly ($P<0.05$), compared to those birds fed CONT, AFWP, and AFJP. The relative weights of liver, spleen and pancreas of the birds fed AFNS were significantly ($P<0.05$) affected by AFB_1. The RBC, HbC and PCV of the birds fed AFNS were significantly ($P<0.05$) lower compared to the birds fed the rest diets. The serum GPx and CAT concentrations were lower ($P<0.05$) in birds fed AFNS compared to those fed the control, AFWP, and AFJP. The LDH of the birds fed AFNS were higher ($P<0.05$) than those fed the rest diets. 350 mg/kg of WP and JP as dietary supplements is recommended in broiler chicken diets that are exposed to dietary AFB_1 contamination.

INTRODUCTION

To address the ongoing animal protein crisis in the tropical region, broiler production was recognized as a solution (Hatab *et al.*, 2019); this could be the cause of the rise in poultry production observed in tropical regions of the world (Renaudeau *et al.*, 2012). Commercial broiler chickens grow quickly, and at around seven weeks of age or fewer, they can weigh two kilogrammes or higher at the market (Tallentire *et al.*, 2016). However, feed as a factor (in terms

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of quality and cost), which accounts for 60–80% of the overall production costs in an intensive production system, has an impact on poultry productivity in the tropics (Aguihe *et al.*, 2015).

Tropical and subtropical locations are prone to high levels of aflatoxin formation as a result of their favourable weather conditions and other environmental factors (Verma, 2004). As a result, there is a high growth rate of *Aspergillus flavus* and *A. parasiticus* contamination on animal feedstuffs,such as maize, peanuts, cotton seed, etc (Ditta *et al.*, 2019). The synthesis of numerous poisonous secondary fungal metabolites, such as aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG1), and G_2 (AFG_2), is typically accompanied by the growth of *A. flavus* or *A. parasiticus* in poultry feedstuffs (Fouad *et al.*, 2019). The riskiest and most prevalent mycotoxin among these secondary metabolites is AFB_1 (Pitt and Miller, 2017). High disease susceptibility, low production, and low income are all risk factors for AFB_1 in poultry (Khlangwiset *et al.*, 2011). Moreover, AFB_1 is the third most significant contributor to liver cancer, particularly in sub-Saharan Africa (Wu *et al.*, 2014). Aflatoxin B_1 can raise the level of Malondialdehyde (MDA) in internal organs and limit the activity of antioxidant enzymes, which results in oxidative damage, cell necrosis, and an increase in apoptosis (Peng *et al.*, 2017).

In addition to stunted growth, anaemia and oxidative stress are also brought on by aflatoxicosis (Celik *et al.*, 2000). For example, the toxicity of aflatoxin results in modifications to haematological parameters. Changes in haematological markers take place even in cases of chronic and subclinical aflatoxicosis before clinical symptoms manifest (Dönmez and Keskin, 2008). As a result, when observed in cases of aflatoxicosis, changes in haematological parameters can aid in the identification of toxications (Dönmez and Keskin, 2009).

Since AFB_1 causes oxidative stress in chickens, numerous detoxification techniques have been proposed to eliminate the mycotoxin from feed (Ismail *et al.*, 2018). For instance, multiple studies have been done to find dietary supplements that could prevent or lessen the effects of oxidative stress on chickens (Alpsoy *et al.*, 2009; Watanabe *et al*., 2020). It has been shown that phytochemicals have antioxidant properties and that consuming phytogens or botanicals rich in antioxidant phytochemicals increases the antioxidant capacity of the serum (Zhang *et al.*, 2015). The antioxidant, antidiabetic and anti-inflammatory properties of Walnut kernel powder (Oloruntola, 2022) and Jácobinia leaf powder (Oloruntola *et al.*, 2022) were recently reported.

The objective of this study was to examine the effects of walnut kernel powder and jácobinia leaf powder dietary supplementations on performance, carcass, blood indices, and antioxidant enzymes of broiler chickens fed AFB_1-contaminated diets.

MATERIALS AND METHODS

A Statement of Animal Standards and Experimental site

The Animal Care and Use Committee of the Department of Animal Science at Adekunle Ajasin University, Akungba Akoko, Nigeria, gave its approval to the broiler care and use process. The feeding experiment took place at the Avian Unit within the Teaching and Research Farm of the Federal

College of Agriculture in Akure, Nigeria.

Walnut kernel powder, Jácobinia leaf powder, Aflatoxin B_1 and Experimental diets

Walnut kernel powder (WP) and Jácobinia leaf powder (JP) were produced as earlier reported by Oloruntola (2022) and Oloruntola *et al.* (2022), respectively. Aflatoxin was produced from the *Aspergillus flavus* (NRRL 3251) pure culture, which was maintained on potato dextrose agar. 500 grammes of maize grits were placed in autoclavable polypropylene bags, which were heated to 121 ºC and then subjected to a pressure of 120 kPa for 60 minutes. The autoclaved maize grit was inoculated with an *A. flavus* spore suspension and then grown for 14 days at a temperature of 28 °C. The maize grit was then dried in a 70 °C oven and pulverised into powder. Thin-layer chromatography was used to quantify aflatoxin B_1 (AF) levels in triplicate in the maize (AOAC, 2010).

For the starter and finisher stages, a baseline diet (Table 1) was created, divided into four halves, and given the following names: CONT: No contamination/ supplementation; AFNS: 0.5 mg/kg AF; AFWP: 0.5 mg/kg AF +350 mg/kg WP; AFJP: 0.5 mg/kg AF+350 mg/kg JP. The description of the experimental diets is as follows:

CONT: No aflatoxin B_1 contamination and no supplementation.

AFNS: The baseline diet was contaminated with 0.5 mg/kg aflatoxin B_1 and not supplemented.

AFWP: The baseline diet was contaminated with 0.5 mg/kg aflatoxin B_1 and supplemented with 350 mg/kg Jácobinia leaf powder.

AFJP: The baseline diet was contaminated with 0.5 mg/kg aflatoxin B_1 and supplemented with 350 mg/kg Walnut kernel powder

The dietary concentration for chicken diets approved by both NAFDAC and the EU (Burel *et al*., 2009) is 25 times less than the 0.5 mg/kg AF/kg dietary contamination level utilized in this research.

Table 1: Composition of the experimental diets

Ingredients (%)	Starter phase	Finisher phase
Maize	50.36	58.36
Rice bran	0.00	3.02
Maize bran	3.00	0.00
Soy oil	1.00	1.00
Soybean meal	38.00	30.00
Fish meal	3.00	3.00
Bone meal	3.00	3.00
Premix	0.31	0.31
Limestone	0.49	0.47
Salt	0.31	0.31
Methionine	0.29	0.29
Lysine	0.24	0.24
Nutrient composition		
ME (Kcal/kg)	3018.10	3108.20
Crude protein (%)	22.17	20.04

ME = Metabolizable energy

Experimental birds and Design

At one day old, 200 Cobb 500 broiler chickens were randomly divided into 4 diets in a completely randomized design, each of which had 5 replicates of 10 chickens. Feed and water were readily available for the six-week experimental period.

Performance, blood indices and antioxidant enzymes determination

Feed intake (FI), body weight (BW), and body weight gain (BWG) were measured at intervals of seven days. Thereafter, the feed conversion ratio (FCR) was calculated as the ratio of the birds' feed consumption to their growth in body weight. On day 42,

four birds chosen randomly per replicate were marked or tagged, and blood samples of roughly 10 ml were taken using a syringe and needle from the brachial vein. For haematological tests, blood samples were administered into bottles containing ethylenediaminetetraacetic acid, and for the measurement of serum antioxidant enzymes, into plain sample vials. The samples from plain bottles were centrifuged, and their sera splitted into a different set of plain bottles and refrigerated at 20 °C before being used to determine serum antioxidant enzyme activities. Red blood cells count (RBC), white blood cells count (WBC), packed cell volume (PCV), and haemoglobin concentration (HbC) were determined according to the procedure of Cheesbrough (2000). The serum levels of catalase (Holovska *et al.*, 2003), lactate dehydrogenase, (Kumar and Gill, 2018) and glutathione peroxidase (Payne and Southern, 2005) were evaluated.

The EU requirements for animal protection during slaughter and killing were followed to butcher 12 birds from each treatment group (2 birds/replicate) (Uijttenboogaart, 1999). The birds were completely deprived access to feed on the evening preceding their slaughter. The birds were then dressed and eviscerated. The weights of the birds' internal organs were determined with a sensitive scale. The dressing % was calculated using the carcass weight to final body weight ratio. As a percentage of the slaughter weight, the relative weights of the heart, liver, lung, pancreas, gizzard, and spleen were also calculated.

Statistical data analysis

The data were subjected to an analysis of variance (ANOVA) using SPSS version 20, and the Duncan multiple range test of the same programme was used to see whether the treatment means differed.

RESULTS

The performance of broiler chickens fed aflatoxin B_1-contaminated diet supplemented with WP and JP are shown in Table 2. The final weight and weight gain of the birds fed AFNS were significantly ($P<0.05$) lower when compared to those birds fed the other diets (CONT, AFWP, and AFJP). However, the final weight and weight gain of the birds fed the control, AFWP, and AFJP were similar ($P>0.05$). The FI and FCR of the broiler birds fed AFNS were significantly ($P<0.05$) lower than those fed the Control, AFWP, and AFJP, although, the FI of the birds on control, AFWP, and AFJP were similar ($P>0.05$); while the FCR of birds fed AFJP was similar ($P>0.05$) to those fed AFWP but

Table 2. Performance of broiler chickens fed aflatoxin B_1-contaminated diet supplemented with Walnut kernel powder (WP) and Jacobinia leaf powder (JP)

Parameters	CONT	AFNS	AFWP	AFJP	SEM	P value
Initial weight (g/bird)	40.65	40.44	40.17	40.86	0.22	0.77
Final weight (g/bird)	2782.04^{ab}	2411.21^{c}	2886.78^{a}	2960.59^{a}	45.57	0.01
Weight gain (g/bird)	2741.39^{ab}	2370.77^{c}	2846.61^{a}	2919.62^{a}	45.54	0.01
Feed intake (g/bird)	4657.08^{a}	4217.67^{b}	4608.74^{a}	4693.15^{a}	45.82	0.01
Feed conversion ratio	1.68^{b}	1.78^{a}	1.61^{bc}	1.60^{c}	0.01	0.01

$^{a-c}$Means within a row with different letters are significantly different ($P<0.05$);
AF: Aflatoxin B_1; CONT: No contamination/supplementation; AFNS: 0.5 mg AF /kg;
AFWP: 0.5 mg AF /kg +350 mg WP /kg; AFJP: 0.5 mg AF /kg +350 mg JP /kg;
SEM: Standard error of means.

was significantly (P<0.05) better than those fed the control.

The dressed percentage and the weights of the liver, pancreas and spleen were significantly (P<0.05) affected by the dietary treatments (Table 3). The Dressed percentage of the birds fed AFNS reduced significantly (P<0.05), compared to those

was similar (P>0.05) to AFWP but significantly (P<0.05) higher than those birds fed CONT and AFJP. The pancreas weight of the birds in the CONT group was similar (P>0.05) to that of the birds in the AFJP group. The RBC, Hbc and PCV of the birds fed AFNS were significantly (P<0.05) lower when compared to the birds fed the control, AFWP, and AFJP (Table 4).

Table 3. Carcass and internal organ relative weight (%SW) of broiler chickens fed aflatoxin B_1-contaminated diet supplemented with Walnut kernel powder (WP) and Jacobinia leaf powder (JP)

Parameters	CONT	AFNS	AFWP	AFJP	SEM	P value
Dressed percentage (%)	75.92^{a}	68.59^{b}	76.22^{a}	74.49^{a}	0.78	0.01
Lung	0.52	0.53	0.52	0.48	0.01	0.50
Liver	2.77^{b}	3.55^{a}	2.34^{bc}	2.82^{b}	0.10	0.01
Heart	0.49	0.45	0.43	0.53	0.01	0.05
Pancrease	0.24^{c}	0.36^{a}	0.30^{b}	0.24^{c}	0.01	0.01
Gizzard	2.24	1.93	1.97	2.31	0.07	0.22
Spleen	0.19^{bc}	0.26^{a}	0.24^{ab}	0.16^{c}	0.01	0.01

$^{a-c}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin B_1;
CONT: No contamination/supplementation; AFNS: 0.5 mg AF /kg; AFWP: 0.5 mg AF /kg +350 mg WP /kg;
AFJP: 0.5 mg AF /kg +350 mg JP /kg; SEM: Standard error of means.

birds fed the Control, AFWP, and AFJP. The relative weights of the liver of the birds fed AFNS was higher (P<0.05) than the birds fed the Control, AFWP, and AFJP. The relative weight of the pancreas of the birds

Furthermore, the HbC of the birds fed AFWP was similar (P>0.05) to those fed AFJP, but was significantly(P<0.05) lower than those fed CONT.

Table 4. Haematological indices of broiler chickens fed aflatoxin B_1-contaminated diet supplemented with Walnut kernel powder (WP) and Jacobinia leaf powder (JP)

Parameters	CONT	AFNS	AFWP	AFJP	SEM	P value
Red blood cell (x10 ^{6}/L)	3.01^{a}	1.52^{b}	3.15^{a}	3.35^{a}	0.18	0.01
Haemoglobin conc. (g/dl)	12.16^{a}	10.16^{c}	11.33^{b}	11.66^{ab}	0.17	0.01
Packed cell volume (%)	32.91^{a}	27.00^{b}	31.75^{a}	35.00^{a}	0.85	0.02
White blood cell (x10^{9}/L)	6.23	6.35	4.50	6.65	0.35	0.12

$^{a-c}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin B_1;
CONT: No contamination/supplementation; AFNS: 0.5 mg AF /kg;
AFWP: 0.5 mg AF /kg +350 mg WP /kg; AFJP: 0.5 mg AF /kg +350 mg JP /kg;
SEM: Standard error of means.

fed AFNS was higher (P<0.05) than those fed control, AFWP and AFJP; while the pancreas weight of birds in CONT and AFJP were similar (P>0.05). The relative weight of the spleen of the birds on AFNS

Table 5 shows the antioxidant enzyme activities of broiler chickens fed aflatoxin B_1-contaminated diet supplemented with WP and JP. The serum GPx and CAT concentrations were lower (P<0.05) in birds

Table 5. Antioxidant enzyme activities of broiler chickens fed aflatoxin B_1-contaminated diet supplemented with Walnut kernel powder (WP) and Jacobinia leaf powder (JP)

Parameters	CONT	AFNS	AFWP	AFJP	SEM	P value
Glutathione peroxidase (u/L)	29.69^{b}	22.43^{c}	44.39^{a}	40.89^{a}	1.99	0.01
Catalase (u/ml)	164.68^{ab}	71.03^{c}	189.72^{a}	182.32^{a}	10.05	0.01
Lactate dehydrogenase (U/L)	183.61^{bc}	301.44^{a}	195.26^{b}	133.86^{c}	15.26	0.01

$^{a-c}$Means within a row with different letters are significantly different ($P<0.05$);
AF: Aflatoxin B_1; CONT: No contamination/supplementation; AFNS: 0.5 mg AF /kg;
AFWP: 0.5 mg AF /kg +350 mg WP /kg; AFJP: 0.5 mg AF /kg +350 mg JP /kg;
SEM: Standard error of means.

fed AFNS compared to those fed the CONT, AFWP, and AFJP. In addition, the GPx concentration of the birds fed AFWP and AFJP was higher ($P<0.05$) than those fed CONT and AFNS. The LDH of the birds fed AFNS were higher ($P<0.05$) than those fed the other diets. The LDH of birds fed AFWP were similar ($P>0.05$) to those fed the CONT but was higher ($P<0.05$) than those fed AFJP.

DISCUSSION

The observed growth retardation, reduced feed intake and feed conversion ratio in birds fed the aflatoxin B_1-contaminated diets that were not supplemented with phytosupplements in this study agreed with El-Katcha *et al.* (2017), who reported a decreased growth performance and lowered feed intake in the birds that were fed a diet containing 1mg AFB_1/kg of feed when compared to the control group. The decreased productivity of the broiler chicken given the AFB_1-contaminated diet may be linked to a reduction in protein and energy use as a result of the birds' declining digestive and metabolic efficiency (Verma *et al.*, 2002). However, the broiler chickens fed the AF-contaminated and WP/JP-supplemented diets in this study showed observed improved performance, which is suggestive of the activities of the phytochemicals or bioactive compounds in WP and JP in preventing the process of decline in digestive and metabolic efficiency brought on by AF (Verma *et al.*, 2002). An earlier study demonstrated that phytogenic antioxidants (curcuminoids derived from turmeric) significantly reduced the negative effects of aflatoxin on the productivity of broiler chicks (El-Katcha *et al.*, 2017).

According to this study, broiler chickens' dressed weight is somewhat influenced by their final weight and slaughter weight, which is supported by the concurrently observed decreased growth performance and decreased dressed percentage in broiler chickens fed AFB_1-contaminated diets and the improvement of these aforementioned parameters in broiler chickens fed the WP/JP-supplemented AFB_1-contaminated diets. This was consistent with the trend observed by Oloruntola *et al.* (2021), that noted a similar pattern in the final weight, body weight gain, slaughter weight, dressed weight, and dressed % of broiler chicken fed phyto-additives.

The liver is regarded as the target organ for AFB_1 since it is the organ where the majority of aflatoxins are bioactivated to the reactive 8,9-epoxide form, which is known to bind DNA and proteins and harm liver structures as well as increase liver weight (Pasha *et al.*, 2007). This explains the observed increased liver weight recorded in AFB_1-treated broiler chickens in this study. However, the similar liver weights between the birds fed the control diet and those exposed to AF and WP/JP

supplementation in this study could be explained by the antioxidative activities of bioactive compounds in WP and JP (Oloruntola, 2022; Oloruntola *et al.*, 2022), which were effective in halting the process of oxidative stress and nucleic acid adducts interference with DNA integrity, genomic stability, DNA and protein functions, and subsequently loss of liver structure and function due to AFB_1 toxication. Similarly, the increased pancreas and spleen weights observed in the AFNS group in this study provided additional evidence of the impact of aflatoxin (AF) on internal organs, including the pancreas and spleen. This finding aligns with previous studies by El-Katcha *et al*. (2017) and Peng *et al*. (2014), which also reported similar effects. The abnormal size of the pancreas in birds fed AFB_1 may affect its functions by causing the decreased apparent digestibility of crude protein and other nutrients and consequently retarded performance (Chen *et al.*, 2016; Fouad *et al.*, 2019). Further evidence for the nutraceutical or antioxidant activities of WP and AJ in preventing the oxidative processes in these organs and resulting loss of function and structure is provided by the weight of the pancreas and spleen of broiler chickens on AFWP and AFJP being comparable to those on control diet.

The reduced RBC, HbC and PCV in birds in AFNS being lower than those birds in the CONT, AFWP and AFJP in this study agreed with reduced erythrocyte and haemoglobin concentration in birds fed 250 μg/day aflatoxin report by Fouad *et al.* (2019). By implication, exposure to aflatoxin results in a decrease in haemoglobin concentrations and total red blood cell counts. This is likely caused by some factors, including a reduction in total iron binding capacity, a decrease in protein synthesis, and haemopoietic cellular defects associated with aflatoxicosis (Fouad *et al.*, 2019). Furthermore, the stable RBC, HBc, and PCV values found in birds on CONT, AFWP, and AFJP, which differ from those found in the control in this study, showed that the phytochemicals in WP and JP can impair the ability of the AF to reduce the total iron binding capacity, reduce protein synthesis, and cause haematopoietic cellular defects as a result of aflatoxicosis.
Aflatoxin B_1 causes more reactive oxygen species and free radicals to develop than the body can expel, which increases lipid peroxidation and results in oxidative damage to the majority of the body's organs (Fouad *et al.*, 2019). This explains the reason behind the reduced serum GPx and CAT concentrations recorded in birds on AFNS. In addition, the resultant improved GPx and CAT recorded AFWP and AFJP birds which were also similar to CONT birds also showed the possible antioxidant properties of phytogenic supplements such as WP and JP (Oloruntola, 2022; Oloruntola *et al.*, 2022). Strong antioxidant and free radical scavenging properties, along with an anti-inflammatory effect, make antioxidant phytochemicals capable of preventing oxidative stress and the associated health complications (Zhang *et al.*, 2015).

The anaerobic metabolic pathway depends on lactate dehydrogenase, a crucial enzyme. Liver illness, anaemia, heart attacks, muscular injuries, bone fractures, malignancies, and infections like encephalitis, meningitis, and encephalitis are just a few of the conditions that can elevate blood levels of LDH (Schumann *et al.*, 2002; Feng *et al.*, 2018). Therefore, the elevated LDH in birds on AFNS is pathological (Feng *et al.*, 2018) and this was ameliorated by WP and JP dietary

supplementation in birds fed AFWP and AFJP. Essential components of plants and phytochemicals lessen necrotic cell death, repair the antioxidant defence system, restrict oxidative stress, avoid tissue inflammation, and stop mitochondrial malfunction (Zhang *et al.*, 2015; Wu *et al.*, 2017).

CONCLUSION

Dietary AFB_1 contamination at 0.5mg/kg of feed decreased the ' growth, feed utilisation, and dressed percentage of broiler chickens. The AF feed contamination significantly increased the relative weights of the liver, pancreas, and spleen, as well as decreased the RBC, PCV, and HbC levels. Additionally, broiler chickens exposed to AF contaminated feed had lower serum activities of antioxidant enzymes. However, dietary supplementation with 350 mg WP and JP/kg of feed was able to counteract the deleterious effects of AF on the performance, carcass weight, internal organs, erythrogram, and antioxidant biochemical indices of the broiler chickens given AFB_1-contaminated diets. Therefore, it is advisable to consider adding 350 mg/kg of WP and JP as dietary supplements to broiler chicken diets that could potentially be exposed to or affected by AF contamination.

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