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Juglans kernel powder and jacobinia leaf powder supplementation influenced growth, meat, brain, immune system and dna biomarker of broiler chickens fed aflatoxin-b1 contaminated diets

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Abstract

This study investigates the impact of Juglans kernel powder (JKP) and Jacobinia leaf powder (JLP) supplementation on Aflatoxin-B1 (AF) exposed broiler chickens. 200 Cobb-500 broiler chicks were grouped to four treatment: CONT: No supplement; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF+ 350 mg/kg JKP; AFJL: 0.5 mg/kg AF+350 mg/kg JLP. On day 42, the broiler chicken's relative growth rate, and dressed percentage were lowest in AFNS compared to the rest treatments. Meat cholesterol was lower in AFNS, AFJK, and AFJL, compared to CONT. Meat catalase in AFNS was lower than those in CONT, AFJK, and AFJL. Meat glutathione peroxidase levels of birds in AFNS are similar to AFJL but were lower than those in CONT, and AFJK. Lipid oxidation, and protein oxidation activities of broiler chickens in AFNS were higher than those in the rest of the treatments. Brain catalase, acetylcholinesterase, and glutathione peroxidase activities of birds in AFNS were lower than CONT, AFJK, and AFJL. Expressions of proinflammatory cytokines, and 8-hydroxy-2'-deoxyguanosine in AFNS were higher compared to other treatments. The immunoglobulins A, E and G of broiler chickens in AFNS were lower than CONT, AFJK, and AFJL. 350 mg/kg JKP or JLP ameliorate the effects of AF contamination on broiler chickens.

Introduction

Commercial broiler chicken production is one of the profitable ventures that bring back profits on capital investment in a relatively short period because broiler chickens are fast and can reach a market weight of about 2 kg in less than 7 weeks of age (Kpomasse et al., 2021; Tallentire et al., 2016).

Feed quality has been identified as a significant challenge, exerting a negative impact on the performance and health of broiler chickens (Houndonougbo et al., 2012). According to Udomkun et al. (2017), one of the main causes of food insecurity is feed contamination by mycotoxins, which harm 25% of the world's crops (Pankaj et al., 2018). Aflatoxins (AFs), a class of very hazardous mycotoxins are known to infect a wide range of foods, including grains like maize and groundnuts (Igbal et al., 2015; Mahato et al., 2019). Since ingestion of AF-contaminated diets by animals or humans produces serious health complications, strict regulations for AFs in feed and food are implemented to maintain public health (Juan et al., 2012; Mahato et al., 2019).

Several hazardous secondary metabolites, including aflatoxins B1, B2, G1, and G2, are frequently produced in response to the growth of *Aspergillus flavus* or *Aspergillus parasiticus* in poultry feeds (Fouad et al., 2019). Aflatoxin B1 is the most harmful and prevalent

mycotoxin among these metabolites (Pitt & Miller, 2017). It is known to be hepatotoxic, cancer-causing, and mutagenic (De Ruyck et al., 2015), and the risks associated with aflatoxin B1 in chickens include low productivity and a high propensity for disease (Fouad et al., 2019). Additionally, it has been hypothesized that Aflatoxin B1 can cause cells to produce intracellular Reactive Oxygen Species (ROS) like superoxide anion, hydroxyl radical, and hydrogen peroxide (An et al., 2017; Towner et al., 2003). Consequently DNA, lipids, and proteins are damaged by oxidation, which causes serious cellular dysfunctions (Forni et al., 2019).

Since the increase in ROS and subsequent oxidative stress and inflammation are closely related to the pathophysiological processes of aflatoxicosis, the use of phytosupplements with well-known antioxidant and anti-inflammatory effects is presently gaining attention (Forni et al., 2019). For example, it is well known that ROS act as physiologic activators of transcription factors like Nuclear Factor B and Activator Protein-1 that, in turn, can modulate the transcription of proinflammatory cytokines like Tumor Necrosis Factor, Interleukin 6, 8, and 1 (Nordberg & Arnér, 2001). Therefore, a fascinating approach for potential clinical applications is the utilisation of phytosupplements with antioxidant and anti-inflammatory action (Forni et al., 2019).

Recently, JKP and JLP were reported as potential phytogenic supplements that possess antioxidant and anti-inflammatory properties and other nutraceutical properties that could be explored to mitigate the negative effects of Aflatoxin dietary contamination (Oloruntola, 2022a; Oloruntola et al., 2022a). Therefore, the objectives of this work are to study the effects of Juglans kernel powder and Jacobina leaf powder supplementation on the growth, carcass, immune system, and DNA biomarkers of broiler chickens fed Aflatoxin B1 contaminated diets.

Materials and Methods

Ethical approval, juglans kernel and jacobinia leaf powder, aflatoxin b1, and experimental diets

The animal care and use procedure was approved by the Animal Care and Use Committee of the Department of Animal Science at Adekunle Ajasin University in Akungba Akoko, Nigeria. The JKP and JLP were produced as described by Oloruntola (2022a) and Oloruntola et al. (2022a), respectively. The pure culture of *Aspergillus flavus* (NRRL 3251), which was grown on potato dextrose agar, produced aflatoxin. The autoclavable polypropylene bags containing 500 g of corn grits were heated to 121 °C and then exposed to a pressure of 120 kPa for 60 min. After being inoculated with an *A. flavus* spore suspension, the autoclaved grit maize was cultivated for seven days at a temperature of 28 °C. Once the fungus had grown, the grit maize was dried in a 70°C oven and ground into powder. Aflatoxin B1 (AF) levels were measured in triplicate using thin-layer chromatography in maize (AOAC, 2010).

A baseline diet (Table 1) was prepared, divided into four halves, and given the designations for the starter and finisher stages:

CONT: No aflatoxin AF contamination and no supplementation.

AFNS: No phytosupplement was added to 0.5 mg/kg of AF-contaminated baseline diet.

AFJK: 350 mg/kg of JKP was added to the 0.5 mg/kg of AF-contaminated baseline diet.

AFJL: 350 mg/kg of JLP was added to the 0.5 mg/kg of AF-contaminated baseline diet.

The 0.5 mg/kg AF/kg dietary contamination utilized in this study is 25 times higher than the dietary concentration for chicken authorized by the National Agency for Food and Drug Administration and Control (NAFDAC) and the European Union (EU) (Burel *et al.*, 2009).

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		
Metabolizable energy (Kcal/kg)	3018.10	3108.20
Crude protein (%)	22.17	20.04

Birds used in experiments and experimental design

At one day of age, 200 Cobb 500 broiler chickens were randomly assigned to four diets, each of which contained five replicas of 10 chicks. Water and feed were continuously accessible during the six-week feeding trial period.

Blood collection and analysis

On day 42, four randomly chosen birds per replication were marked or tagged, and blood samples of roughly 10 ml were taken using a syringe and needle from the brachial vein. For the measurement of pro-inflammatory cytokines and immunoglobulins into plain sample vials. The samples from plain bottles were centrifuged, and their serum was split into a different set of plain bottles and refrigerated at 20°C before being used. The Nuclear Factor Kappa B (NFK B) was determined using a Rat NFKB-p65 ELISA kit (Elabscience Biotechnology Inc. USA); the Tumor Necrosis Factor Alpha (TNF α) was determined with an ELISA kit (Elabscience Biotechnology Inc. USA) while the Interleukin 6 (IL 6) was determined using a Rat IL-6 ELISA kit (Elabscience Biotechnology Inc. USA). The

immunoglobulins A (IgA), E (IgE), G (IgG) and M (IgM) were determined using ELISA kits (Fortress Diagnostics Limited, United Kingdom). The 8-hydroxy-2'-deoxyguanosine (8-OHdG) was determined as described by Zhang et al. (2013).

Relative growth rate and carcass traits

Broiler chickens that were used in the experiment were weighed before the feeding experiment began (day 1) and when the experiment was over (day 42). The relative growth rate (RGR) was estimated (Adebayo et al., 2020) by using the formula:

RGR=[(wt2-wt1)/((w1+w2))/2]*100.

wt1= the broiler chickens' initial weight before the experiment, and wt2= the broiler chicks' weight on the final day of the experiment.

On the 42nd day of the experiment, 14 birds were arbitrarily chosen from each treatment group (two birds/replica), weighed, and slaughtered in accordance with the EU regulation on animal protection during slaughter and killing (Osowe et al., 2022; Uijttenboogaart, 1999). Afterwards, the carcasses were spray-washed and cooled for 30 min at 2 °C. The ratio of the carcass weight to the final body weight was used to estimate the dressing percentage. Also, the relative weights of the heart, liver, lung, pancreas, gizzard, and spleen (percentage of final weight) were computed (Osowe et al., 2022).

Meat and brain analysis

After being slaughtered, a total of 5 birds (one bird per replication) were chosen from each treatment group to be tested for the level of the enzymes catalase, glutathione peroxidase, lipid peroxidation, and protein oxidation, as well as meat cholesterol. Catalase, acetylcholinesterase (AChE), glutathione peroxidase, and ferric ion-reducing antioxidant power (FRAP) in the brain were also examined. A portion of the breast meat from the carcasses was removed, wrapped aerobically in an oxygen-permeable bag, and frozen for 20 days at -18 °C.

The concentration of meat cholesterol was measured spectrophotometrically using commercial kits (Asan Pharm. Co., Ltd., Seoul). The catalase and glutathione peroxidase activities were determined as reported by Muhlisin et al. (2016) and Cichoski et al., (2012), respectively. The thiobarbituric acid (TBA) assay method was used to determine the degree of lipid oxidation in the meat (Tokur et al., 2006). The meat protein oxidation was determined as described by Souza et al. (2013).

Using a high-speed homogenizer, the entire brains of broiler chickens were removed and homogenized in cold saline at 0.9% in a ratio of 1:10 (w/v). The homogenate samples were divided into 1.0 ml aliquots and stored at -18 °C until use after being centrifuged at 2000 rpm for 20 min. The brain catalase (Khan et al. 2012), FRAP (Benzie and Strain, 1996; Sadeghi et al., 2019), and glutathione peroxidase (Khan et al. 2012) activities were determined. The brain AChE activity was determined spectrophotometrically using a modified Ellman's method (Freitas et al., 2016; Silva et al., 2004).

Statistical data analysis

The data were subjected to an analysis of variance (ANOVA) using SPSS v.20, and the Duncan multiple range test of the same program was used to see whether the treatment means differed (Oloruntola et al., 2018).

Results

The comprehensive examination of broiler chickens subjected to AF contamination, coupled with JKP and JLP dietary supplementation, reveals multifaceted impacts on various physiological parameters and meat properties. Figure 1 shows that the relative growth rate (RGR) was lowest in AFNS compared to the rest of the treatments. In addition, the RGR of birds in AFJK and AFJL were similar but significantly higher than the CONT and AFNS.

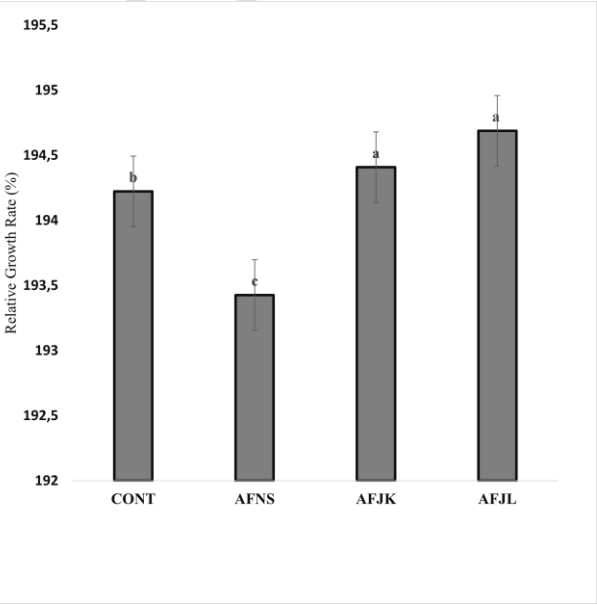

Figure 1. Effects of Justicia kernel powder and Jabobinia leaf powder on the Relative growth rate of broiler chickens fed aflatoxin B1 contaminated diets. CONT: No contamination/supplementation; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF +350 mg/kg JK; AFJL: 0.5 mg/kg AF+350 mg/kg JL.

Table 2 shows the effects of JKP and JLP supplementation on the carcass and internal organs weight (% slaughter weight) of broiler chickens fed an AF-contaminated diet. The dressed percentage of the birds in AFNS was significantly lower than that in CONT and the rest treatments (AFJK and AFJL). There was significant inflammation of the liver, and pancreas of birds in AFNS compared to those in CONT and the rest of the treatments. The enlargement of the spleen recorded in AFNS is similar to AFJK but was significantly higher than those in CONT and AFJL.

The AF dietary contamination, and phytosupplementation have significant effects on the

Table 2. Effects of Juglans kernel powder and Jacobinia leaf powder supplementation on the carcass and internal organs weight (% slaughter weight) of broiler chickens fed Aflatoxin B1-contaminated diet

Parameters	CONT	AFNS	AFJK	AFJL	SEM	P value
Dressed percentage	75.92^{a}	68.59^{b}	76.22^{a}	74.49^{a}	0.78	0.01
Heart	0.48	0.44	0.43	0.53	0.01	0.05
Liver	2.76^{b}	3.56^{a}	2.34^{bc}	2.82^{b}	0.01	0.01
Lung	0.53	0.54	0.52	0.47	0.-02	0.50
Pancrease	0.24^{c}	0.36^{a}	0.30^{b}	0.24^{c}	0.01	0.01
Gizzard	2.23	1.92	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 B1; CONT: No contamination/supplementation; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF +350 mg/kg JK; AFJL: 0.5 mg/kg AF+350 mg/kg JL; SEM: Standard error of means.

Table 3. Effects of Juglans kernel powder and Jacobinia leaf powder supplementation on the meat properties of broiler chickens fed Aflatoxin B1-contaminated diet

Parameters	CONT	AFNS	AFJK	AFJL	SEM	P value
Cholesterol (mmol/l)	5.38^{a}	4.84^{b}	4.00^{c}	4.25^{c}	0.12	0.01
Catalase (kU/ml)	48.65^{a}	32.35^{c}	42.41^{b}	42.46^{b}	1.32	0.01
Glutathione peroxidase (µmole)	208.34^{a}	168.62^{b}	202.05^{a}	178.43 ^{ab}	4.19	0.01
Lipid oxidation (mgMDA/g)	1.21^{b}	1.78^{a}	1.16^{b}	1.28^{b}	0.06	0.01
Protein oxidation (nmol/mg)	99.57^{b}	141.65^{a}	107.04^{b}	105.33^{b}	3.72	0.01

$^{a-c}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin B1; CONT: No contamination/supplementation; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF +350 mg/kg JK; AFJL: 0.5 mg/kg AF+350 mg/kg JL; SEM: Standard error of means.

meat properties in the broiler chickens (Table 3). The meat cholesterol concentration was significantly lower in AFNS, AFJK and AFJL, compared to those in CONT. The meat catalase of birds in AFNS was significantly lower than that in CONT, AFJK, and AFJL. The meat glutathione peroxidase levels of birds in AFNS are similar to AFJL but were lower than those in CONT and AFJK although the glutathione peroxidase of broiler chicken's meat in AFJL was comparable to CONT and AFNS. The lipid oxidation and protein oxidation activities of broiler chickens in AFNS were significantly higher than those in CONT, AFJK, and AFJL.

The effects of JKP and JLP supplementation on the brains of broiler chickens fed AF-contaminated diet are shown in Table 4. The brain catalase, AChE, and glutathione peroxidase activities of birds in AFNS were significantly lower than CONT, AFJK, and AFJL. The brain FRAP of birds in AFNS was lower than those in CONT, AFJK, and AFJL although the FRAP in AFJK and AFJL were lower than CONT.

The NFKB, TNF-α, and IL-6 expressions in AFNS were significantly lower than in CONT, AFJK, and AFJL (Table 5).

Effects of JKP and JLP supplementation on the immunoglobulins of broiler chickens fed AF-contaminated diet are shown in Table 6. The immunoglobulins A, E, and G of broiler chickens in AFNS were lower in AFNS, compared to CONT, AFJK, and AFJL while the immunoglobin M of broiler chickens in AFNS was similar to AFJK but was lower than CONT and AFJL. However, the immunoglobulin M level was higher in AFKL, compare to the rest diets.

Table 4. Effects of Juglans kernel powder and Jacobinia leaf powder supplementation on the brain of broiler chickens fed Aflatoxin B1-contaminated diet

Parameters	CONT	AFNS	AFJK	AFJL	SEM	P value
Catalase (u/mg protein)	7.51^{a}	5.10^{b}	6.68^{a}	6.54^{a}	0.24	0.01
Acetylcholinesterase (u/ml)	0.23^{a}	0.11^{b}	0.21^{a}	0.20^{a}	0.01	0.01
Glutathione peroxidase (U/L)	57.94^{a}	49.13^{b}	56.19^{a}	56.24^{a}	0.99	0.03
FRAP (µM(Fe(II))	74.37^{a}	58.13^{c}	69.06^{b}	69.57^{b}	1.42	0.01

$^{a-c}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin B1; CONT: No contamination/supplementation; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF +350 mg/kg JK; AFJL: 0.5 mg/kg AF+350 mg/kg JL; FRAP: Ferric ion Reducing Antioxidant Power; SEM: Standard error of means.

Table 5. Effects of Juglans kernel powder and Jacobinia leaf powder supplementation on the proinflammatory cytokines of broiler chickens fed Aflatoxin B1-contaminated diet

Parameters	CONT	AFNS	AFJK	AFJL	SEM	P value
NFKB (pg/ml)	26.57^{b}	38.17^{a}	27.99^{b}	26.71^{b}	1.14	0.01
TNF ALFA (pg/ml)	34.47^{b}	63.78^{a}	42.05^{b}	43.57^{b}	3.09	0.01
IL6 (pg/ml)	14.31^{c}	39.53^{a}	22.71^{b}	24.42^{b}	2.18	0.01

$^{a-c}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin B1; CONT: No contamination/supplementation; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF +350 mg/kg JK; AFJL: 0.5 mg/kg AF+350 mg/kg JL; SEM: Standard error of means.

Table 6. Effects of Juglans kernel powder and Jacobinia leaf powder supplementation on the immunoglobulins of broiler chickens fed Aflatoxin B1-contaminated diet

Parameters	CONT	AFNS	AFJK	AFJL	SEM	P value
Immunoglobulin A (mg/dl)	219.54[a]	171.43[b]	243.99[a]	230.67[a]	8.34	0.01
Immunoglobulin E (IU/ml)	1073.83[a]	931.01[b]	1068.58[a]	1105.87[a]	15.66	0.01
Immunoglobulin G (mg/dl)	316.48[a]	210.78[b]	317.17[a]	329.87[a]	11.26	0.02
Immunoglobulin M (mg/dl)	373.64[b]	330.21[c]	348.35[bc]	410.25[a]	7.80	0.01

[a-c]Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin B1; CONT: No contamination/supplementation; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF +350 mg/kg JK; AFJL: 0.5 mg/kg AF+350 mg/kg JL; SEM: Standard error of means.

The effects of JKP and JLP on the serum 8-OHdG of broiler chickens fed AF-contaminated diets were depicted in Figure 2. The expression of 8-OHdG in AFNS was significantly higher than CONT, AFJK, and AFJL. The 8-OHdG in AFJK and AFJL were similar to CONT.

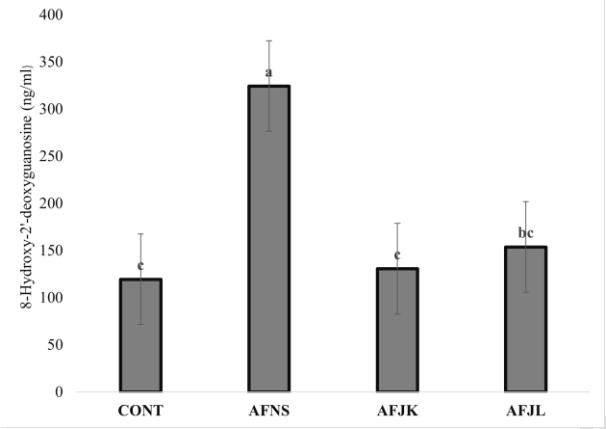

Figure 2. Effects of Justicia kernel powder and Jabobinia leaf powder on the serum 8-Hydroxy-2'-deoxyguanosine of broiler chickens fed aflatoxin B1 contaminated diets. CONT: No contamination/supplementation; AFNS: 0.5 mg/kg AF; AFJK: 0.5 mg/kg AF +350 mg/kgJK; AFJL: 0.5 mg/kg AF+350 mg/kg JL.

Discussion

Examining JKP and JLP supplementation in broiler chickens exposed to Aflatoxin-B1, this discussion explores comprehensive effects on growth, meat, brain, immune system, and DNA biomarkers. The observed lower relative growth rate in this study's experimental birds fed 0.5 mg/kg of AF was comparable to the reduced body weight gain in broiler chickens documented in response to AF dietary contamination by Nazarizadeh et al. (2019). Aflatoxicosis resulted in stunted growth and may have been caused by gastrointestinal dysfunction, which is typically accompanied by reduced feed efficiency (Sarma et al., 2017). The improved relative growth rate observed in the experimental birds fed AF-contaminated diets supplemented with JKP and JLP (AFJK and AFJL) in this study, however, indicates the potential of these phytosupplements to counteract growth-suppressing effects of dietary AF contamination in broiler chickens by improving the nutrient digestion and absorption (Hashemi & Davoodi, 2010; Valenzuela-Grijalva et al., 2017). Previous studies have indicated phytosupplements as growth promoters in animals and particularly broiler chickens (Olarotimi et al., 2022; Oloruntola 2022b; Valenzuela-Grijalva et al., 2017). According to this study, JKP and JLP supplements boosted growth performance in broiler birds fed diets contaminated with AF. Specifically, broiler chicks fed diets contaminated with AF showed enhanced body weight gain as a result of JKP and JLP supplementation. This improvement may be attributed to a variety of biological activities, including the antioxidant, antibacterial, and flavor-enhancing effects of phytogenic supplements (Valenzuela-Grijalva et al., 2017).

The observed reduced dress percentage recorded in AFNS in this study could also be due to the same factor that affected their relative growth rate. Furthermore, the animals' relative internal organ weights may have increased abnormally, which could be a sign that their internal organs are reacting to a toxin in their diet (Ayodele et al., 2016). As recorded in this study, the increased liver, pancreas, and spleen relative weights of the experimental birds due to aflatoxicosis were linked to the carcinogenic, mutagenic, immunosuppressive, and teratogenic activities of AF causing significant interference with the normal protein synthesis and inhibition of myriad metabolic systems, and consequently, causing pathological processes in various organs such as the heart, kidney, and liver (Mohammed & Metwally, 2009). The pancreas (El-Haleem et al., 2011) and spleen (Li et al., 2019) are also among the common organs affected by aflatoxicosis.

There is currently an increased global preference for low-cholesterol chicken meat by consumers and more dietary modifications are being adopted to decrease the fat and cholesterol contents of poultry meat (Ponte et al., 2004). By implication, the observed reduced meat cholesterol concentration of birds in AFNS, AFJK, and, AFJL in this study is of benefit. The reduced meat cholesterol concentration in broiler chickens fed aflatoxin-contaminated feed could be due to gastrointestinal dysfunction resulting in reduced nutrients e.g., fat utilization and consequently low concentration of fat and cholesterol in their meat (Sarma et al., 2017). Additionally, the phytogenic supplements in AFJK and AFJL may contain bioactive substances like tannin and saponins that prevent the absorption of fat and an excessive buildup of lipids in the meat (Oloruntola et al., 2022b; Thinh et al., 2018).

Catalase, glutathione peroxidase, and superoxide dismutase are antioxidant enzymes that are active in meat's enzymatic defense systems (Min et al., 2008).

The decreased catalase, and glutathione peroxidase activities in the meat of the birds fed a diet contaminated with AF were consistent with earlier studies that explained that aflatoxicosis caused the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase to be downregulated, increasing the byproducts of lipid peroxidation (Da Silva et al., 2018). Previous reports show that JKP and JLP have antioxidant activities and nutraceutical values (Oloruntola, 2022a; Oloruntola et al., 2022a). This may explain the improved meat catalase and glutathione peroxidase activities in AFJK and AFJL birds' meat when compared to AFNS. Recent studies suggest that several phytosupplements have advantageous antioxidant properties and may significantly contribute as natural substitutes for synthetic antioxidant feed additives in enhancing the meat's antioxidant status (Abbas et al., 2015; Lee et al., 2017).

The elevated birds' meat lipid oxidation and protein oxidation activities in AFNS agreed with Da Silva et al. (2018) while similar lipid oxidation and protein oxidation activities found in the meat of broiler chickens fed AF-contaminated diets being supplemented with JKP and JLP and those fed the control diet in this study point to the presence of antioxidants and the potency of these phytosupplements (JKP and JLP) in stabilizing or delaying the lipid and protein oxidation processes in the meat of broiler chickens. According to earlier research, using dietary antioxidants can delay the oxidation process. (Cortinas et al., 2005; Smet et al., 2008). For instance, Cortinas et al. (2005) found positive oxidative stability after dietary supplementation with tocopheryl acetate. Additionally, polyphenols and flavonoids regulate oxidation by stopping or restricting chain reactions after radicals are created (Keppler et al., 2020).

AF induces neurotoxicity by causing DNA damage, apoptosis, and an interruption of the S-phase cell cycle (Huang et al., 2020). This may help to an extent explain why broiler chickens fed AFNS had reduced catalase, acetylcholinesterase, glutathione peroxidase, and ferric ion-reducing antioxidant power. Increased lipid peroxidation, increased oxidative pathways, and lower levels of antioxidant enzymes have all been associated with AF (Gugliandolo et al., 2020). However, the stable catalase, acetylcholinesterase, glutathione peroxidase, and ferric ion-reducing antioxidant power in AFJK and AFJL, when compared to CONT, further support the antioxidant and nutraceutical activities of JKP and JLP and their neuroprotective potentials (Kumar & Khanum, 2012; Oloruntola, 2022a; Oloruntola et al., 2022a).

By affecting the function of receptors for the main inhibitory neurotransmitters, phytosupplements from medicinal plants play a crucial part in preserving the chemical balance of the brain (Kumar & Khanum, 2012). Some phytosupplements have been demonstrated to have antioxidant and/or anti-inflammatory properties in a range of peripheral systems. Anti-inflammatory herbal medicine and its contents are now being demonstrated to be an effective neuroprotector against many brain disorders, as increasing data suggest that neuroglia-derived chronic inflammatory responses play a pathogenic function in the central nervous system (Kumar & Khanum, 2012; Pueyo & Calvo, 2009).

The observed amplified serum NFKB, TNF-α, and IL-6 in birds AFNS could be the expression of the mycotoxins' ability to cause or worsen inflammation through a variety of molecular processes, including the activation of inflammasomes and the generation of reactive oxygen species (Brown et al., 2021; Zhen & Zhang, 2019). It has also been demonstrated that food contamination with AF raises the levels of pro-inflammatory cytokines (Kraft et al., 2021). In addition, the stabilized serum NFKB, TNF-α, and IL-6 of birds in AFJK and AFJL could be due to the anti-inflammatory activities and other nutraceutical properties of JKP and JLP, the phytosupplement used in this study (Oloruntola, 2022a; Oloruntola et al., 2022a). A number of compounds originating from plants have lately been theorized to act as anti-inflammatory agents by controlling the production of proinflammatory microRNAs (Saleh et al., 2021). When consumed, bioactive substances originating from plants, such as phenolic compounds, which include flavonoids and tannins, glucosinolates, alkaloids, and terpenoids, get involved in a variety of biological processes in the body, including redox reactions, cell signaling, and inflammation (Perez-Gregorio & Simal-Gandara, 2017). As a result, natural products are increasingly showing promise in the treatment of a variety of inflammatory disorders arising from aflatoxicosis and related cases (Gautam & Jachak, 2009; Mahoney & Molyneux, 2004).

The depressed immunoglobulins A, E, G, and M of birds in AFNS agreed with Soltani et al. (2019), who recorded lower immunoglobulins G and M in broiler chickens fed AF-contaminated diet, compared to those fed the control diet. The inhibition of protein synthesis, which lowers the production of immunoglobulin as a result of aflatoxicosis, could be the cause of the decreased immunological responses of broiler chickens in AFNS (Soltanin et al., 2019; Sur & Celik, 2003). Furthermore, the stable immunoglobulins A, E, G, and M of broiler chickens in AFJK and AFJL, when compared to those in CONT in this study, further unveils the immunomodulatory properties of phytosupplements (Oloruntola et al., 2016). It has been demonstrated that phenolic compounds also have an impact on humoral immunity by promoting the release of certain immunoglobulins (Allam et al., 2016). Previous studies shows that serum immunoglobulins M and G levels are significantly enhanced by the phenolic compounds in phytosupplements (Allam et al., 2016; Maheshwari et al., 2022).

The best non-invasive indicator of DNA oxidative damage is 8-OHdG, which is the most representative product of oxidative alterations in DNA (Valavanidis et al., 2009; Guo et al., 2016). The amplified expression of serum 8-OHdG of broiler birds in AFNS indicates the possibility of DNA oxidative damage being precipitated

by AF exposure. As is popularly known, AF is mostly metabolized by cytochrome P450 enzymes producing the genotoxic metabolite 8,9-epoxide-AFB1 (AFBO) (Guengerich et al., 1998; Feng et al., 2016). AF-DNA adducts can be formed when AFBO binds to DNA. Consequently, AF-DNA adducts may obstruct regular transcription and replication, leading to damage in double-stranded DNA (Feng et al., 2016). The levels of 8-OHdG found in the serum of broiler chickens in AFJK and AFJL are comparable to those in CONT, suggesting that JKP and JLP, taken as dietary supplements, have antioxidant and other nutraceutical properties that support the delay of reactive oxygen species' detrimental effects on DNA integrity and prevent DNA damage (Ye et al., 2023). In addition, it has been demonstrated that a number of phytosupplements prevent tumor onset and growth by causing DNA damage (Ye et al., 2023). For instance, dietary polyphenols can shield the organism from reactive oxygen species' negative effects on DNA integrity (Azqueta & Collins, 2016).

Conclusion

The 0.5 mg/kg AF dietary contamination produced a retarded relative growth rate, depressed dress percentage and inflammation of the liver and pancreas, which were ameliorated by 350 mg/kg JKP and JLP supplementations while the 0.5 mg/kg AF dietary contamination with or without JKP or JLP supplementation caused a reduced meat cholesterol level. The reduced meat catalase, glutathione peroxidase and increasing lipid peroxidation and protein peroxidation activities being caused by AF dietary contamination are prevented by both JKP and JLP dietary supplementations. In addition, the brain AChE and glutathione peroxidase activities and the serum IgA, IgE, IgG, NFKB, TNF-α and IL-6 were reduced as a result of 0.5 mg/kg AF exposure while the expression of 8-OHdG was also depleted by AF dietary contamination. Therefore, 350 mg/kg JKP or JLP supplementations are recommended to improve the growth, meat oxidative status, immune system, and health status of broiler chickens when exposed to AF dietary contaminated diets.

Ethical Statement

The animal care and use procedure was approved by the Animal Care and Use Committee of the Department of Animal Science at Adekunle Ajasin University in Akungba Akoko, Nigeria.

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