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Juglans regia kernel powder supplementation in Broiler Chickens fed Aflatoxin-contaminated diets: Effect on growth, serum chemistry indices, immunoglobulin and pro-inflammatory cytokines

Running title: Walnut kernel in chickens fed an aflatoxin-infected feed

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ABSTRACT

The study aimed to investigate the impact of *Juglans regia* kernel powder (JKP) on broiler chickens subjected to Aflatoxin (AF)-contaminated diets during a 42-day feeding trial conducted in February and March 2022. A total of 240 one-day-old broiler chickens were divided into four dietary groups: Diet 1 (Control), Diet 2 (0.5 mg/kg AF), Diet 3 (0.5 mg/kg AF +250 mg/kg JKP), and Diet 4 (0.5 mg/kg AF+500 mg/kg JKP). Birds on Diet 2 exhibited a significantly lower (P=0.01) relative growth rate compared to other diets. JKP supplementation at 250 mg/kg (Diet 3) and 500 mg/kg (Diet 4) mitigated the negative impact of AF on growth. Birds on Diet 2 showed significantly lower (P=0.01) serum concentrations of total protein, albumin, and globulin compared to those on Diets 1, 3, and 4. Elevated levels of aspartate aminotransferase (AST) and creatinine in Diet 2 indicated liver and kidney damage. Alanine transaminase (ALT) concentrations in Diet 2 were higher (P=0.01) than Diets 1 and 4. Birds fed diet 2 had lower glucose levels (P=0.01) than diets 1 and 4. IgA levels in birds fed Diet 2 were lower (P =0.03) than those in the birds fed Diet 4. Birds fed diet 2 had considerably (P<0.05) lower IgE and IgG levels than birds fed diets 1 and 4. Nuclear Factor Kappa B (NFK B) was higher (P=0.01) in birds fed Diet 2 compared to other diets. Interleukin 6 (IL 6) concentration was significantly (P=0.01) higher in the birds fed Diet 2 than in the rest

diets. A recommended dietary supplementation of 250 mg/kg JKP is suggested based on observed ameliorative effects.

Keywords: aflatoxicosis, botanicals, broiler chickens, immunity, inflammation.

INTRODUCTION

According to reports, the recent surge in animal output in tropical and subtropical nations has been attributed to an increase in the human population (Godfray *et al*., 2010). Additionally, broiler chicken production has been identified as a potential solution to the issue of animal protein deficiency in these regions (Hatab *et al*., 2019). However, broiler chickens face challenges in reaching their genetic potential in tropical and subtropical climates due to factors such as the scarcity of feedstuffs, heat stress, and contaminated feed (Kpomasse *et al*., 2019).

Aflatoxin (AF), the most common mycotoxin, is produced by *Aspergillus flavus*, *A. nomius*, and *A. parasiticus* (Morrison *et al*., 2017). *A. flavus* produces four toxins (AFB1, AFB2, AFG1, and AFG2) with similar chemical structures, but AFB1 is the most potent hepatotoxin and a recognized hepatocarcinogen (Quezada *et al*., 2000). The prevalence of warm and humid conditions favorable for aflatoxin growth, combined with the limited effectiveness of feed processing techniques in removing aflatoxins from contaminated diets due to their thermal resistance, contributes to the prevalence of aflatoxicosis in the tropics and subtropics (Medina *et al*., 2017; Mahato *et al*., 2019).

Aflatoxicosis in poultry induces anorexia, lethargy, stunted growth, reduced fertility, and microbial stress, leading to impaired gut health, economic losses, and toxicity (Sarma *et al*., 2017). Furthermore, aflatoxin damage extends to the liver and kidneys, resulting in impaired immune function and an upregulation of proinflammatory gene expression (Quezada *et al*., 2000; Li *et al*., 2022). It was reported that AF induces the generation of intracellular Reactive Oxygen Species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide in mammalian cells (Sohn *et al*., 2003; An *et al*., 2017). This suggests the potential of antioxidants to ameliorate the negative effects of AF toxicity in animals when fed AF-contaminated diets.

The potential of medicinal plant-derived antioxidant dietary intake in ameliorating the damage caused by oxidative stress through inhibiting the initiation or propagation of oxidative chain reactions and quenching singlet oxygen and reducing agents has been reported (Cai *et al*., 2004; Baiano and del Nobile, 2015; Adegbeye *et al*., 2020). Broadly speaking, these natural antioxidants have anti-inflammatory, antiviral, antibacterial, and anticancer activities (Xu *et al*., 2017).

Juglans regia Linn is a potential nutraceutical and medicinal plant used traditionally to address various maladies, including diarrhea, stomachaches, arthritis, asthma, and endocrine problems like diabetes mellitus, thyroid dysfunctions, and cancer (Taha and Al-wadaan, 2021). As documented by Oloruntola (2022), *Juglans regia* kernel powder contains saponins (43.49 mg/g), alkaloids (120.80 mg/g), flavonoids (14.72 mg/g), tannins (1.69 mg/g), phenol (35.93 mg/g), and steroids (4.84 mg/g), contributing to its nutraceutical properties. Recent study (Oloruntola, 2022) have highlighted *Juglans regia* kernel powder's anti-inflammatory, antioxidant, and anti-diabetic effects, encouraging its usage as a nutritional supplement for feed.

Including green husk walnut powder (Mousavi Razi *et al*., 2017) and walnut leaves (Popescu *et al*., 2020) in the diet has been reported to enhance the function of the broiler immune system and promote gastrointestinal tract health. However, research on the dietary supplementation of *Juglans regia* kernel powder in broiler nutrition is relatively scarce. Therefore, this study aims to investigate the effects of *Juglans regia* kernel powder dietary supplementation on the growth, serum chemistry indices, immunoglobulin, and pro-inflammatory cytokines of broiler chickens fed aflatoxin-contaminated diets.

MATERIALS AND METHODS

Animal Ethics, *Juglans regia* kernel powder, Aflatoxin B1, Experimental diets and Birds

The broiler care and use procedures have obtained approval from the Department of Animal Science's Animal Care and Use Committee at Adekunle Ajasin University, Akungba Akoko, Nigeria. *Juglans regia* kernel powder (JKP) was produced, as previously detailed by Oloruntola (2022). The *Juglans regia* fruits were sourced from villages in Akungba Akoko, Nigeria. Raw kernels were carefully extracted, finely chopped, sparingly scattered, and air-dried in the shade for 14 days. Subsequently, the dried kernels were milled to form *Juglans regia* kernel powder (JKP), which was then stored for subsequent laboratory analysis.

The *Aspergillus flavus* (NRRL 3251) pure culture, maintained on potato dextrose agar, served as the source of the aflatoxin. Autoclavable polypropylene bags containing 500 grams of maize grits were heated to 121 degrees Celsius and exposed to a pressure of 120 kPa for 60 minutes. Following inoculation with an *A. flavus* spore suspension, the autoclaved grit maize was cultivated for seven days at a temperature of 28°C. After the fungus developed, the grit maize was dried in a 70°C oven and ground into powder.

In formulating experimental diets with 0.5mg/kg AFB1 contamination, 100g of AFB1 cultured maize was carefully blended with 1kg of broiler feed and subsequently analyzed for

AFB1 concentration. The analysis indicated an AFB1 concentration of 17mg/kg. Consequently, these findings were utilized to calculate the necessary amount of cultured maize required for 1kg of broiler feed to achieve the targeted 0.5mg/kg AFB1 concentration. The amount of aflatoxin (AF) in the blend of maize and broiler feed was measured in triplicate using thin-layer chromatography (AOAC, 2010).

A baseline diet (Table 1) for the starter and finisher stages was produced following the recommendations of the National Research Council (NRC, 1994). Subsequently, thin-layer chromatography was employed to check the baseline diet for any AF that may have been present (AF was not present in any significant amount). The proximate composition of the baseline diets was investigated (AOAC, 2010), and the diets were split into four equal parts. Each part was sufficiently contaminated with AF-maize powder, added JKP, and labeled as necessary: Diet 1: Control; Diet 2: 0.5 mg/kg AF; Diet 3: 0.5 mg/kg AF +250 mg/kg JKP; Diet 4: 0.5 mg/kg AF+500 mg/kg JKP. The 0.02 mg/kg limit allowed by NAFDAC, the EU, the USFDA, the CFIA, and ANAC was 25 times lower than the 0.5 mg AF/kg feed concentration in the chicken diet used in this study (Burel *et al*., 2009).

A total of 240 Cobb 500 broiler chickens that were 1 day old were randomly assigned to 4 diets, each having 6 replicates of 10 chickens. The experiment consisted of two phases: 1-21 days and 22-42 days. For the entire six-week testing period, both feed and water were freely available.

Determination of Relative Growth Rate, Serum Chemistry Indices, Immunoglobulin, and Pro-inflammatory Cytokines

Measurement of Relative Growth Rate (RGR)

At the onset of the feeding experiment (day 1) and upon its conclusion (day 42), the weights of the broiler chicks were meticulously measured. The Relative Growth Rate (RGR) was estimated using the formula published by Adebayo *et al*. (2020):

$RGR= [(w_2-w_1)/((w_1+w_2))/2]*100.$

Where:

w represents the initial weight of the broiler chickens before the experiment,

w represents the weight of the broiler chicks on the final day of the experiment.

Three randomly selected birds per replication were tagged, and approximately 10 ml of blood samples were obtained using a syringe and needle from the brachial vein. The blood was drawn into plain bottles, allowed to stand at room temperature for around 30 minutes, centrifuged at 3,000 rpm for 10 minutes, and the serum was then decanted into new plain bottles. The labeled serum samples were stored at -20 °C until required for analysis of chemistry indices, immunoglobulins, and pro-inflammatory cytokines.

Serum Chemistry Indices Analysis

A Reflectron® Plus 8C79 (Roche Diagnostic, GmbH Mannheim, Germany) with commercial kits was employed to measure total protein, albumin, aspartate aminotransferase (AST), alanine transferase (ALT), cholesterol, creatinine, and glucose (Oloruntola *et al*., 2018). The discrepancies between total protein and albumin were utilized to determine globulin.

Immunoglobulin and Pro-inflammatory Cytokines Analysis

Immunoglobulins A (IgA), E (IgE), G (IgG), and M (IgM) were determined using ELISA kits from Fortress Diagnostics Limited, United Kingdom. Nuclear Factor Kappa B (NFK B) was determined using a Rat NFKB-p65 ELISA kit from Elabscience Biotechnology Inc. USA. Tumor Necrosis Factor Alpha (TNF α) was determined with an ELISA kit, also from Elabscience Biotechnology Inc. USA, while Interleukin 6 (IL 6) was determined using a Rat IL-6 ELISA kit from the same manufacturer.

Statistical Data Analysis

The obtained data were subjected to analysis of variance (ANOVA) using SPSS version 20. To identify differences in treatment means, the Duncan multiple range test from the same statistical program was employed (Oloruntola *et al*., 2018).

RESULTS

Figure 1 illustrates the impact of *Juglans regia* kernel powder (JKP) supplementation on the relative development of broiler chickens fed diets contaminated with aflatoxin (AF). Broiler chickens on diet 2 (AF-contaminated) exhibited a significantly lower ($P<0.05$) relative growth rate compared to those on control (diet 1) and other diets. Birds on diet 4 (AF + 500 mg/kg JKP) displayed a relative growth rate comparable ($P>0.05$) to diet 3 (AF + 250 mg/kg JKP) but significantly higher ($P<0.05$) than diet 1.

The results of JKP supplementation on the serum chemistry indices are presented in Table 2. Birds on diet 2 showed significantly lower ($P<0.05$) serum concentrations of total protein, albumin, and globulin compared to diets 1, 3, and 4. Additionally, diet 2 resulted in significantly higher ($P<0.05$) levels of aspartate aminotransferase (AST) and creatinine compared to the control and other diets. Alanine transaminase (ALT) concentrations in diet 2 were comparable ($P>0.05$) to diet 3 but significantly higher ($P<0.05$) than diets 1 and 4. Broiler chickens on diet 2 had glucose levels comparable ($P>0.05$) to diet 3 but significantly lower ($P<0.05$) than diets 1 and 4.

Table 3 shows the impact of JKP supplementation on the immunoglobulin levels of broiler chickens fed diets contaminated with AF. Birds on diet 2 had significantly lower ($P<0.05$) IgA, IgE, and IgG levels compared to birds on diets 1 and 4. IgA levels in diet 2 were comparable to diets 1 and 3 but significantly lower than diet 4.

The effects of JKP supplementation on pro-inflammatory cytokines are presented in Table 4. Nuclear Factor Kappa B (NFK B) and Interleukin 6 (IL 6) concentrations were significantly higher ($P<0.05$) in birds on diet 2 compared to diets 1, 3, and 4. IL 6 concentrations were similar in birds fed the control (diet 1) and diet 4.

DISCUSSION

Juglans regia kernels are rich in antioxidants, including polyphenols, omega-3 fatty acids, and melatonin, providing potential benefits for anti-inflammatory responses and cardiovascular health (Bhat *et al*., 2023). Constituents such as ellagic acid, gallic acid, Alpha-Linolenic Acid (ALA), and melatonin contribute to the nutraceutical profile of *Juglans regia* kernels, making them a promising dietary supplement (Shah *et al*., 2018).

Aflatoxin feed contamination (0.5 mg/kg) significantly reduced the relative growth rate of broiler chickens, aligning with previous studies (Denli *et al*., 2004; Denli *et al*., 2009). Due to the degradation of the digestive and metabolic efficiency of the birds exposed to AF dietary contamination, the retarded growth rate was connected to decreased energy and poor protein utilisation (Verma *et al*., 2002; Denli *et al*., 2009). However, supplementation with JKP, especially at 250 and 500 mg/kg, mitigated the negative effects of AF contamination on growth, suggesting a protective role for JKP in the digestive system and physiological processes. The use of medicinal or herbal plants parts in controlling or preventing cases of toxicity has been reported (Khafaga and Bayad, 2016; Aboelhassan *et al*., 2018). Certain components present in *Juglans regia* kernel, including bioactive compounds like saponins, alkaloids, flavonoids, tannins, phenols, and steroids, may contribute to aflatoxin-binding properties (Oloruntola, 2022). These compounds may interact with aflatoxins, potentially reducing their absorption and mitigating their adverse effects on the gastrointestinal tract (Pathaw *et al*., 2022). Nevertheless, this assertion is contingent upon further and more comprehensive research.

The blood total protein test determines the quantity of all proteins, specifically blood globulin and albumin (Tothova *et al*., 2016) and is one of the sensitive early biomarkers of poultry exposure to aflatoxin B1 (Quezada *et al*., 2000). Also, significant clinical problems such as inflammatory illnesses, liver disorders, kidney disorders, malnutrition, and others were linked to low total protein levels (Tothova *et al*., 2016).

The reduction in blood concentrations of total protein, albumin, and globulin observed in this investigation is consistent with the finding of Safameher (2008) that broiler chickens fed diets containing 0.5 to 2.0 ppm AFB1/kg indicate a decrease in total serum protein concentration. The blocking of RNA synthesis, followed by the inhibition of protein synthesis in the liver, and ultimately the reduction in plasma protein concentration could be the reason for the decreased serum protein concentration brought on by aflatoxin exposure (Del Bianchi *et al*., 2005). Furthermore, complications with the liver or kidneys (Tothova *et al*., 2016) could be responsible for the reported decrease in serum total protein concentration in this study. This is supported by concurrent elevated serum AST, ALT, and creatinine recorded in the same group of birds (diet 2). The elevated serum AST and ALT concentrations recorded in birds fed diet 2 indicate liver damage. This finding aligns with the observations of Tessari *et al*. (2010), who documented elevated AST levels in birds fed 50 and 200 μg AFB1/kg. Additionally, Valchev *et al*. (2014) reported increased ALT activity in broiler chickens fed 0.5 mg/kg AFB1.

In addition, records on the toxic effects of aflatoxin on blood parameters exhibited through increased creatinine and uric acid were reported (Valchev *et al*., 2014). Hence, the observed elevation in serum creatinine concentration among birds fed aflatoxin-contaminated feed (diet 2) in this study underscores the potential peril of aflatoxin dietary contamination on the normal physiological and anatomical functions of the kidney (Valchev *et al*., 2014).

In a nutshell, the production of a reactive metabolite called AFB1- 8,9-epoxide, which is formed quickly by the action of at least five members of the mixed-function oxidase family, is the cause of aflatoxin's renal toxicity. AFB1-8,9-epoxide reacts with DNA to yield the 8,9-dihydro-8-(N7-guanyl)-9- hydroxy aflatoxin B1 adduct (AFB1-N7-Gua), which has been positively correlated with DNA strand breaks, hepatic tumor development, and the development of renal lesions (O’Brien and Dietrich, 2004).

According to this study, broiler chickens fed AF-contaminated diets had lower blood glucose levels, possibly attributed to aflatoxin's hepatotoxic effects, which cause problems with lipid and carbohydrate metabolism (Rosa *et al*., 2001; Basmacioglu *et al*., 2005). This outcome was consistent with data from Basmacioglu *et al*. (2005), who noted hypoglycemia in broiler chickens fed a diet contaminated with 2 mg AF/kg feed.

Free radicals and reactive oxygen species (ROS) produced by mycotoxins harm cells (Marin and Taranu, 2012). Aflatoxin-induced ROS generation can harm the cells of target organs like the liver and kidney. In addition to an increase in lipid peroxidation metabolites in the liver and kidney (Alpsoy and Yalvac, 2011) and a decrease in the cellular total antioxidant in birds, there is a considerable shift in blood biochemical indices after this increase (Sirajudeen *et al*., 2011). Therefore, the observed ameliorative activities of JKP in the birds fed diets contaminated with AF (0.5mg/kg) and supplemented with JKP (250 and 500 mg/kg) diets in this study about the serum total protein, albumin, globulin, AST, ALT, creatinine, and glucose could be an outcome of the nutraceutical and antioxidant activity of JKP. It has been claimed that JKP has antioxidant, anti-inflammatory, and anti-diabetic characteristics and is a helpful nutraceutical feed additive (Oloruntola, 2022).

JKP's ameliorative effects on broiler chickens fed diets contaminated with aflatoxin in this study were consistent with those of curcumin (Damiano *et al*., 2022) and aloe vera powder (Seifi *et al*., 2022) on poultry/birds fed diets containing aflatoxin.

It was discovered that aflatoxins impair the innate and acquired responses of the immune system (Weaver *et al*., 2013). The decreased concentrations of IgA, IgE, and IgA observed in broiler chickens given a diet contaminated with aflatoxin in this study may be caused by the dysregulation of dendritic cells' ability to present antigens and impaired cell-mediated immunity as a result of aflatoxin exposure (Mehrzad *et al*., 2014). However, the improved levels of IgA, IgE, and IgA in birds fed a supplemented diet in this study unveil the immunomodulatory properties of phytochemicals or bioactive compounds in the JKP. Inferentially, JKP supplementation stops the mechanisms leading to immune system dysfunction typically linked to aflatoxin dietary contamination. As previously explained, several dietary phytochemicals interact with immunological signal transduction pathways connected to inflammation to exhibit immune modulatory actions (Zhao *et al*., 2021).

The triggered Nuclear Factor Kappa B (NF-κB) and interleukin 6 (IL-6) observed in the birds fed the aflatoxin-contaminated diet in this study could be associated with the typical expression of aflatoxicosis because exposure to aflatoxin frequently results in elevated reactive oxygen species (ROS), oxidative stress, lipid peroxidation, apoptosis, mitochondrial dysfunction, necrosis, and inflammatory response (Dai *et al*., 2022). For instance, NF-κB is one of the several pathways that have been shown to support AFB1-mediated toxicity in mammalian cells (Dai *et al*., 2022), and according to Karunaweera *et al*. (2015), the activation of NF-κB requires the degradation of the inhibitor kappa B alpha and mediates the production of more than 500 genes, including tumor necrosis factor-alpha (TNF-alpha) and IL-6 (Yamashita *et al*., 2014).

As recently reported, the administration of low doses of aflatoxin may also upregulate the expression of NF-κB, TNF-α, and IL-6, causing a significant inflammatory response in the liver tissues (Guo *et al*., 2022; Dai *et al*., 2022). However, the identical or similar NF-κB and

IL-6 gene expression observed in the birds fed aflatoxin-contaminated diets supplemented with JKP and the control diet in this study further demonstrates the nutraceutical properties and the activities of bioactive components of JKP. JKP achieves this by inhibiting the activation of TLR4/MyD88, which is followed by the activation of NF-κB and its downstream IL-6, and TNF-α genes' expression (Li *et al*., 2022; Guo *et al*., 2022). This outcome agrees with Li *et al*. (2022) findings, which showed that curcumin supplementation slowed the expression of the NF-κB and IL-6 genes.

CONCLUSIONS

Dietary supplementation with JKP at 250 and 500 mg/kg demonstrated ameliorative effects on broiler chickens exposed to aflatoxin B1. The improvements in growth rate, serum chemistry indices, immunoglobulins, and pro-inflammatory cytokines suggest the potential of JKP as a nutraceutical feed supplement. A recommended dietary supplementation of 250 mg/kg JKP is suggested for optimal broiler chicken production.

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Table 1. Composition of the experimental diets.

Ingredients (%)	Starter phase	Finisher phase
Rice bran	0.00	3.02
Maize	50.36	58.36
Maize bran	3.00	0.00
Soy oil	1.00	1.00
Fish meal	3.00	3.00
Soybean meal	38.00	30.00
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
Available phosphorus	0.48	0.43
Calcium	1.03	1.04
*Crude fibre	3.52	3.58
*Crude fat	4.23	2.38
*Crude protein	22.17	20.04

*Analyzed composition

**1kg of vitamin-mineral premix contains Vitamin D3 - 2,000,000IU, Vitamin K - 2,250mg, Vitamin A - 10,000,000IU, Vitamin E - 20,000IU, Thiamine B1 - 1,750mg, Niacin - 27,500mg, Pantothenic acid - 7,500mg, Biotin - 50mg, Choline chloride - 400g, Riboflavin B2 - 5,000mg, Pyridoxine B6 - 2,750mg, Antioxidant - 125g, Magnesium - 80g, Iodine - 1.2g, Selenium - 200mg, Cobalt - 200mg, Zinc - 50mg, Iron - 20g, Copper - 5g.

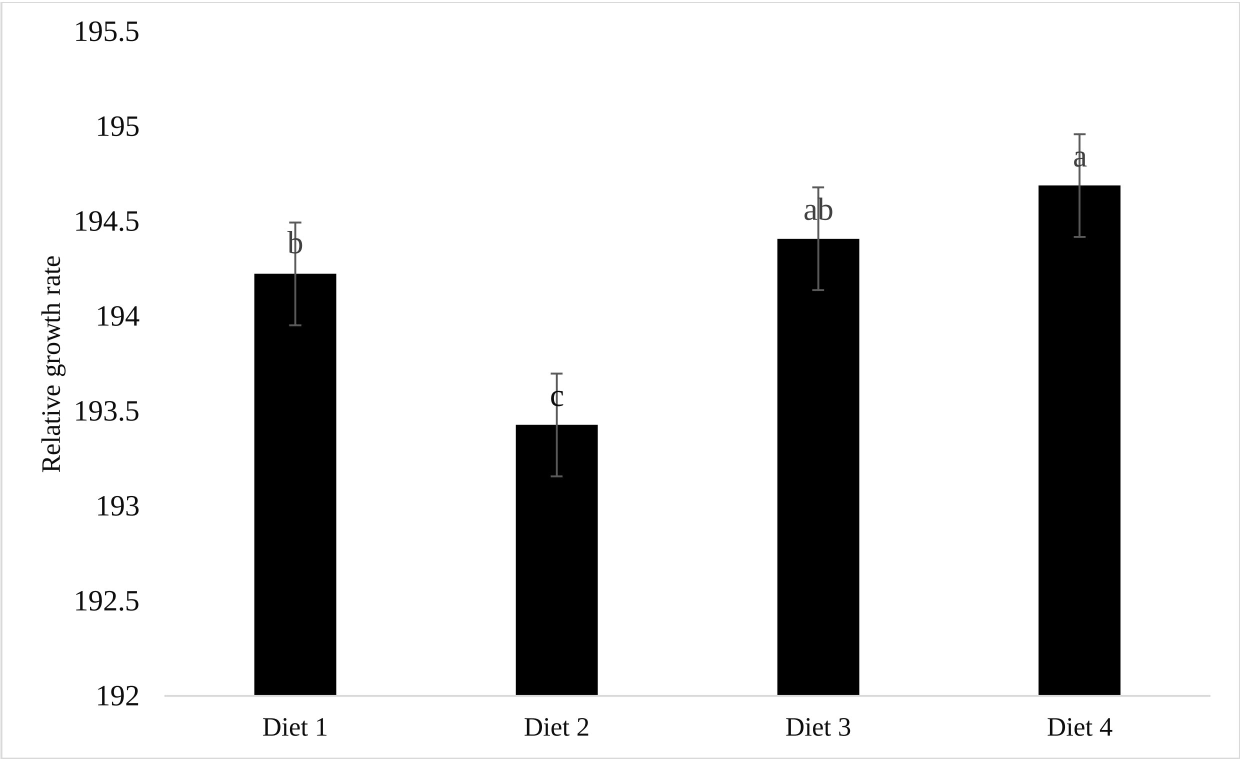

Figure 1. Effects of *Juglans regia* kernel powder supplementation on growth of broiler chickens fed Aflatoxin B1 contaminated diets. AF: Aflatoxin; Diet 1: Control; Diet 2: 0.5 mg/kg AF; Diet 3: 0.5 mg/kg AF+250 mg/kg JKP; Diet 4: 0.5 mg/kg AF+500 mg/kg JKP.

Table 2. Effects of *Juglans regia* kernel powder supplementation on serum chemistry of broiler chickens fed Aflatoxin-contaminated diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Total protein (mmol/l)	39.80^{a}	26.40^{b}	37.00^{a}	39.47^{a}	1.76	0.01
Albumin (mmol/l)	21.35^{a}	13.75^{b}	20.45^{a}	20.52^{a}	1.17	0.04
Globulin (mmol/l)	18.45^{a}	12.65^{b}	16.55^{a}	18.95^{a}	0.87	0.01
Aspartate aminotransferase (IU/L)	87.05^{b}	111.20^{a}	94.30^{b}	85.05^{b}	3.45	0.01
Alanine transaminase (IU/L)	46.20^{c}	52.65^{a}	50.65^{ab}	49.45^{b}	0.75	0.01
Cholesterol (mmol/l)	5.05	5.40	5.65	5.30	0.08	0.07
Creatinine (mmol/l)	45.24^{b}	53.22^{a}	35.21^{c}	36.86^{c}	2.30	0.01
Glucose (mmol/l)	17.64^{a}	14.10^{b}	15.51^{ab}	17.16^{a}	0.46	0.01

$^{a-c}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin; Diet 1: Control; Diet 2: 0.5 mg/kg AF; Diet 3: 0.5 mg/kg AF +250 mg/kg JKP; Diet 4: 0.5 mg/kg AF+500 mg/kg JKP; SEM: Standard error of means.

Table 3. Effects of *Juglans regia* kernel powder supplementation on immunoglobulins of broiler chickens fed aflatoxin-contaminated diets.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Immunoglobulin A (mg/dl)	218.80^{ab}	170.69^{b}	221.64^{ab}	266.34^{a}	12.49	0.03
Immunoglobulin E (mg/dl)	1071.50^{a}	931.52^{b}	1047.93^{a}	1089.23^{a}	20.81	0.01
Immunoglobulin G (mg/dl)	315.65^{a}	212.06^{b}	297.68^{a}	336.67^{a}	15.64	0.02
Immunoglobulin M (mg/dl)	371.41	330.88	353.21	343.51	7.44	0.28

$^{a-b}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin; Diet 1: Control; Diet 2: 0.5 mg/kg AF; Diet 3: 0.5 mg/kg AF +250 mg/kg JKP; Diet 4: 0.5 mg/kg AF+500 mg/kg JKP; SEM: Standard error of means.

Table 4. Effects of *Juglans regia* kernel powder supplementation on pro-inflammatory cytokines of broiler chickens fed aflatoxin-contaminated diets.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Nuclear Factor Kappa B (pg/ml)	26.93^b	38.37^a	27.92^b	28.06^b	1.59	0.01
Tumour necrosis factor alpha (pg/ml)	34.82	66.13	43.82	40.58	4.91	0.09
Interleukin 6 (pg/ml)	14.43^c	39.82^a	27.31^b	18.11^c	3.18	0.01

$^{a-c}$Means within a row with different letters are significantly different (P<0.05); AF: Aflatoxin; Diet 1: Control; Diet 2: 0.5 mg/kg AF; Diet 3: 0.5 mg/kg AF +250 mg/kg JKP; Diet 4: 0.5 mg/kg AF+500 mg/kg JKP; SEM: Standard error of means.