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Mitigating Potential of Three Phytogenic Feed Additives in Broilers Exposed to Dietary Aflatoxin

Research Article

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ABSTRACT

A feeding trial was researched to evaluate the mitigating potential of three phytogenic additives on performance, visceral organs, hematological and some biochemical indices of broilers exposed to dietary aflatoxin. A total number of 192 one-day-old unsexed Abor Acre broiler chicks were used in a 2 × 4 factorial design of two levels of aflatoxin (0 and 500 ppb) and three different phytogenic powdered additives (Garlic, Ginger and Turmeric) at 2 g/kg and control diet. Phytogenic additives of ginger and turmeric (T3 and T4) to diet without aflatoxin increased the feed conversion ratio and the cost of raising 1kg of live broiler chicken (P<0.0001). Dietary aflatoxin in broiler chickens reduced the performance and digestibility of crude protein and crude fiber while phytogenic additives intervention improved the feed intake, weight gain, feed conversion ratio, cost of producing 1kg of broilers, digestibility of crude protein and crude fiber (P<0.0001). The inclusion of aflatoxin affected the values of liver, kidney, proventriculus, bursa of fabricius, intestine, white blood cells (WBC), neutrophils, red blood cell (RBC), hemoglobin (HGB), packed cell volume (PCV), platelet, urea, creatinine, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Inclusion of phytogenic additives (garlic, ginger and turmeric) at 2 g/kg to the diet with aflatoxin improved the values of the parameters. The study concluded that aflatoxin in diets induced detrimental effects on growth performance, nutrient digestibility, hematological and serum biochemical indices and there was a mitigating effect of the phytogenic additives in case of aflatoxicosis.

KEY WORDS aflatoxin, blood indices and phytogenic powder, digestibility, performance.

INTRODUCTION

The poultry industry has gained considerable attention as one of the world's most important livestock economic base. However, it is faced with the major challenge of contaminants that reduce profit margin, feed efficiency and hamper birds' wellness. Mycotoxins are secondary fungal metabolites and a worldwide threat to food safety and security. Fungal growth in raw material for feed is inevitable under aerobic conditions. Almost 200 species of fungi producing mycotoxins have been documented. Majority of these mycotoxin-forming fungi are of three genera: *Fusarium*, *Penicillium* and *Aspergillus*. There are more than 500 mycotoxins produced by these known fungi, although only some of these exert toxigenic properties. The most common ones are aflatoxins, ochratoxin and fumonisins (Skalická and Koréneková, 2016). Aflatoxins are a group of toxigenic secondary metabolites characterized by furanocoumarins derived from polyketides, and are primarily of the *Aspergillus* spp. such as *A. flavus*, *A. parasiticus*. Aflatoxins B1,

G1, and their B2 and G2 dihydroxy derivatives are natural contaminants of feeds (Dohnal *et al*. 2014; Filazi *et al* 2017). There are variety of effects caused by aflatoxins in poultry, they are: reduction in weight gain and feed utilization (Patil *et al*. 2013; Skalická and Koréneková, 2016), decrease in the size of egg and production, increased deposit of fat and damage of the liver, decreased serum protein levels, changes in organ weight, poor pigmentation, decreased activity of several enzymes involved in digestion of fiber, protein and lipids (Manafi and Khosravinia, 2013; Murugesan *et al.* 2015). Decontamination strategies in feed commodities to reduce mycotoxins are different and based on biological, chemical, and physical approaches. Chemical remedy strategies include the conversion of mycotoxin through several chemical reactions. Peroxidation, ammonization, and the use of ozonation on one or more mycotoxins are reported to be successful but with a clear insight of toxicity that affects the product's palatability and nutritional quality (Faris *et al*. 2020). Hence, the use of organic, safe and biodegradable natural phytogenic additives which provide a synergistic approach as protectants of aflatoxin contamination and further stimulate pathways that elicit the natural defense systems (Gacem *et al*. 2020; Meng *et al*. 2020). Essential oils, spices, herbs and crude extracts are known as phytogenic, and have presented outstanding alternatives for the discovery of bio-fungicides and nutraceuticals for mitigating aflatoxicosis and related infections (Kim *et al*. 2005; Razzaghi-Abyaneh *et al*. 2009; Makhuvele *et al.* 2020). The phytogenic phenolic compounds which inhibited the production of aflatoxin were syringaldehyde, and sinapic acid, while acetosyringones were the phenolic compounds which stopped growth in *A. flavus* and also the production of aflatoxin (Hua *et al*. 1999; Mahmoud, 1999; Kim *et al*. 2006; Makhuvele *et al.* 2020). The curcuminoid pigments present in the dried root powder and turmeric rhizomes (*Curcuma longa*) were reported to have protective tendencies against aflatoxin (Soni *et al*. 1997; Pauletto *et al*. 2020). Garlic, in contact phase, was reported to be more effective in inhibiting growth, spores and aflatoxin B1 production of *A. flavus* (Ismaiel *et al*. 2012). It also exhibited antioxidant properties by its ability to scavenge free radicals, reduce oxidative stress and mutation and lipid peroxidation (Shaarawy *et al*. 2009). The research work (Hassan *et al*. 2019) clearly indicated that ginger is effective as an antifungal agent, reducing aflatoxins contamination in stored maize grains. It acts as a scavenger of free radicals in several ways, prevents or breaks the lipid peroxidation chain, possesses anti-inflammatory properties, enhances the antioxidant defense mechanism and modulates detoxifying enzymes (Arpit Saxena and Raja Fayad, 2013). The effectiveness of these phytogenic additives is still subject of research in the situation of aflatoxicosis in broilers. The aim of this study is to determine the potential of garlic (*Allium sativum*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) on the performance, nutrient digestibility, some organs' characteristics and the blood parameters of broilers exposed to dietary aflatoxin.

MATERIALS AND METHODS

Location of study

This research was carried out at the Poultry Pavilion, Teaching and Research Farm, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria, situated in the Guinea Savanna belt with longitude 4˚ 35'E and latitude 8˚ 20'N. It is at an approximate altitude of 310 m above level.

Sourcing and processing of phytogenic additives

Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*) rhizomes and Garlic (*Allium sativum*) bulbs were sourced from Ilorin, Kwara State, Nigeria, and confirmed at the Herbarium, University of Ilorin, Kwara State, Nigeria. The phytogenic materials were washed with water (pH=6.7), and disinfected with 2% sodium hypochlorite in water solution for about one hour. The materials, afterwards, were rinsed with sterile distilled water to eliminate residual sodium hypochlorite and dried in shade for 14 days (25±2 ˚C). The shade-dried materials were ground and passed through a 1 mm sieve, and the phytogenic additives used were free of aflatoxin.

Aflatoxin preparation

Toxigenic *Aspergillus parasiticus* obtained from Mycotoxin Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria, was used for aflatoxin production. Aflatoxin was produced in rice (Shotwell *et al.* 1966). The autoclaved culture rice was dried, ground to powder and 500ppb of total aflatoxin quantified were used in experimental diets. Aflatoxin in fungal culture rice and experimental diets were directly quantified on TLC plates with a scanning demonstration (canning TLC scanner 3 with way (ATS 1.4.2 software)).

Experimental birds, management and diets

This experiment was laid out in a 2×4 factorial design of inclusion of aflatoxin at two different levels (0 and 500 ppb) in basal diet and four types of phytogenic additives (Control, Garlic, Ginger and Turmeric). Mixed sexes of 192 one-day-old broiler chicks of Abor Acre strain with average body weight of 40 ± 0.28 that were sourced from a commercial hatchery located in Ibadan, Oyo State, Nigeria and allotted randomly to eight treatments, three replicates of eight birds each, were raised in a regulated metabolic cage and provided with water and fed *ad libitum* throughout the experimental trial.

Feed ingredients (maize, wheat offal, soybean meal, full fat soya bean, fish meal) used for the preparation of basal diet were screened for the presence of aflatoxin. Only feed ingredients free of aflatoxin were used for the preparation of experimental diets. All the diets were isocaloric (3200 kcal/kg) and isonitrogenous (23% CP) (AOAC, 2000) as shown in Table 1. Vaccination and other routine practices were duly followed all through the experimental period. The mouldy rice containing known quantities of each of aflatoxin, ginger, garlic and turmeric were used in the preparation of experimental diets and they are as follows: T1: basal diet only, T2: basal diet + garlic (2g/kg), T3: basal diet + ginger (2 g/kg), T4: basal diet + turmeric (2 g/kg), T5: basal diet + aflatoxin (500 ppb), T6: basal diet + aflatoxin (500 ppb) + garlic (2 g/kg), T7: basal diet + aflatoxin (500 ppb) + ginger (2 g/kg), T8: basal diet + aflatoxin (500 ppb) + turmeric (2 g/kg).

Feed intake, weight gain and feed conversion ratio estimation

Initial weights of the birds were measured on day 1 and subsequent weight of broilers and feed intake were measured weekly until 5 weeks of age. The average daily feed intake and daily body weight gain per bird were calculated. Feed conversion ratio (FCR) was calculated as the ratio between average daily feed intake and average daily body weight gain per bird. The economic benefit was estimated as feed cost per kg of live broiler weight which was calculated by multiplying feed cost (₦/kg diet) with the FCR (Salami *et al*. 2018).

Nutrient digestibility and proximate composition

At third week ending, nutrient digestibility trial was carried out using the total collection method (AOAC, 2000). Weighed quantities of feed were supplied to the birds and excreta samples collected from them over a 72-hour period. Samples of excreta were oven-dried at 70^{o}C, weighed and ground prior to proximate analysis. Both samples of feeds and excreta were passed through 1mm sieve for determining of proximate composition. Total nitrogen in the feed and excreta samples was determined by the Kjeldahl procedure. Crude fat was determined by Soxhlet extraction (BP 60-80 ˚C) AOAC (2000).

$$\text{Digestibility \%} = [(N_{intake} - N_{excreta}) / N_{intake}] \times 100 \quad ....(1)$$

Where:

N_{intake} and $N_{excreta}$: concentration (g/kg) of nutrients ingested and voided in excreta, respectively.

Blood collection and analysis

At the end of the experiment, birds were fasted for 6 hours and 6 birds (2 per replicate) were randomly selected from each treatment. A 5 mL blood sample was collected into heparinized bottles (for complete blood count) and non-heparinized bottles (for serum samples) from the jugular vein of each bird. The blood was drawn carefully with the use of 4ml syringe fitted with a 24-guage sterile hypodermic needle. Blood samples were incubated at 37 ˚C for 2 h, centrifuged at 1500 × g for 10 min and serum was separated and stored in 1.5 mL centrifuge tubes at −20 ˚C until analysis. The full blood counts were analyzed using an auto hematological analyzer at the Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria. Indices such as serum creatinine, urea nitrogen, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined with the ELISA technique and using the ELISA Reader BioTek, Elx 800 and Elabscience® commercial kits.

Absolute and relative organ weight

After blood collection, the same birds were weighed individually, anaesthetized with carbon dioxide and euthanized by cervical dislocation. Broiler organs including liver, kidney, gizzard, proventriculus and bursa of fabricius were collected from the birds, excised, weighed and expressed as a percentage of bird's body weight (Bowes and Julian, 1988).

Statistical analysis

All data were analyzed by two-way (ANOVA) using the Statistical Analysis System Software package, version 9.4 (SAS, 2004). The following statistical method was applied: aflatoxin, additives and the interaction between aflatoxin and additives were considered as sources of variation. Paired treatment means were separated using the least significant difference method at a significant level of $P < 0.05$ and the tendency of effects were observed when $0.05 \leq P \leq 0.10$.

$$R_{ijk} = \mu + A_i + P_j + AP_{ij} + E_{ijk} \quad (2)$$

Where:

R_{ijk}: any observation made in the experiment.

μ: population means.

A_i: effect of aflatoxin (i=0, 500 ppb).

P_j: effect of additives (j=control, garlic, ginger and turmeric).

AP_{ij}: effect of aflatoxin and additives.

E_{ijk}: experimental error.

RESULTS AND DISCUSSION

There were interactive effects of aflatoxin and phytogenic additives on performance indices for broiler chickens ($P<0.0001$) (Table 2).

Table 1 Gross feed composition of experimental diets

Ingredients (%)	Treatments							
	1	2	3	4	5	6	7	8
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Full fat soya	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Fish meal (72%)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Wheat offal	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Broiler premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Grit	2.00	-	-	-	2.00	-	-	-
Garlic	-	2.00	-	-	-	2.00	-	-
Ginger	-	-	2.00	-	-	-	2.00	-
Turmeric	-	-	-	2.00	-	-	-	2.00
Total	100	100	100	100	100	100	100	100
Aflatoxin (500 ppb)	-	-	-	-	500	500	500	500
Analyzed nutrients								
Calculated energy (kcal/kg)	3200.17							
Dry matter (%)	90.67							
Crude protein (%)	23.01							
Crude fiber (%)	5.65							
Ether extract (%)	6.23							
Calcium (%)	1.13							
Phosphorus (%)	0.89							

[1] Broiler premix contained the following: univit. 15 Roche: 1500 IU; vitamin A: 1500 IU; vitamin D: 3000 IU; vitamin E: 3.0 g; vitamin B_2: 0.3 g; vitamin B_6: 8.0 mg; vitamin B_{12}: 8.0 g; vitamin B_3: 3.0 g; Ca-pantothenate: 50 mg; Fe: 1000 g; Al: 0.2 g; Cu: 3.5 mg; Zn: 0.15 mg; I: 0.02 g and Se: 0.01 g.

Phytogenic additive of garlic to diet of broiler with no aflatoxin showed decrease in feed intake (FI) when compared with diets T1, T3 and T4 (P<0.0001). Values of weight gain (WG) were comparable for diets with no aflatoxin. Feed conversion ratio (FCR) for T2 was reduced (P<0.0001) compared with T1, T3 and T4 which were comparable for birds on diets without aflatoxin. The cost of producing 1kg of broilers increased with diets T3 and T4 (P<0.0001), a decrease for T2 (P<0.0001) when compared with T1.

Broilers on diets with aflatoxin T5 had a decrease in FI, WG, increased FCR and cost of producing 1kg of broilers for broilers on diet. Phytogenic additives intervention increased feed intake, weight gain and reduced FCR and cost of producing 1kg of broilers (P<0.0001).

There were interactive effects of aflatoxin and phytogenic additives (P<0.0001) on nutrient digestibility in broiler chickens (Table 3). Dietary inclusion of phytogenic additives to diets without aflatoxin were comparable for crude protein digestibility while that of crude fiber and ether extract were improved by Ginger (T3) and Turmeric (T4) inclusion (P<0.0001), respectively. Dietary aflatoxin (500 ppb) in broilers diet reduced (P<0.0001) the digestibility of crude protein and crude fiber while phytogenic additives improved (P<0.0001) the digestibility of the same protein and fiber. Ether extract digestibility in diet with aflatoxin was comparable with phytogenic additives of garlic (T6) and ginger (T7) while there was a significant improvement with turmeric (T8).

Effect of dietary inclusion of phytogenic additives with or without aflatoxin on organs of broilers expressed as a percentage of their body weights (Table 4) showed no interactive effect for gizzard and lung. For gizzard (P>0.0945) and lung (P>0.1434) there was no difference in broilers on diets with or without aflatoxin. The values for phytogenic additives were comparable for both gizzard and lung. There was significant interaction between aflatoxin and phytogenic additives for liver (P<0.0234), kidney (P<0.0212), proventriculus (P<0.0001), bursa of fabricius (P<0.0010) and intestine (P<0.0004) in broiler chickens. The inclusion of aflatoxin (500 ppb) in the diets was observed to have significant (P<0.05) effect on the organ weights as values were increased for liver, kidney, proventriculus, bursa of fabricius and intestine.

Table 2 Effects of dietary inclusion of phytogenic additives with or without aflatoxin on performance of broiler chickens (5 weeks)

Treatments	Aflatoxins (ppb)	Additives	Feed intake (g/bird/day)	Weight gain (g/bird/day)	Feed conversion ratio	FC per live broiler [+](₦)[1]
T1	0	Control	96.94[a]	52.10[a]	1.87[b]	931.44[e]
T2	0	Garlic	85.94[b]	53.50[a]	1.62[c]	856.15[f]
T3	0	Ginger	104.92[a]	54.93[a]	1.91[b]	1004.74[c]
T4	0	Turmeric	98.07[a]	51.38[a]	1.91[b]	1033.37[b]
T5	500	Control	63.60[d]	27.51[c]	2.31[a]	1154.40[a]
T6	500	Garlic	79.11[c]	43.65[b]	1.81[b]	954.66[b]
T7	500	Ginger	97.31[a]	54.96[a]	1.77[bc]	932.27[e]
T8	500	Turmeric	93.75[a]	53.93[a]	1.74[c]	940.81[d]
Main Effects						
	Additives (Ad)					
A	Control		80.27[c]	39.82[c]	2.09[a]	1042.92[a]
B	Garlic		82.53[c]	48.58[b]	1.60[c]	905.41[c]
C	Ginger		101.12[a]	54.95[a]	1.84[b]	968.51[b]
D	Turmeric		95.91[b]	52.66[a]	1.83[b]	987.09[b]
	Aflatoxin (Af) (ppb)					
	0		96.47	52.98[a]	1.83[b]	956.43[b]
	500		93.44	45.01[b]	1.91[a]	995.54[a]
P-values						
Treatments			< 0.0001	< 0.0001	< 0.0001	< 0.0001
Additives (Ad)			< 0.0001	< 0.0001	< 0.0001	< 0.0001
Aflatoxin (Af)			0.0726	0.0020	0.0056	< 0.0001
Ad × Af			< 0.0001	< 0.0001	< 0.0001	< 0.0001
SEM			1.825	1.105	0.074	38.461

FC: feed cost and [+]₦ (1 Nigeria Naira=0.0028 USA Dollars).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of dietary inclusion of phytogenic additives with or without aflatoxin on nutrient digestibility in broilers at 21-24 days of age

Treatments	Aflatoxins (ppb)	Additives	Crude protein (%)	Crude fiber (%)	Ether extract (%)
T1	0	Control	73.44[ab]	64.94[cd]	54.13[ab]
T2	0	Garlic	73.58[ab]	59.87[d]	51.49[b]
T3	0	Ginger	77.55[a]	72.22[ab]	50.76[b]
T4	0	Turmeric	76.39[a]	66.97[bc]	55.8a[b]
T5	500	Control	43.97[c]	34.65[e]	51.23[b]
T6	500	Garlic	70.57[b]	62.37[cd]	51.14[b]
T7	500	Ginger	74.94[ab]	75.97[a]	51.01[b]
T8	500	Turmeric	73.65[ab]	73.21[ab]	57.77[a]
Main effects					
	Additives (Ad)				
A	Control		58.71[c]	49.80[d]	52.68[b]
B	Garlic		72.08[b]	61.12[c]	51.32[b]
C	Ginger		76.24[a]	74.10[a]	50.88[b]
D	Turmeric		75.02[a]	70.09[b]	56.79[a]
	Aflatoxin (Af) (ppb)				
	0		75.24[a]	66.00[a]	53.05
	500		65.78[b]	61.55[b]	52.79
P-values					
Treatments			< 0.0001	< 0.0001	0.0010
Additives (Ad)			< 0.0001	< 0.0001	< 0.0001
Aflatoxin (Af)			< 0.0001	0.0002	0.7768
Ad × Af			< 0.0001	< 0.0001	0.0317
SEM			1.878	2.261	2.189

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of dietary inclusion of phytogenic additives with or without aflatoxin on organs of boiler chickens expressed as percentage body weight (5 weeks)

Treatments	Aflatoxins (ppb)	Additives	Liver	Kidney	Proventriculus	Gizzard	Lung	Bursa of fabricius	Intestine
T1	0	Control	1.49^{b}	0.45^{b}	0.61^{b}	2.60^{c}	0.35^{b}	0.03^{b}	3.67^{c}
T2	0	Garlic	2.20^{b}	0.65^{bc}	0.54^{b}	2.61^{c}	0.45^{ab}	0.04^{b}	4.31^{bc}
T3	0	Ginger	2.05^{b}	0.69^{bc}	0.51^{b}	2.66^{b}	0.58^{a}	0.05^{b}	4.31^{bc}
T4	0	Turmeric	1.78^{b}	0.65^{bc}	0.49^{b}	2.65^{b}	0.47^{ab}	0.07^{b}	3.73^{c}
T5	500	Control	5.08^{a}	0.92^{a}	7.12^{a}	2.64^{b}	0.47^{ab}	0.15^{a}	8.57^{a}
T6	500	Garlic	1.97^{b}	0.70^{bc}	0.69^{b}	2.67^{b}	0.47^{ab}	0.14^{ab}	5.16^{b}
T7	500	Ginger	1.97^{b}	0.76^{ab}	0.49^{b}	2.74^{a}	0.42^{ab}	0.05^{b}	4.49^{bc}
T8	500	Turmeric	1.70^{b}	0.54^{cd}	0.54^{b}	2.17^{d}	0.47^{ab}	0.05^{b}	3.98^{c}
Main effects									
	Additives (Ad) (2g/kg)								
A	Control		3.29^{a}	0.69^{a}	0.86^{a}	2.62^{ab}	0.41^{b}	0.09^{a}	6.12^{a}
B	Garlic		2.06^{b}	0.68^{a}	0.60^{b}	2.64^{ab}	0.46^{ab}	0.09^{a}	4.74^{b}
C	Ginger		2.01^{b}	0.73^{a}	0.50^{b}	2.70^{a}	0.49^{a}	0.05^{b}	4.45^{b}
D	Turmeric		1.74^{b}	0.59^{b}	0.52^{b}	2.41^{b}	0.47^{ab}	0.06^{b}	3.86^{c}
	Aflatoxin (Af) (ppb)								
	0		1.88^{b}	0.61^{b}	0.54^{b}	2.63	0.46	0.05^{b}	4.03^{b}
	500		2.67^{a}	0.73^{a}	0.70^{a}	2.55	0.46	0.10^{a}	5.55^{a}
P-values									
Treatments			0.0001	0.0002	< 0.0001	0.0015	0.0434	0.0010	< 0.0001
Additives (Ad)			< 0.0001	0.0013	0.0001	0.0202	0.0306	< 0.0001	< 0.0001
Aflatoxin (Af)			< 0.0001	< 0.0001	< 0.0001	0.0945	0.1434	0.0001	< 0.0001
Ad × Af			0.0234	0.0212	< 0.0001	0.3452	0.0811	0.0010	0.0004
SEM			0.263	0.060	0.105	0.217	0.051	0.017	0.314

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Inclusion of phytogenic additives at 2 g/kg (T6, T7 and T8) to the diet with aflatoxin reduced the percentage values for liver, kidney, proventriculus and bursa of fabricius.

Effect of dietary inclusion of phytogenic additives with or without aflatoxin on blood hematology (Table 4) showed no interactive effect for monocytes (P>0.9452), mean corpuscular volume (MCV) (P>0.9872) and mean corpuscular hemoglobin concentration (MCHC) (P>0.3452). Dietary aflatoxin increased the value of monocyte (P<0.0001) but there was a decrease in the values of MCV (P<0.0001) and MCHC (P<0.0001). The effect of phytogenic additives (B, C and D) reduced the value for monocytes (P<0.0001), the same trend was observed for MCV (P<0.0001) although only for garlic and turmeric. MCHC (P<0.0001) values were increased with phytogenic additives intervention.

There was significant interaction between aflatoxin and phytogenic additives for WBC (P<0.0134), neutrophils (P<0.0012), lymphocyte (P<0.0001), RBC (P<0.0111), HGB (P<0.0116), PCV (P<0.0042), MCH (P<0.0123) and platelet (P<0.0001) in broiler chickens. Dietary inclusion of aflatoxin significantly influences the values of WBC, neutrophils, RBC, HGB, PCV and platelet. Inclusion of phytogenic additives to diet with aflatoxin had a positive influence on the parameters also.

There was significant (P<0.05) interaction amongst diets with and without the inclusion of aflatoxin and phytogenic additives for urea, creatinine, ALT, AST and ALP as shown in Table 6. They were all significantly influenced by the dietary treatments (P<0.05). Diet T5 elevated the serum biochemical indices (P<0.05) compared to other experimental diets.

The principal effects of experimental diets with and without the inclusion of phytogenic additives and aflatoxin on the serum biochemical parameters of broiler chickens was shown to be significant. The diets B, C and D reduced (P<0.05) the serum indices significantly while the serum indices for birds on diets with aflatoxin (500 ppb) were significantly (P<0.05) increased.

Table 5 Effect of dietary inclusion of phytogenic additives with or without aflatoxin on blood haematology of boiler chickens (0-5 weeks)

Treatments	Aflatoxins (ppb)	Additives	WBC 10^9/L	Neutrophils (%)	Lymphocyte (%)	Monocyte (%)	RBC 10^{12}/L	HGB (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (%)	Platelet 10^{10}/L
T1	0	Control	7.60^{c}	29.50^{a}	68.00^{b}	2.50^{dc}	4.45^{a}	10.35^{cd}	36.50^{abc}	82.02^{c}	13.61^{bc}	28.36^{c}	2.06^{d}
T2	0	Garlic	6.35^{d}	31.00^{a}	67.00^{bc}	2.00^{dc}	3.22^{b}	13.35^{b}	39.50^{abc}	122.67^{b}	16.43^{a}	33.80^{b}	2.10^{cd}
T3	0	Ginger	6.50^{d}	29.00^{ab}	69.50^{ab}	1.50^{c}	3.45^{b}	12.50^{bc}	41.00^{ab}	118.84^{b}	13.88^{bc}	30.49^{bc}	1.54^{c}
T4	0	Turmeric	5.50^{d}	27.50^{c}	70.00^{ab}	2.50^{cd}	4.19^{a}	16.40^{a}	43.00^{a}	102.63^{c}	15.70^{ab}	38.14^{a}	1.44^{c}
T5	500	Control	16.15^{a}	17.00^{e}	53.50^{d}	29.50^{a}	0.86^{d}	6.35^{c}	21.50^{c}	250.00^{a}	14.06^{b}	29.53^{bc}	3.11^{a}
T6	500	Garlic	9.35^{b}	25.00^{cd}	62.50^{c}	12.50^{b}	2.98^{c}	8.80^{dc}	33.50^{cd}	112.42^{b}	14.36^{b}	26.27^{c}	2.67^{b}
T7	500	Ginger	8.60^{bc}	24.50^{cd}	66.00^{bc}	9.50^{c}	3.37^{b}	8.15^{dc}	29.00^{d}	86.05^{c}	12.20^{bc}	28.10^{c}	2.64^{b}
T8	500	Turmeric	8.35^{bc}	22.50^{d}	73.00^{a}	4.50^{d}	3.76^{b}	8.50^{dc}	35.50^{bcd}	94.41^{d}	10.92^{c}	23.94^{d}	2.25^{c}
Main effects													
	Additives (Ad) (2g/kg												
A	Control		11.88^{a}	23.25^{c}	60.75^{d}	16.00^{a}	2.65^{d}	8.35^{b}	29.00^{c}	109.43^{ab}	13.84^{ab}	28.79^{b}	2.59^{a}
B	Garlic		7.85^{b}	28.00^{a}	64.75^{c}	7.25^{b}	3.10^{c}	11.08^{b}	36.50^{ab}	117.74^{a}	15.59^{a}	30.36^{ab}	2.39^{b}
C	Ginger		7.55^{b}	26.75^{b}	67.75^{b}	5.50^{c}	3.41^{b}	10.33^{b}	35.00^{b}	102.64^{b}	13.05^{b}	29.51^{ab}	2.09^{c}
D	Turmeric		6.93^{c}	25.75^{bc}	71.50^{a}	3.50^{d}	3.98^{a}	12.45^{a}	39.25^{a}	98.62^{b}	13.31^{b}	31.72^{a}	1.84^{d}
	Aflatoxin (Af) (ppb)												
	0		6.49^{b}	29.25^{a}	68.63^{a}	2.13^{b}	3.83^{a}	13.15^{a}	40.00^{a}	104.44^{b}	14.90^{a}	32.88^{a}	1.78^{b}
	500		10.69^{a}	22.25^{b}	63.75^{b}	14.00^{a}	2.74^{b}	7.95^{b}	29.88^{b}	109.05^{a}	12.89^{b}	26.61^{b}	2.67^{a}
P-values													
Treatments			< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Additives (Ad)			< 0.0001	0.0020	0.0003	< 0.0001	0.0023	< 0.0001	< 0.0001	< 0.0001	0.0021	0.001	0.0103
Aflatoxin (Af)			< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0012	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Ad × Af			0.0134	0.0012	< 0.0001	0.9452	0.0111	0.0116	0.0042	0.9872	0.0123	0.3452	< 0.0001
SEM			0.386	1.581	1.649	0.984	0.378	0.867	2.404	4.431	1.391	1.633	5.879

WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin and MCHC: mean corpuscular hemoglobin concentration.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This trial was carried out to evaluate the mitigating potential of three phytogenic additives on performance, digestibility of nutrients, visceral organs, blood and serum parameters of broilers exposed to dietary aflatoxin. Birds that consumed diet with inclusion of aflatoxin at 500 ppb were observed to have the lowest intake of feed, WG and FCR. This research agrees with previous reports of how performance of poultry is manifestly obstructed after they are fed diets containing aflatoxin level that exceeds (500 ppb) the hygiene regulations for animal feeds.

Broilers are one of the domesticated animals that are sensitive to aflatoxin and their performance is significantly affected when they ingest aflatoxin above the required limits of 20µg approved in poultry diet (Denli *et al.* 2003). Aflatoxin has the capacity to cause growth depression, reduction in FI and poor feed conversion (Afzal and Zahid, 2003; Manafi *et al.* 2014; Mohajeri *et al.* 2018; Akande *et al.* 2019). Reduced feed consumption is suggestive of decreased appetite after ingesting aflatoxin and this might be a protective mechanism adopted by birds to consume less aflatoxin.

This reduction might be as a result of high absorption of aflatoxin in the gastrointestinal tract which was evident in the reduced nutrient digestibility in this study. The metabolism of aflatoxin in liver generates toxic metabolites which inhibits protein synthesis hereby cumulating in a reduction in the intake of feed which eventually affects the WG (Yang *et al.* 2012; Filho *et al.* 2016).

The WG promoting effect exerted by turmeric in the diets with and without aflatoxin could be linked to curcumin which is a major antioxidant ingredient of turmeric which has been implicated to inhibit the aflatoxin to aflatoxicol in the liver by biotransformation (Gowda *et al.* 2008; Abdulbaqi *et al.* 2018; Limaye *et al.* 2018). Garlic exerted a positive effect on diets with aflatoxin and are largely due to the anti-nutritional factors (ANFs) such as saponin and flavonoid which are present in Garlic (Otunola *et al.* 2010). They have therapeutic advantages in a diseased situation (Soetan, 2008). Also, improvement in WG of birds on diets with inclusion of garlic at 2 g/kg might be due to allicin which influence better absorption of nutrients (Fayed *et al.* 2011; Gautam *et al.* 2017).

Table 6 Effect of dietary inclusion of phytogenic additives with or without aflatoxin on serum biochemistry of boiler chickens (0-5 weeks)

Treatments	Aflatoxins (ppb)	Additives	Urea mM/L	Creatinine mg/mL	ALT U/L	AST U/L	ALP U/L
T1	0	Control	85.26^{b}	1.45^{b}	81.00^{b}	166.61^{b}	48.60^{d}
T2	0	Garlic	60.33^{c}	0.60^{c}	64.25^{cd}	106.95^{cd}	18.00^{e}
T3	0	Ginger	70.06^{d}	0.68^{bc}	52.25^{d}	95.03^{d}	20.00^{e}
T4	0	Turmeric	59.48^{c}	0.90^{c}	53.00^{d}	89.01^{d}	20.50^{e}
T5	500	Control	116.74^{a}	2.69^{a}	128.25^{a}	293.83^{a}	98.50^{a}
T6	500	Garlic	96.43^{b}	1.44^{b}	83.00^{b}	152.11^{b}	70.50^{c}
T7	500	Ginger	76.47^{cd}	0.77b^{c}	83.67^{b}	123.83^{c}	58.90^{c}
T8	500	Turmeric	66.12^{ed}	0.63^{c}	72.83^{b}	116.24^{c}	66.67^{bc}
Principal effects							
	Additives (Ad) (2 g/kg)						
A	Control		101.00^{a}	2.07^{a}	73.55^{a}	104.63^{a}	230.22^{a}
B	Garlic		78.62^{b}	1.02^{b}	44.25^{b}	73.63^{b}	129.53^{b}
C	Ginger		73.26^{c}	0.76^{b}	39.45^{c}	67.96^{bc}	109.43^{b}
D	Turmeric		62.80^{d}	0.73^{b}	43.83^{bc}	62.92^{c}	102.63^{c}
	Aflatoxin (Af) (ppb)						
	0		68.90^{b}	0.91^{b}	26.78^{b}	62.63^{b}	114.40^{b}
	500		88.94^{a}	1.38^{a}	73.39^{a}	91.94^{a}	171.50^{a}
P-values							
Treatments			< 0.0001	0.0201	< 0.0001	< 0.0001	< 0.0001
Additives (Ad)			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Aflatoxin (Af)			0.0012	< 0.0001	0.0001	0.0194	< 0.0001
Ad × Af			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SEM			4.060	0.278	3.296	5.650	7.373

ALT: alanine transaminase; AST: aspartate transaminase and ALP: alkaline phosphatase.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

For the diet with aflatoxin, supplementation with ginger led to increase in body weight due to increase in feed intake (FI). This might be possible because ginger contains volatile oils such as zingibaine, zingiberol, gingrol and resin which speed digestion and enhance protein digesting enzymes (Demirca *et al.* 2005; Javid *et al.* 2019). The phytogenic additives improved the digestibility of nutrients for crude protein and crude fiber. Lee *et al.* (2004) and Jang *et al.* (2007) observed that phytogenic extracts such as turmeric, garlic and ginger improve the activity enzymes especially those related to digestion. Furthermore, it has been reported that phytogenic additives enhance liver and metabolism functions (Al-Kassie, 2009).

Aflatoxin exerted potentiated distressing effect on bursa of fabricius, liver, kidney, gizzard, proventriculus and intestine weight when fed. Although, Huff *et al.* (1992) did not report any influence of the feeding of aflatoxin on bursa weight in broilers, the increase in weight of bursa of fabricius in the diet containing aflatoxin might be linked to the hypertrophy of liver to enhance its detoxification role (Kumar *et al.* 2014). In aflatoxin fed birds, the change in weight observed in the kidneys, liver, proventriculus, gizzard and intestine aligned with the observations of (Kumar and Balachandran, 2009; Kumar *et al.* 2014; Fouad *et al.* 2019).

The inflation in the intestine might have resulted in venous congestion in the systemic circulation leading to anoxia and poor supply of nutrients to the vital organs like liver and kidneys, which would have further reduced their function and caused degeneration of their cells. Feeding phytogenic additives in combination with the aflatoxin was suggestive of beneficial effects aiding the function and performance of the organs (Gholami-Ahangaran *et al.* 2016; Saei *et al.* 2017; Limaye *et al.* 2018).

Results of hematology revealed that hemoglobin content, red blood cell, and packed cell volume were reduced and this is suggestive that the reduction observed in the hemoglobin levels may be as a result of the reduced synthesis of protein in the case of aflatoxicosis (Juma *et al.* 2015). The reduction in the mean values of hematology tissue and the increased value of WBC and increase of its component in the present study indicate the deleterious effects of aflatoxin, and the result is in accordance with the studies of (Samuel *et al.* 2009). The ability of phytogenic additives to improve the feed intake and digestibility and synthesis of protein may be the reasons for the improvement of complete blood count in diets with garlic, ginger and turmeric.

The toxicity of aflatoxin in this trial was expressed as significant alterations in serum indices, this is observed in the elevation of hepatic enzyme activities (ALT and AST)

and uric acid and creatinine in chickens receiving aflatoxin. This is also suggestive of hepatic and renal damage and leakage of the same enzymes in the bloodstream (Nazarizadeh and Pourreza, 2019). This finding and previous findings about the effect of aflatoxin on liver enzyme activity emphasize previous report in literature in which liver and kidney are the primary target organs in the metabolism of aflatoxin. The phytogenic exerted the protective properties to mop up some of the leakages in the serum (Limaye *et al.* 2018; Mohajeri *et al.* 2018; Javid *et al.* 2019; Negera and Washe, 2019).

CONCLUSION

The present study is about feeding broilers with or without aflatoxin contaminated diets from 1 to 35 d of age. Dietary inclusion of ginger and turmeric without aflatoxin increased the FCR and the cost of raising 1 kg of broilers. Aflatoxin in diet induced detrimental effects on growth performance, nutrient digestibility, hematological and serum biochemical indices. There was a mitigating effect of the phytogenic additives for performance, nutrient digestibility, some of the organs, hematological, and serum biochemical changes associated with aflatoxin toxicity. The data from this trial suggests that the three phytogenic additives may mitigate some of the toxic effects of aflatoxin in growing broilers and might prove to be beneficial in case of aflatoxicosis in young broiler chicks.

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