Mitigating dietary aflatoxins in Wistar rat using selected phyto-antioxidant sources

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Abstract

This study was conducted to investigate the mitigation of dietary aflatoxin in Wistar rats using extract from selected phyto-antioxidant sources. A total of one hundred and twenty Wistar rats weighing between 180-190g (80 females and 40 males) at six weeks old were allotted to eight treatments with 15 rats per treatment (10 females and 5 males), in a completely randomized design. The treatments were Treatment 1 (Normal diet + no extract and no aflatoxin), Treatment 2 (Aflatoxin contaminated diet + no extract), Treatment 3 (Aflatoxin contaminated diet + 100mg/kg BW Carrot extract), Treatment 4 (Aflatoxin contaminated diet + 200mg/kg BW Carrot extract), Treatment 5 (Aflatoxin contaminated diet + 100mg/kg BW Ginger extract), Treatment 6 (Aflatoxin contaminated diet + 200mg/kg BW Ginger extract), Treatment 7 (Aflatoxin contaminated diet +100mg/kg BW Garlic extract), Treatment 8 (Aflatoxin contaminated diet+ 200mg/kg BW Garlic extract). Body weight gain and feed conversion ratio of Wister rats (T8) administered 200 mg/kg BW was significant ($p<0.05$), with higher weight gain in male (206.00g) and female (199.70g). However, eviscerated and organs weights of both male and female Wistar rats was similar ($p>0.05$) across the treatments. Haematological and serum biochemical indices among the treatments was not significant ($p>0.05$), except for the globulin in male Wistar rats that differed significantly ($p<0.05$) with the value (5.00g/dL) being higher in T7. In conclusion, 200mg/kg body weight of garlic extract improved growth rate of Wistar rats, without any deleterious effect on haematological and serum biochemical parameters. Therefore, 200mg/kg body weight garlic extract mitigated the adverse effect of aflatoxin contaminated feed in Wistar rats.

Keywords: Wistar rat, phyto-antioxidants, aflatoxin, plant extract.

Atténuer les aflatoxines alimentaires chez le rat 'Wistar' à l'aide de certaines sources phyto-antioxydantes

Résumé

Cette étude a été menée pour étudier l'atténuation de l'aflatoxine diététique chez les rats de 'Wistar' tout en employant l'extrait des sources phyto-antioxydantes choisies. Un total de cent vingt rats Wistar pesant entre 180 et 190 g (80 femelles et 40 mâles) à l'âge de six semaines ont été attribués à huit traitements avec 15 rats par traitement (10 femelles et 5 mâles), dans une conception complètement randomisée. Les traitements étaient le traitement 1 (régime normal + aucun extrait et aucune aflatoxine), traitement 2 (régime contaminé par l'aflatoxine + aucun extrait), traitement 3 (régime contaminé par l'aflatoxine + extrait de carotte BW de 100mg/kg), traitement 4 (régime contaminé par l'aflatoxine + extrait de carotte BW de 200mg/kg), traitement 5 (Aflatox alimentation contaminée par l'aflatoxine + extrait de gingembre BW 100mg/kg), Traitement 6 (régime contaminé à l'aflatoxine + extrait de gingembre BW 200mg/kg), Traitement 7 (régime contaminé par l'aflatoxine +100mg/kg extrait d'ail BW), Traitement 8 (régime contaminé à l'aflatoxine+ 200mg/kg extrait d'ail

BW). Le gain de poids corporel et le rapport de conversion des aliments pour animaux des rats wisters (T8) administrés 200 mg/kg BW étaient significatifs (p<0,05), avec un gain de poids plus élevé chez les rats Wistar mâles (206,00 g) et femelles (199,70 g). Cependant, les poids éviscérés et organes des rats Wistar mâles et femelles étaient similaires (p>0,05) à travers les traitements. Les indices biochimiques hématologiques et sériques parmi les traitements n'étaient pas significatifs (p>0,05), à l'exception de la globuline chez les rats wistar mâles qui différait considérablement (p<0,05) avec la valeur (5,00 g/dL) étant plus élevée dans T7. En conclusion, le poids corporel de 200mg/kg de l'extrait d'ail a amélioré le taux de croissance des rats de Wistar, sans n'importe quel effet délétère sur les paramètres biochimiques hématologiques et sériques. Par conséquent, l'extrait d'ail de poids corporel de 200mg/kg a atténué l'effet défavorable de l'alimentation contaminée d'aflatoxin chez les rats de Wistar.

Mots-clés : Rat Wistar, phyto-antioxydants, aflatoxine, extrait de plante.

Introduction

Globally, aflatoxin has been the major toxic substance affecting adequate production of animal protein. Adverse effects of aflatoxin on animal health and production amongst others include liver damage, gastro-intestinal dysfunction, decreased feed utilization and efficiency, decrease in egg production, poor egg-shell quality in poultry and inferior carcass quality (Devegowda *et al*., 1998; Charoenpornsook and Kavisarai, 2006). Consumption of high levels aflatoxin contaminated cereals and oilseeds diets have been implicated in reduction of growth and overall health of animals. Four major aflatoxins produced in feedstuffs are Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2. Research also indicated that only four species of fungi produce aflatoxins namely, *Aspergillus parasiticus, Aspergillus nominus, Aspergillus pseudotamarii, Aspergillus flavus* (Kurtzman *et al*., 1987, Payne, 1998; Ito *et al*., 2001; Cortyl, 2008). However, only *Aspergillus flavus* and *Aspergillus parasiticus* are of economic importance. Since animal feedstuffs are major input needed for feed formulation to meet the nutritional requirements of livestock, contamination by aflatoxins could affect growth and performance. Hence, the approach of mitigating the adverse effect of aflatoxin contamination of feed is paramount. Several approaches of mitigating aflatoxin contamination in food and feed such as the use of microbes and enzymes capable of degrading tricothecene have been documented (Wilson *et al.,* 2017). In addition, Gomez-Espinosa *et al*. (2017) reported the use of neutral electrolyzed water to prevent aflatoxicosis in turkey poults. However, none of these approaches has been able to mitigate dietary contamination of feed by aflatoxin using Wistar rats as an experimental animal.

Phytotheraphy has been the back bone of medicine in which various herbs and their extract containing active ingredient of therapeutic significance are used. Therefore, it is necessary to understand the effects of phytochemical feed additives in terms of cost effectiveness and comparatively less side effect (Mahima *et al.,* 2012, 2013) when compared with antibiotic feed additive. The consumption of phytochemical rich foods such as fruits, vegetables, spice are associated with a reduced risk of diseases mediated by oxidative stress and inflammation such as certain cancers, atherosclerosis and neurodegenerative diseases Halliwell, (1994). Several plants and their constituents are rich source of antioxidant and play a significant role in prevention of disease progression process thus qualifying the leaves as a potential animal feed supplement (Moyo *et al.,* 2011). However, there are several naturally existing phyto-antioxidant sources, especially ginger, carrot, and garlic in which studies have shown some effective potential. Garlic (*Allium sativum*) is the most important specie of the onion genus, *Allium* belonging to the family *Alliaceae* (Eric, 2010). Intact garlic bulbs contain alliin (S-allyl cystein sulfoxide), the precursor of allicin (S-allyl-2propenthiosulphate), which is hydrolyzed by enzyme allinase upon crushing to its active form, and the allicin (S-allyl-2propenthiosulphate) has the agent responsible for garlic's potent antibacterial properties. Garlic has been shown to increase feed palatability and thus feed intake (Horton *et al*., 1991). Allicin is the most potentially active component of garlic that is responsible for its characteristic odour, flavor as well as most of its biological properties (Chowdhury *et al.,* 2002). Tollba and Hassan (2003) found that garlic as a natural feed additive, improved broilers growth, feed conversion ratio (FCR) and decreased mortality rate. The whole use of plant (bulb) and plant extract singly have been documented with positive effect. Ginger (*Zingiber officinale*), on the other hand, is a rhizomatous herbaceous perennial herb, and a member of the *Zingiberaceae* family. The rhizome of ginger (*Zingiber officinale)* are considered to have natural medicinal properties, including anti-inflammatory (Ammon *et al* 1993), antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal (Antony *et al.,* 1999), antiviral, antifibrotic, antivenom, anti-ulcer, hypotensive, and hypocholesterolemic (Al-Yahya *et al.,* 1989), anti-hypertensive (vasolidator), antithrombotic, hypoglycaemic, hypolipidemic, and anti-pyretic properties because they contains a number of monoterpenoids, sesquiterpinoids, curcuminoids, sesquiterpene-Zingiberene, and pungent phenol compounds such as gingerols and shagoals (Soni *et al*., 1997). Different researchers have worked on the effect of ginger on growth performance in broilers, and controversial results were reported. Taylor (2001) observed that the use of ginger powders significantly increased body weight and improved feed conversion compared to birds fed with control diet. In contrast, Zhang *et al* (2009) examined the effect of processed ginger on growth performance and showed that ginger additive had no significant effect on the feed efficiency, while body weight gain of birds fed ginger supplement were higher than control groups. Carrot (*Daucus carota*) is one of the popular root vegetables, the presence of high concentration of antioxidant carotenoids especially β-carotene may account for the biological and medicinal properties of carrots. Carrots have been reported to have diuretic, N-balancing properties and are effective in the elimination of uric acid (Anon, 1952). Numerous animal experiments and epidemiological studies have indicated that carotenoids inhibit carcinogenesis in mice and rats and may have anti-carcinogenic effects in humans. In biological systems, β-carotene functions as a free radical-trapping agent and single oxygen quencher and have antimutagenic, chemopreventive, photoprotective and immunoenhancing properties (Deshpande *et al*., 1995). Studies have shown that the presence of α- and β-carotene in blood has a protective effect against atherosclerosis (D'Odorico *et al*., 2000). However, the use of phyto-antioxidant to mitigate dietary aflatoxin has not received much attention. Hence, their use in aflatoxin contaminated diet for Wistar rat requires investigation. This research was therefore designed to assess the efficiency of the extract of Ginger (*Zingiber officinale*), Garlic (*Allium sativum*), and Carrot (*Daucus carota*) in mitigating dietary aflatoxin using Wistar rat as a model.

Materials and methods

Experimental site and processing of test samples

The experiment was conducted at the Rat House of the Department of Animal Science, University of Ibadan, Nigeria. Fresh samples of Garlic, ginger and carrot were purchased from Bodija market in Ibadan. Part of the fresh samples were first subjected to phytochemical analysis to quantify the tannins, saponins, flavonoids, alkaloids, terpenoids, carotenoids, and antioxidant using ORAC (Oxygen Radical Absorbance Capacity).

Preparation of extract

Fresh samples of ginger, garlic and carrot were sliced into surface area, oven dried and milled into powder. Carrot was first soaked and rinsed in salt solution of 20g in 2litres of water (2% NaCl solution) to maintain its colour because of heat. The extraction was carried out using ethanol as described (Mohan, 2004). A liter of 80% ethanol solution was mixed with 200g of powdered ginger, garlic and carrot, respectively. The mixtures were kept for 5 days in tightly sealed vessels at room temperature of $22^{0}C$ and kept away from sunlight. The mixtures were stirred several times daily with a sterile glass rod. At the fifth day, each mixture was filtered using muslin cloth and the residue adjusted to the required concentration (500ml of 80% ethanol for the residue of 200g each of the ginger, garlic and carrot powdered materials) with the ethanol for further extraction. Extraction of the residue was repeated 3-5 times until a clear colourless supernatant liquid was obtained indicating that no more extraction from the plant material was possible. Thereafter, a 2-liter aliquot of extracted liquid each was subjected to rotary evaporation for 1-2 hours using Brinkmann Rotavapor Model to remove the ethanol. A known quantity (5mls) of the semi-solid extract produced was subjected to antioxidant analysis and later kept in a deep freezer at a temperature of $-80^{\circ}C$ until further use.

Experimental animals and management

One hundred and twenty (120) Wistar rats with body weight (BW) of 180-190g at six weeks old were used for the experiment (80 females and 40 males) in a 70 day feeding trial. The Wister rats were allotted into eight treatments groups of 15 rats (10 females and 5 males) in a completely randomized design. Animals were acclimatized to individual cubicles for at least three weeks before the start of the experiment. The female rats were replicated into 2 (5 rats per replicate) and 5 male rats in each cubicle. The dietary treatments as indicated below were fed once daily to the rats according to their body weight. Clean and cool water was offered *ad libitum*. Rats were administered extracts orally three times in a week according to their BW as highlighted below using cannula. Weight gain were determined weekly before early morning feeding. Feed intake was obtained by subtracting the leftover feed from the quantity served. Weight gain of the rats was determined by subtracting the initial weight from the final weight gain. Feed conversion ratio was obtained by dividing the average daily feed intake by the average body weight gain.

Experimental diet

T1= Normal diet + No extract and no aflatoxin

T2= Aflatoxin contaminated diet + No extract

T3= Aflatoxin contaminated diet +100mg/kg BW Carrot Extract

T4= Aflatoxin contaminated diet + 200mg/kg BW Carrot Extract

T= 5Aflatoxin contaminated diet + 100mg/kg BW Ginger Extract

T6= Aflatoxin contaminated diet + 200mg/kg BW Ginger Extract

T7= Aflatoxin contaminated diet + 100mg/kg BW Garlic Extract

T8= Aflatoxin contaminated diet + 200mg/kg BW Garlic Extract

Table 1: Ingredient composition of experimental diets (%)

Control group		Aflatoxin contaminated	Diet
Ingredients	**Quantity (kg)**	**Ingredients**	**Quantity (kg)**
Maize	40.00	Maize	35.20
------	------	Contaminated maize	4.80
Soya bean	40.00	Soya bean	40.00
Wheat offal	13.15	Wheat offal	13.15
Di-calcium phosphate	2.50	Di-calcium phosphate	2.50
Limestone	2.50	Limestone	2.50
Salt	0.65	Salt	0.65
Methionine	0.60	Methionine	0.60
Lysine	0.60	Lysine	0.60
Total	**100.00**	**Total**	**100.00**
Determined analysis		**Determined analysis**	
Energy(Kcal/kg ME)	2600	Energy (Kcal/kg ME)	2600
Crude protein (%)	20	Crude protein (%)	20
-----	-----	Aflatoxin (ppb)	200

ppb = part per billion

Blood collection and evaluations

At the end of feeding trial, three rats from each replicate of female and male were bled individually from the retro-orbital venous plexus according to the standard procedure for haematology and serum biochemical analysis (Girling *et al,* 2015). Blood samples were collected into separate sterilized glass tubes containing EDTA (ethylene-diamine-tetra-acetic acid) and another glass tube without anticoagulant for haematological and serum biochemical assay, respectively. Blood samples for serum were centrifuged and serum was decanted and freeze stored until analysis. Blood samples collected were analyzed for Packed cell volume, haemoglobin, red blood cell (RBC), white blood cell (WBC), platelets, lymphocyte, neutrophils, monocytes and eosinophils as outlined in Ewuola and Egbunike, (2008). The blood samples collected for serum biochemical analysis were centrifuged at 3500 revolutions per minute (rpm) for 15 mins. The serum was collected and analyzed for the following parameters; aspartate amino-transferase (AST), alanine amino-transferase (ALT), and alkaline-phosphate (ALP), activities were determined using spectrophotometric methods (McComb *et al*, 1988), Serum total protein was determined by Biuret method (Kohn and Allen, 1995), while albumin was determined using the BCG (Bromocresol green) method (Peter *et al*, 1982), Serum urea was determine by Urease method and creatinine by Foin-wu filtrate methods (Toro and Ackermann, 1975).

Statistical analysis

Data collected were subjected to ANOVA according to the procedure of SAS (1999) and means were separated using Duncan's Multiple Range Test (Steel and Torrie, 1990).

Results

Growth indices of the Wistar rats administered extract from selected phyto-antioxidant sources

Growth indices of the male Wistar rats fed with selected phyto-antioxidant source**s** is presented in Table 2. There were significant differences ($p<0.05$) in the values obtained for the total feed intake and feed conversion ratio across the treatment groups. The rats in T4 had the highest feed conversion ratio of 5.15, while those in T8 had the least (4.28). Results of the body weight gain was significantly ($p < 0.05$) higher in the Wistar rats fed T8 (206.00g) than others while those fed T2 had the least (193.95g) body weight gain. Growth indices of the female wistar rats administered extract from selected phyto-antioxidant source is presented in Table 3. There was no significant difference in the value obtained for the total feed intake. The body weight gain showed significant difference ($p<0.05$) with T8, T3, T4, T5 and T6 having higher significant values of 199.70g, 196.10g, 197.10g, 196.80g and 198.70g, respectively than T1 (153.25) and T2 (151.70). The feed conversion ratio was significantly different ($p<0.05$) across the treatment groups with rats administered T4 having higher significant value (4.61) than other treatment groups, while the T8 rats had the least (3.98) feed conversion ratio.

The eviscerated and organ weight of the Wistar rats administered extract from selected phyto-antioxidant sources

Relative eviscerated and internal organs weight of the Wistar rats administered extract from selected phyto-antioxidant sources is shown in Table 4 and 5. There was no significant difference in the eviscerated weight of the Wistar rats and their internal organs among the treatment groups.

Table 2: Growth indices of male Wistar rats administered extract from selected phyto-antioxidant sources

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	P-value
TFI (g/day)	39.50±2.25[a]	34.00±0.45[b]	36.00±5.75[ab]	35.50±5.75[a]	38.50±2.65[a]	37.00±5.90[ab]	37.00±2.75[ab]	38.50±160[ab]	0.12
IBW (g/day)	183.50±1.50	183.10±2.30	182.75±0.55	185.20±2.80	186.75±1.05	183.60±1.40	183.80±1.20	186.50±0.90	0.54
BWG (g/day)	203.20±2.80	193.95±0.05	200.75±1.05	198.80±0.80	200.70±0.00	199.25±0.75	200.80±0.30	206.00±0.60	0.25
FCR	4.71±0.45[ab]	4.85±0.02[c]	4.54±0.03[bc]	5.15±0.09[a]	5.08±0.09[ab]	4.96±0.07[ab]	4.97±0.04[ab]	4.28±0.04[ab]	0.07

[abc]= Means on the same row with different superscripts are significantly ($P < 0.05$) different.

TFI= Total feed intake; IBW= Initial body weight; BWG= Body weight gain; FCR= Feed conversion ratio; T1= No Aflatoxin and extract (control); T2 = Diet + Aflatoxin + No extract; T3= Aflatoxin + 100mg/kg BW Carrot Extract; T4 = Aflatoxin + 200mg/kg BW Carrot Extract l; T5= Aflatoxin + 100mg/kg body weight ginger extract; T6= Aflatoxin + 200mg/kg body weight ginger extract; T7= Aflatoxin + 100mg/kg body weight garlic extract; T8= Aflatoxin + 200mg/kg body weight garlic extract

Table 3: Growth indices of female Wistar rats administered extract from selected phyto-antioxidant sources

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	P-value
TFI(g/day)	39.50±0.50[a]	34.00±1.00[c]	36.50±1.50[abc]	35.00±1.00[bc]	38.00±1.00[ab]	37.00±1.00[abc]	37.00±1.00[abc]	38.50±1.50[ab]	0.09
IBW (g/day)	180.10±1.05	180.00±0.55	185.20±2.80	184.30±1.20	186.20±1.05	187.15±0.90	181.35±0.55	186.30±1.40	0.46
BWG(g/day)	183.25±3.05[b]	181.70±1.70[b]	196.10±1.10[a]	197.10±1.10[a]	196.80±1.80[a]	198.70±1.70[a]	185.55±2.05[ab]	199.20±0.20[a]	0.01
FCR	4.06±0.05[c]	4.46±0.08[ab]	4.39±0.15[ab]	4.61±0.10[a]	4.06±0.06[bc]	4.23±0.07[ab]	4.26±0.06[bc]	3.98±0.08[c]	0.01

[abc]= Means on the same row with difference superscripts are significantly different ($P < 0.05$)

TFI= total feed intake; BWG= Body weight gain; FCR = feed conve rsion ratio; T1= No Aflatoxin and extract (control); T2= Aflatoxin + No extract, T3= Aflatoxin + 100mg/kg BW carrot extract; T4= Aflatoxin + 200mg/kg BW carrot extract; T5= Aflatoxin + 100mg/kg BW ginger extract; T6= Aflatoxin + 200mg/kg BW ginger extract; T7= Aflatoxin + 100mg/kg BW garlic extract; T8= Aflatoxin + 200mg/kg BW garlic extract.

Table 4: Relative eviscerated and organ weights (%) of the male Wistar rats administered extract from selected Phyto-antioxidant sources

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM
Left kidney	0.45 ± 0.04^{a}	0.44 ± 0.06^{b}	0.46 ± 0.03^{ab}	0.53 ± 0.05^{ab}	0.43 ± 0.09^{b}	0.47 ± 0.02^{ab}	0.45 ± 0.01^{ab}	0.61 ± 0.04^{a}	0.23
Right kidney	0.51±0.05	0.45±0.06	0.36±0.17	0.54±0.10	0.44±0.04	0.47±0.02	0.41±0.02	0.59±0.03	0.54
Spleen	0.64 ± 0.11^{ab}	0.48 ± 0.11^{b}	0.53 ± 0.10^{b}	0.62 ± 0.07^{b}	0.66 ± 0.10^{ab}	0.68 ± 0.05^{ab}	0.58 ± 0.01^{b}	0.99 ± 0.22^{a}	0.12
Adrenal Gland	0.02 ± 0.00^{b}	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}	0.04 ± 0.01^{ab}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{ab}	0.04 ± 0.01^{a}	0.03
Lungs	1.54±0.15	1.17±0.10	1.68±0.35	3.09±0.55	1.730.±0.55	1.54±0.26	2.04±0.01	1.81±0.26	0.58
Liver	5.95 ± 0.23^{a}	5.07 ± 0.43^{b}	6.84 ± 0.26^{b}	4.92 ± 0.15^{b}	4.86 ± 0.36^{b}	5.22 ± 0.19^{ab}	5.08 ± 0.04^{b}	5.63 ± 0.32^{ab}	0.08
Left Testis	1.49±0.27	1.28±0.07	1.35±0.07	1.19±0.15	1.58±0.08	1.51±0.14	1.22±0.09	1.63±0.15	0.28
Right Test	1.31±0.17	1.30±0.05	1.43±0.07	1.23±0.13	1.58±0.07	1.44±0.04	1.31±0.10	1.58±0.18	0.28
GIT	13.41 ± 1.87^{a}	6.37 ± 0.50^{b}	9.41 ± 0.95^{ab}	8.49 ± 1.27^{ab}	9.38 ± 1.47^{ab}	12.80 ± 1.60^{a}	11.86 ± 1.27^{a}	12.11 ± 2.45^{a}	0.06
Heart	0.43±0.23	0.45±0.43	0.41±0.15	0.48±0.09	0.51±0.36	0.42±0.19	0.43±0.04	0.52±0.32	0.41

[ab]= Means on the same row with different superscripts are significantly ($P < 0.05$) different.

GIT = gastro intestinal tract, T1= No aflatoxin + No extract (control), T2 = Diet + Aflatoxin + No extract, T3 = Aflatoxin +100mg/kg BW carrot extract, T4 = Aflatoxin + 200mg/kg BW carrot extract, T5 = Aflatoxin + 100mg/kg BW ginger extract, T6 = Aflatoxin + 200mg/kg BW ginger extract, T7 = Aflatoxin + 100mg/kg BW garlic extract, T8 = Aflatoxin + 200mg/kg BW garlic extract

Table 5: Relative eviscerated and organ weights (%) of female Wistar rats administered extract from selected Phyto-antioxidant sources

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	SEM
Left kidney	0.49±0.07	0.39±0.02	0.46±0.03	0.47±0.02	0.42±0.03	0.44±0.06	0.34±0.02	0.41±0.01	0.13
Right kidney	9.37±8.91	0.44±0.02	0.52±0.03	0.47±0.02	0.46±0.04	0.45±0.07	0.37±0.03	0.43±0.02	0.58
Spleen	$0.59±0.06^{ab}$	$0.58±0.05^{ab}$	$0.61±0.09^{a}$	$0.81±0.09^{a}$	$0.46±0.07^{b}$	$0.56±0.14^{ab}$	$0.63±0.07^{a}$	$0.58±0.09^{ab}$	0.30
Liver	5.00±0.49	4.27±0.28	4.36±0.25	4.77±0.47	3.76±0.25	4.60±0.63	3.75±0.22	4.39±0.45	0.28
Lung	1.44±0.22	2.08±0.37	1.63±0.13	1.24±0.09	1.97±0.60	1.54±0.15	1.54±0.34	2.16±0.12	0.36
Rep org.	1.75±0.40	1.19±0.24	0.79±0.29	1.41±0.27	1.16±0.51	1.34±0.81	1.31±0.58	1.25±0.15	0.89
GIT	13.32±2.64	11.76±0.08	12.45±1.71	11.76±0.66	12.54±1.26	10.55±1.56	11.18±1.71	10.62±0.62	0.92
Adrenal	0.06±0.02	0.05±0.02	0.05±0.01	0.05±0.01	0.04±0.01	0.05±0.01	0.04±0.01	0.05±0.00	0.61
Heart	0.41±0.04	0.43±0.04	0.42±0.02	0.39±0.02	0.47±0.02	0.35±0.04	0.36±0.02	0.37±0.02	0.35

^{abcd}= Means on the same row with different superscripts are significantly ($P < 0.05$) different.

GIT = gastro intestinal tract, RP = reproductive organ. T1= No aflatoxin + No extract (control) , T2 = Diet + Aflatoxin + No extract, T3= Aflatoxin + 100mg/kg BW carrot extract, T4 = Aflatoxin + 200mg/kg BW carrot extract, T5 = Aflatoxin + 100mg/kg BW ginger extract, T6 = Aflatoxin + 200mg/kg BW ginger extract, T7 = Aflatoxin + 100mg/kg BW garlic extract, T8 = Aflatoxin + 200mg/kg BW garlic extract.

Haematology and serum indices of wistar rats administered extract from selected phyto-antioxidant sources

The haematology parameters of female Wistar rats administered extract from selected phyto-antioxidant sources are presented in Table 6. There were no significant differences (P>0.05) in packed cell volume, haemoglobin, RBC, WBC, lymphocytes, eosinophils, mean corpuscular volume and mean corpuscular haemoglobin across the treatment groups. The serum indices of the female wistar rats administered extract from selected phyto-antioxidant sources are presented in Table 7. There were no significant differences (P>0.05) in all the serum indices examined among the treatments. The haematology parameters and serum indices of male wistar rats administered extract from selected phyto-antioxidant sources are presented in Table 8 and 9. There were no significant differences in all the parameters monitored except for globulin with T7 having higher value of 5.00g/dL than other treatment groups.

Table 6 : Haematological indices of female Wistar rats administered extract from selected phyto - antioxidant sources

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	P-value
PCV (%)	47.00±3.78	49.00±0.85	43.00±5.48	49.50±1.80	48.17±1	50.50±2.99	49.50±2.01	50.50±1.82	0.73
HB (g/dL)	15.01±1.12	15.37±0.68	13.98±1.75	16.10±0.48	15.85±0.57	16.18±0.88	16.04±0.65	16.73±0.67	0.57
RBC ($x10^6/\mu L$)	7.61±0.67	7.85±0.37	7.03±0.87	8.18±0.25	7.97±0.32	8.19±0.37	8.04±0.33	8.43±0.24	0.57
WBC ($x10^3/\mu L$)	5.40±33.3^{ab}	5.64±34.6^{ab}	4.65±20.20^{b}	5.39±1.93^{ab}	4.65±2.42^{bc}	5.97±3.72^{ab}	4.89±67.8^{ab}	4.89±5.09^{ab}	0.13
Platelet($x10^3/\mu L$)	130.00±8.34^{ab}	115.17±3.86^{bc}	138.17±8.58^{ab}	135.83±5.23^{ab}	125.83±8.56^{b}	140.17±4.59^{ab}	154.60±1.74^{a}	106.60±6.62	0.01
Lymphocyte (%)	73.83±1.33	73.83±1.66	66.17±4.00	73.33±1.67	72.17±1.40	73.67±1.58	73.20±0.66	72.50±1.15	0.13
Neutrophil (%)	23.17±1.45^{b}	22.67±1.74^{b}	30.33±3.90^{a}	25.50±1.38^{ab}	24.50±1.57^{a}	23.50±2.39^{b}	23.40±0.60^{b}	22.67±1.20^{b}	0.18
Monocyte (%)	2.00±0.26^{ab}	1.33±0.21^{b}	1.50±0.22^{b}	1.83±0.17^{ab}	1.50±0.22^{b}	1.83±0.31^{ab}	1.60±0.40^{b}	2.50±0.34^{a}	0.09
Eosinophil (%)	1.00±0.26	2.00±0.52	2.00±0.52	1.33±0.56	1.83±0.54	1.50±0.56	1.80±0.37	2.33±0.21	0.55
MCV (fL)	62.28±1.56	62.31±1.04	60.89±1.39	60.53±0.89	60.49±0.79	61.45±1.00	59.93±0.66	59.92±1.28	0.67
MCH (Pg)	19.94±0.50	19.61±0.29	19.78±0.43	19.70±0.20	19.91±0.20	19.72±0.23	19.95±0.24	19.85±0.53	0.99
MCHC (%)	32.03±0.33^{bc}	31.50±0.60^{c}	32.50±0.38^{abc}	32.57±0.24^{abc}	32.92±0.22^{ab}	32.10±0.24^{bc}	33.29±0.23^{a}	33.12±0.27^{ab}	0.01

^{ab}= means on the same row with different superscripts are significantly (P<0.05) different.
PCV= packed cell volume; HB= haemoglobin; RBC= red blood cell; WBC= white blood cell; T1= No aflatoxin + No extract (control); T2 =Diet + Aflatoxin + No extract; T3= Aflatoxin + 100mg/kg BW carrot extract; T4 = Aflatoxin + 200mg/kg BW carrot extract; T5 = Aflatoxin + 100mg/kg BW ginger extract; T6 = Aflatoxin + 200mg/kg BW ginger extract; T7 = Aflatoxin + 100mg/kg BW garlic extract;T8 = Aflatoxin + 200mg/kg BW garlic extract.

Table 7: Serum indices of female Wister rats administered extract from selected phyto-antioxidant sources

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	P-value
Total protein (g/dL)	6.77±0.39	7.00±0.12	7.06±0.22	6.53±0.03	7.08±0.44	6.64±0.22	7.06±0.24	6.33±0.29	0.48
Albumin (g/dL)	2.73±0.12	2.90±0.15	3.10±0.12	3.63±0.12	3.02±0.22	2.88±0.17	3.03±0.14	2.58±0.08	0.21
Globulin (g/dL)	4.03±0.29	4.10±0.06	3.97±0.12	3.90±0.10	4.06±0.24	3.76±0.09	4.03±0.11	3.75±0.23	0.79
Arginine ratio	0.67±0.03	0.70±0.06	0.73±0.03	0.63±0.07	0.70±0.03	0.74±0.04	0.68±0.03	0.67±0.04	0.69
AST (uL)	42.00±1.15	41.00±0.58	42.00±1.33	37.33±0.33	41.00±2.49	39.60±1.54	42.00±2.12	38.33±1.52	0.48
ALT(uL)	30.33 ± 1.20^{ab}	29.33±0.67^{ab}	32.00±1.15^{a}	26.67±1.20^{b}	30.00±1.87^{ab}	28.80±1.11^{ab}	30.50±1.32^{ab}	28.00±1.21^{ab}	0.34
ALP (uL)	108.00 ±12.22	109.00±11.06	97.33±12.12	182.00±98.50	102.80±9.16	96.80±5.27	110.25±4.82	94.00±5.42	0.46
BUN (mg/dL)	16.67±0.52	16.57±0.29	17.37±0.12	16.23±0.12	17.06±0.44	16.66±0.32	17.15±0.28	16.70±0.19	0.43
Creatinine (mg/dL)	0.57±0.03	0.60±0.00	0.63±0.03	0.53±0.03	0.60±0.04	0.58±0.04	0.58±0.03	0.53±0.02	0.47

^{ab}= Means on the same row with different superscripts are significantly (P<0.05) different.
AST = a spartate amino transferase; ALT = alanine amino transfe rase; ALP = alkaline ph osphate; BUN =Blood urea nitrogen; T1= No aflatoxin + No extract (control), T2 = Diet + Aflatoxin + No extract, T3= Aflatoxin + 100mg/kg BW carrot extract, T4 = Aflatoxin + 200mg/kg BW carrot extract, T5 = Aflatoxin + 100mg/kg BW ginger extract, T6 = Aflatoxin + 200mg/kg BW ginger extract, T7 = Aflatoxin + 100mg/kg BW garlic extract, T8 = Aflatoxin + 200mg/kg BW garlic extract.

Table 8 : Haematological indices of the male Wistar rats administered extract from selected phyto - antioxidant sources

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	P-value
PCV (%)	45.00±1.90	51.25±2.25	50.00±1.15	50.25±1.03	44.50±10.02	43.75±5.12	51.25±3.61	49.60±2.40	0.79
HB (g/dL)	14.96±0.56	15.83±0.49	16.67±0.35	16.40±0.31	14.40±3.36	14.15±1.81	16.68±1.29	16.76±0.82	0.74
RBC ($x10^6/\mu L$)	7.55±0.34	8.34±0.29	8.50±0.16	$8.46±0.09^a$	7.23±1.39	7.23±0.99	8.38±0.51	8.27±0.31	0.66
WBC ($x10^3/\mu L$)	4.41±5.97	5.88±1.11	7.61±3.34	7.59±2.49	7.15±7.41	4.58±6.17	4.09±1.96	4.29±1.69	0.27
Platelet ($x10^3/\mu L$)	$110.18±5.33^{ab}$	$109.57±2.12^b$	$104.76±2.00^a$	$110.72±1.08^{ab}$	$111.20±1.82^{ab}$	$118.70±1.94^{ab}$	$113.95±2.14^{ab}$	$111.92±4.85^{ab}$	0.43
Lymphocyte (%)	71.20±1.53	69.75±3.71	73.67±1.20	72.75±1.11	69.75±5.36	71.00±1.73	74.25±0.85	74.40±1.21	0.76
Neutrophil (%)	26.00±1.76	28.00±3.85	23.67±1.67	24.50±0.87	26.50±4.87	25.75±1.55	23.00±1.47	23.00±1.58	0.81
Monocyte (%)	2.20±0.37	1.50±0.29	1.67±0.67	1.00±0.41	1.25±0.48	2.00±0.41	1.50±0.29	1.20±0.37	0.40
Eosinophil (%)	$0.60±0.40^b$	$0.75±0.48^{ab}$	$1.00±0.58^{ab}$	$1.75±0.48^{ab}$	$2.50±0.50^{ab}$	$1.25±0.63^{ab}$	$1.25±0.63^{ab}$	$1.40±0.51^{ab}$	0.28
MCV (fL)	596.27±3.71	614.18±7.43	588.15±3.42	594.38±11.26	581.98±34.06	612.67±18.18	610.76±14.14	599.16±10.86	0.79
MCH (Pg)	19.84±0.27	20.19±0.21	19.60±0.50	19.40±0.33	19.06±1.49	19.68±0.34	19.84±0.35	20.24±0.30	0.85
MCHC (%)	$33.27±0.27^{ab}$	$32.89±0.50^{ab}$	$33.34±0.15^{ab}$	$32.64±0.07^{ab}$	$32.63±0.73^{ab}$	$32.17±0.73^b$	$32.51±0.60^{ab}$	$33.79±0.24^a$	0.27

^{ab}= means on the same row with difference superscripts are significantly (P < 0.05) different.
PCV = packed cell volume, HB = haemoglobin, RBC = red blood cell, WBC= white blood cell, T1= No Aflatoxin + No extract (control) , T2 = Diet + Aflatoxin + No extract, T3= Aflatoxin + 100mg/kg BW carrot extract, T4 = Aflatoxin + 200mg/kg BW carrot extract, T5 = Aflatoxin + 100mg/kg BW ginger extract, T6 = Aflatoxin + 200mg/kg BW ginger extract, T7 = Aflatoxin + 100mg/kg BW garlic extract, T8 = Aflatoxin + 200mg/kg BW garlic extract

Table 9 : Serum indices of male Wistar rats administered extract from selected phyto -antioxidant sources

Parameters	T1	T2	T3	T4	T5	T6	T7	T8	P-value
Total protein (g/dL)	6.96±0.05	6.93±0.41	7.07±0.12	7.37±0.31	7.28±0.69	7.58±0.40	8.15±0.24	7.54±0.30	0.34
Albumine (g/dL)	2.64±0.05	2.85±0.20	2.90±0.06	3.15±0.17	3.10±0.37	3.08±0.13	3.15±0.26	2.80±0.14	0.46
Globulin (g/dL)	$4.32±0.09^b$	$4.08±0.27^b$	$4.17±0.09^b$	$4,15±0.14^b$	$4.18±0.32^b$	$4.50±0.27^{ab}$	$5.00±0.07^a$	$4.74±0.19^{aab}$	0.04
Arginine ratio	$0.58±0.02^b$	$0.70±0.00^a$	$0.67±0.03^{ab}$	$0.73±0.03^a$	$0.70±0.04^{ab}$	$0.65±0.03^{ab}$	$0.65±0.65^{ab}$	$0.56±0.02^b$	0.01
AST (uL)	42.20±1.39	40.25±2.50	41.67±0.08	43.75±2.36	44.00±3.03	42.75±2.29	46.00±0.58	43.60±1.78	0.67
ALT (uL)	$30.60±1.33^{ab}$	$28.75±1.65^b$	$30.33±1.20^{ab}$	$31.25±180^{ab}$	$33.00±2.35^{ab}$	$32.00±1.68^{ab}$	$34.250.25^a$	$32.20±1.66^{ab}$	0.41
ALP (uL)	101.40±462	115.75±3.30	111.33±8.11	115.00±3.76	109.50±11.51	110.00±6.49	112.75±6.02	113.60±4.81	0.76
BUN(mg/dL)	$17.10±0.11^{ab}$	$16.53±0.43^b$	$16.53±0.27^b$	$16.90±0.56^{ab}$	$17.60±11.40^{ab}$	$17.60±0.36^{ab}$	$17.95±0.43^b$	$17.36±0.41^{ab}$	0.17
Creatinine (mg/dL)	0.60±0.00	0.65±0.04	0.68±0.00	0.63±0.05	0.63±0.05	0.68±0.03	0.65±0.03	0.66±0.02	0.55

^{ab}= Means on the same row with difference superscripts are significantly (P < 0.05) different.
AST = aspartate aminotransferase, ALT = alanine amino transferase ALP = alkaline phosphate, BUW = T1= No aflatoxin + No extract (control) , T2 = Diet + Aflatoxin + No extract, T3= Aflatoxin + 100mg/kg BW carrot extract, T4 = Aflatoxin + 200mg/kg BW carrot extract, T5 = Aflatoxin + 100mg/kg BW ginger extract, T6 = Aflatoxin + 200mg/kg BW ginger extract, T7 = Aflatoxin + 100mg/kg BW garlic extract, T8 = Aflatoxin + 200mg/kg BW garlic extract

Discussion

The Wistar male and female rats exposed to 200mg Garlic extract had the best feed conversion ratio and weight gain than other treatment groups which suggest that the garlic extract had significant positive effect on the growth performance of the Wister rats better than both the positive and the negative control groups. It implies that phyto-antioxidant in garlic help inducing growth rate than other treatment groups. This agrees with the report of Rahman *et al*. (2011) that ginger flavonoids contained anti-oxidant activity which may probably reduce stress in the aflatoxin contaminated wistar rats. It has been reported previously that feed intake was not affected (Onimisi *et al*., 2007), feed conversion ratio was reduced (Moorthy *et al*., 2009), while water consumption was stimulated by the inclusion of ginger in the diet of broiler chickens (Onimisi *et al*., 2007). Several phytochemicals have been tested in early stages and have been shown to have anabolic effect (Devi *et al.,* 2015; González-Ríos *et al.,* 2016). These findings agreed with the report of Halliwell (1994) that the consumption of phyto-chemical rich foods such as fruits, vegetables, spice are associated with a reduced risk of diseases mediated by oxidative stress and inflammation such as certain cancers, atherosclerosis and neurodegenerative diseases. It also corroborated the report of Jiang *et al*. (2007) and Li *et al.* (2015) who reported positive results on the growth performance of animal feed supplemented with phyto-chemicals. Non-significant effect of the extracts on eviscerated and organs weight implies that they were not adversely affected. Research has shown that several phytochemicals have been previously reported to have an anabolic activity (Jiang *et al.,* 2007; Herrera *et al.,* 2011; Macías-Cruz 2014; Devi *et al.,* 2015; González-Ríos *et al.,* 2016), garlic improved meat and carcass quality (Kim *et al*., 2009). Phytochemicals in other spices such as ginger rhizome have been reported to stimulate growth performance (Ademola *et al*., 2009), and in biological systems, β-carotene functions as a free radical-trapping agent and single oxygen quencher and have anti-mutagenic, chemo-preventive, photo-protective and immune-enhancing properties (Deshpande *et al*., 1995). Serum indices of Wistar rats administered with selected Phyto-antioxidant extract that was not significantly affected among the treatments possibly affirm that the three extracts did not adversely affect the organ functions and serum variables. According to Keller *et al.* (2005) contamination of medicinal plants with mycotoxins can contribute to adverse human health problems and therefore represents a special hazard. Low levels of aflatoxin exposure require continuous consumption for several weeks to months in order for signs of liver dysfunction to appear (Bingham *et al.,* 2003). In the past decade carotenoids such as β-carotene have attracted considerable attention because of their possible protective effect against some types of cancers (Bast *et al*., 1996; Santo *et al*., 1996; Van, 1996), Ginger, the rhizome of the *Zingiber officinale*, plays an important role in prevention of diseases. But the exact mechanism of action in diseases management is not fully understood. It is thought that the extract act as anticancer due to various constituents such as vallinoids, viz. -gingerol and -paradol, shogaols, zingerone, and galanals A and B in ginger (Aggarwal, and Shishodia, 2006) and also Allicin has been found as the major biologically active component of garlic as reported by Cavallito and Bailey (1994), allicin is the key ingredient responsible for the broad- spectrum of anti-bacterial activity in garlic. The haematological parameters examination is among the methods which may contribute to the detection of some changes in health and physiological status, which may not be apparent during physical examination but which affects the fitness of the animal (Bamishaiye *et al*., 2009). In this present study it was found that the haematological parameters of the female wistar rat studied revealed no significant differences in the values obtained which suggests that the aflatoxin did not have any deleterious effect on the parameters when mitigated with phyto-antioxidant. There was no significant difference found in the haematological indices of the male and female wistar rats administered extract from selected phyto-antioxidant sources, which suggests that the aflatoxin contaminated diet did not have any abysmal effect on the haematological parameters of the wistar rats examined when mitigated with any of the phyto-antioxidants used in this study, which corroborate the report of (Chineke *et al*., 2006) which stated that asides from genotype, age, sex, differences in haematological parameter may be attributed to nutritional, environmental and hormonal factors.

Conclusion

The result of this study has shown that the phyto-chemical present in th plant extract administered to the wistar rats mitigated the adverse effect of the aflatoxin contaminated feed and improved the growth of the wistar rats. Wistar rats that were administered selective phyto-antioxidant plant extracts at different graded levels were found to perform better than the control groups. Therefore, the selected phyto-antioxidants especially 200mg/kg body weight of garlic extract could be used to mitigate aflatoxin effect in livestock feed without any deleterious effects on haematological parameters.

Recommendations

The selected phyto-chemicals especially 200mg/kg body weight garlic extract should be used to mitigate aflatoxin effect in livestock feed without any deleterious effects on performance, carcass yield, haematological parameters and the consumers. **Hi**gher graded level of concentration of the extract should also be further researched into.

Conflict of interest

Authors declared that no conflict of interest exist concerning this manuscript.

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