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Effect of Yeast Beta-Glucans on Aflatoxin Absorption In The Gut of Broiler Chickens

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Abstract

The presence of aflatoxins in poultry feed may reduce productivity below sustainable level when ingested. Their presence in poultry diet is considered inevitable, however, there is no known strategy to completely preclude the uptake of aflatoxins in chicken. Therefore, effects of yeast beta-glucans on gastrointestinal tract (GIT) aflatoxins absorption to minimise liver damage and in preventing the adverse effects of aflatoxins in broiler chickens were investigated. Aspergillus flavus 3228 inoculated maize was used to produce basal diet (BD) of 270.0±16.0 ppb aflatoxins. A 42 day feeding trial was conducted with 210, one-day old Arbor Acres broiler chicks (unsexed). The chicks were randomly allotted to seven dietary treatments of three replicates each which comprised of Negative Control (NC- aflatoxin-free diet), BD; BD containing beta-glucans at 125ppm (BD125), 250ppm (BD250), 375ppm (BD375), 500ppm (BD500) and 625ppm (BD625). Experimental design was completely randomised. Body weight gain (BWG) and feed conversion ratio were measured and absorbed aflatoxin determined. Six birds per treatment were sacrificed for ileal digesta collection to estimate GIT adsorbed and absorbed aflatoxins determination. Data were analysed using descriptive statistics and Anova at $\alpha_{0.05}$. Beta-glucans significantly reduced GIT aflatoxin absorption from 98.86±0.48% (BD) to 20.69±8.47% (BD375). However, beta-glucans did not significantly improve BWG and also did not reduce weight gain either. In conclusion, beta-glucans addition to aflatoxin-contaminated poultry feed reduced aflatoxins absorption but additional mitigation approach is required as a complementary strategy with beta-glucans to enhance and ensure effective mitigation of aflatoxins effects from the inevitably absorbed fraction of the toxin in broiler chickens.

Keywords: Aflatoxins absorption, gastrointestinal tract, productivity, mitigation, ileal digesta

Effet des bêta-glucanes de levure sur l'absorption des aflatoxines dans l'intestin des poulets de chair

Résumé

La présence d'aflatoxines dans les aliments pour volailles peut réduire la productivité en deçà du niveau durable lorsqu'elles sont ingérées. Leur présence dans l'alimentation des volailles est considérée comme inévitable, cependant, il n'existe aucune stratégie connue pour empêcher complètement l'absorption d'aflatoxines chez le poulet. Par conséquent, les effets des bêta-glucanes de levure sur l'absorption des aflatoxines du tractus gastro-intestinal (TGI) afin de minimiser les dommages au foie et de prévenir les effets indésirables des aflatoxines chez les poulets de chair ont été étudiés. Du maïs inoculé Aspergillus flavus 3228 a été utilisé pour produire un régime de base (RB) de 270,0 ± 16,0 ppb d'aflatoxines. Un *essai d'alimentation de 42 jours a été mené avec 210 poussins de chair Arbor Acres âgés d'un jour (non sexés). Les poussins ont été répartis au hasard dans sept traitements diététiques de trois répétitions chacun, qui comprenaient un contrôle négatif (régime sans aflatoxine NC), RB ; RB contenant des bêta-glucanes à 125 ppm (RB125), 250 ppm (RB250), 375 ppm (RB375), 500 ppm (BD500) et 625 ppm (RB625). La conception expérimentale a été complètement randomisée. Le gain de poids corporel (GPC) et le taux de conversion alimentaire ont été mesurés et l'aflatoxine absorbée a été déterminée. Six oiseaux par traitement ont été sacrifiés pour la collecte de digesta iléal afin d'estimer la détermination des aflatoxines adsorbées et absorbées par TGI. Les données ont été analysées à l'aide de statistiques descriptives et d'Anova à α0,05. Les bêta-glucanes ont considérablement réduit l'absorption des aflatoxines GIT de 98,86 ± 0,48 % (RB) à 20,69 ± 8,47 % (RB375). Cependant, les bêta-glucanes n'ont pas significativement amélioré le GPC et n'ont pas non plus réduit le gain de poids. En conclusion, l'ajout de bêta-glucanes aux aliments pour volailles contaminés par les aflatoxines a réduit l'absorption des aflatoxines, mais une approche d'atténuation supplémentaire est nécessaire en tant que stratégie complémentaire aux bêta-glucanes pour améliorer et assurer une atténuation efficace des effets des aflatoxines provenant de la fraction inévitablement absorbée de la toxine chez les poulets de chair.*

Mots clés : Absorption des aflatoxines, tractus gastro-intestinal, productivité, atténuation, digesta iléal

Introduction

Aflatoxins are a group of polyketide-derived toxic secondary metabolites of the *Aspergillus* species of fungi (Payne and Brown, 1998; Alshannaq and Yu, 2017),that naturally contaminate human food, animal feeds and feed materials. The ubiquitous nature of fungi makes the presence of secondary metabolites (mycotoxins) such as aflatoxins to be unavoidable in animal feeds and feed materials (Oyegunwa *et al*., 2015; Alshannaq and Yu, 2017). Besides, aflatoxin contaminated produce that are rejected from human food chain find their way into livestock feeds and feeding stuff.

Ingestion of aflatoxins lead to stunted growth, immune suppression and liver tissue damage which predisposes animals to infectious diseases and ultimately results to increased mortality in farm animals. Being a compound of low molecular weight, the implications of this are that aflatoxins devoid immunogenicity, that is their presence cannot elicit antibody production and also makes their absorption in the gastrointestinal tract a passive one by simple diffusion across concentration gradient between the gut digesta and the blood stream (Gratz, 2007).In view of this, the addition of mycotoxin adsorbent to an already contaminated feed or feed materials during feed production remains the most practicable and acceptable approach to reducing the adverse effects of aflatoxins and mycotoxins in general, in animal production (Galvano *et al*., 2001). Mycotoxins binders commonly in use in animal feeds are from both inorganic and organic substances. Organic mycotoxin binders are derived from both synthetic polymers such as cholestryamine and polyvinylpyrrolidone and from natural indigestible carbohydrates such as cellulose, cell wall polysaccharides of yeast (Vila-Donat *et al*., 2018), bacteria and algae (Murugesan *et al*., 2015).

Baker's yeast (*Saccharomyces cerevisiae*) cell wall component is made up of 1 - 3-β-D-glucan (50- 60% and 1 - 6-β-D-glucan 5 - 10% of the yeast cell wall dry weight (Kliss *et al*., 2002). The 1 - 3 - β- D-glucan has been reported to be the component responsible for complexing beta-glucans with mycotoxin (Bzducha-Wróbel *et al*., 2019).

The *in vitro* ability of yeast cell wall to adsorb aflatoxin has been demonstrated by Kolawole *et al*. (2019) and the potential of yeast β-glucans to bind aflatoxin *in vitro* was also postulated by (Jouany *et al*., 2005). However, scanty information exists on *in vivo* trial of yeast beta-glucans to bind aflatoxin in broiler chicken. A large number of available information in literature about the use of yeast beta-glucans in animal nutrition is in its immunomodulatory potentials. Therefore, the ability and efficacy of yeast beta-glucans in reducing the absorption of ingested aflatoxins in the gastrointestinal tract (GIT) and in preventing its adverse effects on growth of broiler chicken were investigated.

Materials and methods

A total of 210, one-day-old Arbor Acres broiler chicks (mixed sexes) from a reputable commercial hatchery in Ibadan, South West of Nigeria were randomly allotted into seven dietary treatments. The experimental diets were contaminated with inoculated maize grains from *Aspergillus flavus*- isolate 3228, obtained from the Plant Pathology Unit of International Institute of Tropical Agriculture (IITA), Ibadan, and cultured by adapting the method of Athenkeng *et al*. (2008). The cultured maize grains were used to formulate a basal diet (BD) with aflatoxins level of 270.0±16.0 ppb total aflatoxins per kg of feed.

Each dietary treatment was replicated thrice with 10 chicks in each replicate. Diet 1 was the negative control- NC (aflatoxins and β-glucan-free diet) while diet 2 (the basal diet with aflatoxin but without β- glucans) was the positive control. Aflatoxin-free diet was produced by using maize grains harvested from aflasafe® treated maize plots. Aflatoxin level in NC was zero, that is, below the limit of detection (LOD) of High performance thin layer chromatography-HPTLC (i.e. 1ppb total aflatoxins), the analytical method used. The test diets were formulated to be isonitrogenous and isocaloric, to ensure that observed treatment differences are not ascribed to differences in the nutrients' levels of the experimental diets. The starter diet contained 22% CP and 3,000kcal/kg ME, while the finisher diet contained 19% CP and 3,100kcal/kg ME (approximately). Feed and water were offered *ad libitum* and the experiment lasted for 42 days.

Experimental diets layout and composition

The birds were randomly allotted to seven dietary treatments as shown in the layout below:

NC = Negative control (0ppb Aflatoxins; 0 ppm beta-glucans)
BD = Basal diet
BD125 = BD + 125ppm beta-glucans
BD250 = BD + 250ppm beta-glucans
BD375 = BD + 375ppm beta-glucans
BD500 = BD + 500ppm beta-glucans
BD625 = BD + 625ppm beta-glucans

Data collection

Data on daily feed intake and weekly body weight were taken for FCR and weekly body weight gain determination. Daily mortality was also recorded throughout the duration of the experiment. Two birds per replicate were taken to the metabolic cage from day 37 for five days. Total faecal outputs were collected daily and used in estimating total ileal digesta derivable from daily feed intake. At day 42, two birds within the average body weight per replicate were selected and sacrificed, then cut open for ileal digesta collection. The ileal digesta collected were oven-dried at 60 ºC to a constant weight and thereafter air-dried at room temperature (27 - 28 ºC) for three days. This was necessary as the aflatoxin level in the feed samples were done on air-dried basis and not on dry matter basis.

Feed samples were analysed for proximate composition according to the Association of

Table 1: Gross composition (g/100g) of experimental diets

Ingredients	% Inclusion	
	Starter diet	Finisher diet
Maize (Aflasafe®)	55.20	62.45
Inoculated Maize	3.80	3.80
Wheat Offals	1.00	1.00
Soyabean Cake (48%)	36.50	29.00
Limestone	1.75	2.00
DCP	1.00	1.00
Table salt	0.25	0.25
Broiler Premix	0.25	0.25
L–Lysine	0.10	0.10
L–Methionine	0.15	0.15
Beta-Glucan	+	+
Aflatoxins (Crude)	++	++
Total	**100.00**	**100.00**
Nutrient Analysis		
Dry Matter (%)	89.74	89.91
MetabolisableEnergy	3,000.73	3,103.05
(kcal/kg)	23.05	19.01
Crude Protein (%)	3.54	3.35
Crude Fibre (%)	3.70	4.90
Ether Extract (%)	59.44	62.65
Nitrogen Free Extract (%)	1.40	1.50
Calcium (%)	0.54	0.51
Non-Phytate Phosphorus (%)	0.54	0.51
L – Lysine* (%)	1.30	1.11
L – Methionine* (%)	0.51	0.45
Total Aflatoxins (ppb)	270.00	270.00

*_ Calculated. + - Present in all treatment diets except diets 1 and 2, ++ - Presents in all treatment diets except diet 1, DCP - Di – Calcium phosphate.
Premix composition/kg: Vit. A (5 MIU); Vit. D3 (2 MIU); Vit. E (20g); Vit. K3 (1g); Vit. B1 (0.4g); Vit. B2 (2.4g); Vit. B6 (1.8g); Vit. B12 (8µg); Niacin (17.6g); Pantothenic acid (4.8g); Folic acid (0.4g); Biotin (40µg); Mn (24.8g); Zinc (20g); Fe (10g); Cu (4g); Iodine (0.52g); Se (0.1g), Co (0.2g); Antioxidant (BHT) 50g

Official Analytical Chemists (Cundiff, 1995). Feed and ileal digesta samples aflatoxins contents were extracted using AOAC method 968.22 (AOAC, 1990) and quantified with high performance thin layer chromatography (HPTLC) with a scanning densitometer, CAMAG TLC, Scanner 3 at the Mycotoxins Laboratory of IITA, Ibadan, as described by Ramesh *et al.* (2013). Generally, mycotoxins distribution within produce or commodity is usually uneven (Alshannaq and Yu, 2017), therefore, a systematic sampling procedure as described by the EU, under the Commission Regulation (EC) No. 401/2006 (Shephard, 2016), was used to ensure a representative aflatoxin concentration in the sampled materials relative to the whole bulk of the contaminated feed.

Performance indices assessed to evaluate the potentials of beta-glucans in preventing the adverse effects of aflatoxins were daily feed intake, average body weight gain and feed conversion ratio (FCR).

Adsorbed and absorbed aflatoxin estimation

The adsorbed (unabsorbed) aflatoxin was determined using the general standard procedure of balance study (Young, 1986; Fern *et al*., 2015). Average daily total aflatoxin intake (DTAI) was determined by multiplying average daily feed intake by the concentration of aflatoxins in the feed (cAIF). Total aflatoxins in ileal digesta or the relative quantity of adsorbed aflatoxins (rAdA) was determined by multiplying the estimated average daily ileal digesta output (eDIDO) by the concentration of aflatoxin in the ileal digesta (cAIID).

Therefore,

(i)Total daily aflatoxin intake (TDAI= Daily feed intake x cAIF

(ii)Relative amount of adsorbed aflatoxin (rADA)=(eDIDO x cAIID)

(iii)Adsorbed aflatoxin (%) =

$$\frac{(\text{rAdA})}{\text{DTAI}} \times 100\%$$

(iv)Relative amount of aflatoxin absorbed (rAbA)=(DTAI – rAdA)

(v)Absorbed aflatoxin (%)= $\frac{(\text{rAdA})}{\text{DTAI}} \times 100\%$

"Note: Estimated ilea digesta from daily feed intake was calculated to be 23% of feed consumed. A metabolic cage feeding trial was conducted for five days, for faecal collection and the percentage daily faecal output after drying was found to be 23% (on the average) of the daily feed consumed."

Experimental design and statistical analysis

The experimental design was completely randomised. Data collected were analysed with descriptive statistics and one-way ANOVA, using (SAS, 2012) software package, version 9.20. Significant treatment means were separated with Duncan Multiple Range Test (DMRT) of the same software. Treatment means declared as being significant were based on 0.05 level of probability.

Results and discussion

The effect of varied inclusion levels of beta-glucans on aflatoxins absorption in broiler chicken fed aflatoxin-contaminated diets is presented in Table 2. It was observed that varying the levels of beta-glucans did not ($P>0.05$) influence average daily feed intake (DFI) of chicks. Chicks on NC diet had significantly ($P<0.05$) reduced DTAI (0.00) compared to the other dietary treatments. Estimated daily ileal digesta outputs-eDIDO (g/b/d) were similar across the dietary treatments. Higher ($P<0.05$) amount of TAID (µg/b/d) was recorded in chicks offered BD375 (5.44) diet compared to that of chicks on the other treatment diets. Chicks fed NC and BD had similar ($P>0.05$) low levels of aflatoxins in their ileal digesta, though NC diet was aflatoxin-free. Percentage of adsorbed or unabsorbed total aflatoxin (TA) was significantly higher ($P<0.05$) in chicks fed BD375 (79.30%) diet compared to that of chicks on the other treatment diets. Ckicks fed NC (0.00%) and BD (1.14%) diets had similar ($P>0.05$) percentages of adsorbed TA, and were significantly ($P<0.05$) lower below those of chicks on other treatment diets. However, it was also observed that chicks on BD250ppm (53.86%) diet had significantly ($P<0.05$) higher percentage of adsorbed TA compared to chicks on BD500 (35.56%) and BD625 (24.61%) diets. Chicks fed BD (6.93µg/b/d) and BD125 (6.65µg/b/d) diets had significantly ($P<0.05$) higher relative quantity of total aflatoxin absorbed (TAAb) compared to the other dietary treatments. Similar values ($P>0.05$) of relative quantity of TAAb were observed in chicks on BD250 (3.28µg/b/d) and BD375 (1.42µg/b/d) diets, and were lower significantly ($P<0.05$) below that of chicks fed other beta-glucans diets. The highest ($P<0.05$) percentage of absorbed TA was observed in chicks on BD (98.86%) diet, in comparison to the other dietary treatments. However, the least ($P<0.05$) and significantly reduced percentage of absorbed TA was observed in chicks on BD375 (20.70%) diet compared to chicks fed the other beta-glucans diets. Observations however, revealed some deviations from the findings of Jouany *et al*. (2005), who did not stipulate any limitation in the ability of yeast beta-glucans to bind with aflatoxin B_1. It was observed that beyond 375ppm beta-glucans inclusion, there was a decline in the level of GIT aflatoxin adsorbed.

The reason for the reduction in aflatoxin adsorption could be because yeast beta-glucans belongs to the group of polysaccharides known as Non-Starch Polysaccharides or NSP. A characteristic of this group of carbohydrates is that they increase gut viscosity in monogastrics following their ingestion. Increased gut viscosity promotes increase in gut enteritis, which will encourage lesions to develop within the GIT. This will probably encourage "leaky gut" scenario and more aflatoxins will infiltrate into the blood stream. Another consequence of increased gut viscosity is that the digesta passage rate within the GIT will be slowed down, enabling the aflatoxins-contaminated digesta to have more contact with absorptive area of the GIT, hence allowing further diffusion of aflatoxins into the blood stream. It has been reported that aflatoxins absorption is by passive mechanism, meaning that its absorption is across concentration gradient (Gratiz, 2007).

Increase in gut viscosity leading to enteric lesions and reduction in digesta passage rate are the two possible reasons why yeast beta-glucans could not adsorb more than 79% of the ingested aflatoxins and also why levels of yeast beta-glucans higher than 375ppm inclusion resulted in reduced level of adsorbed aflatoxins. Kolawole *et al*. (2019) obtained an average of 58% of aflatoxins binding by yeast cell wall-based binder. Therefore, yeast beta-glucans may not be comprehensive enough as a sole adsorbent. However, the very low inclusion rate of yeast beta-glucans is an advantage in ration formulations, especially in broilers feed, as it allows for the inclusion of more nutrients supplying ingredients.

The figure below is the correlation graph between varied inclusion levels of beta-glucans and absorbed aflatoxin in the GIT of broiler chickens fed aflatoxins-contaminated diets. It showed a curvilinear relationship between levels of beta-glucans inclusion and levels of absorbed aflatoxins.

There was a linear relationship between adsorbed aflatoxins and beta glucans as absorbed aflatoxins reduced as the level of beta glucans increased up to 375ppm and thereafter further increase in the level of beta-glucans resulted in a decline in the level of adsorbed aflatoxins and absorbed aflatoxins started rising for the reasons earlier stated.

The performance of broiler chickens fed varied inclusion levels of beta-glucans on aflatoxin-contaminated diets over 42 days feeding period is shown in Table 3. Beta-glucans inclusion to the contaminated diets did not produce significant differences ($P>0.05$) in feed intake, bodyweight gain and feed conversion ratio (FCR).

Table 2: Effect of varied inclusion levels of beta-glucans on total aflatoxins absorption in the GIT of broiler chickens fed aflatoxin - contaminated diets

		Beta-glucans inclusion rate (ppm)							
Parameters	NC	BD	BD125	BD250	BD375	BD500	BD625	SEM	P-value
Ave. DFI (g/b/d)	31.93±7.74	25.98±3.76	28.54±1.75	26.36±3.59	25.43±0.86	30.42±1.71	28.75±5.06	2.39	0.44
Ave. DTAI (µg/b/d)	0.00±0.00^{b}	7.01±1.01^{a}	7.70±0.47^{a}	7.11±0.97^{a}	6.86±0.23^{a}	8.21±0.46^{a}	7.76±1.37^{a}	0.45	<0.0001
eDIDO (g/b/d)	7.34±1.78	5.96±0.87	6.57±0.40	6.06±0.83	5.85±0.19	6.99±0.39	6.61±1.17	0.55	0.45
cAIID (µg/kg)	0.00±0.00^{f}	13.33±5.77^{f}	159.33±49.24^{e}	632.00±102.72^{b}	929.00±122.29^{a}	416.67±23.71^{c}	289.33±26.03^{d}	37.29	<0.0001
TAID/Bird (µg/b/d)	0.00±0.00^{f}	0.08±0.02^{f}	1.05±0.36^{e}	3.83±0.47^{b}	5.44±0.54^{a}	2.92±0.33^{c}	1.91±0.41^{d}	0.21	<0.0001
Adsorbed TA (%)	0.00±0.00^{f}	1.14±0.48^{f}	13.64±4.15^{e}	53.86±8.81^{b}	79.30±10.38^{a}	35.56±2.01^{c}	24.61±2.21^{d}	3.18	<0.0001
Rel. TAAb (µg/b/d)	0.00±0.00^{e}	6.93±1.03^{a}	6.65±0.41^{a}	3.28±0.96^{c}	1.42±0.77^{d}	5.29±0.16^{b}	5.85±0.99^{ab}	0.42	<0.0001
Absorbed TA (%)	0.00±0.00^{g}	98.86±0.48^{a}	86.36±4.14^{b}	46.13±8.81^{e}	20.69±8.47^{f}	64.43±2.02^{d}	75.39±2.21^{c}	2.89	<0.0001

^{abcdefg}Treatment means within the same rowhaving different superscript differed significantly (P< 0.05), Ave. - Average, DFI - Daily Feed Intake, DTAI - Daily Total Aflatoxins Intake, eDIDO - Estimated Daily Ilea Digesta Output, cAIID - Concentration of Aflatoxins in Ilea Digesta, TAID - Total Aflatoxins in Ilea Digesta, TA - Total Aflatoxins, Rel. TAAb - Relative Total Aflatoxins Absorbed, SEM - Standard error of mean, BD- Basal Diet, NC-Negative control, PC- Positive control (0ppm beta-glucans), P-value- probability level

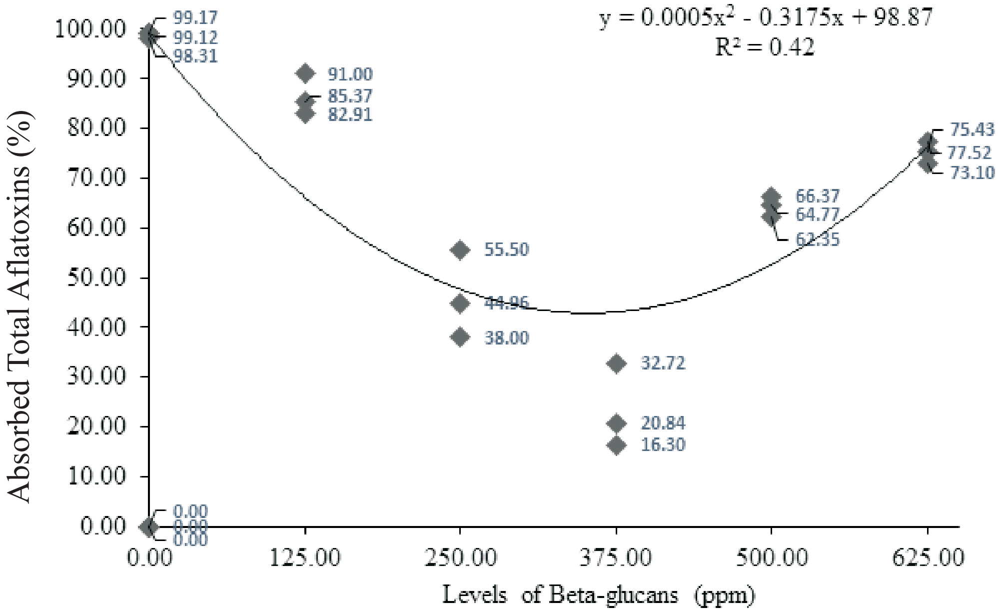

Figure 1. Relationship between varying levels of beta-glucans supplementation and aflatoxins absorption in the GIT of broiler chickens fed aflatoxin-contaminated poultry diets.

Table 3: Effect of varied inclusion levels of beta -glucans on performance of broiler chicken fed aflatoxin -contaminated diet from 0 to 42 days

		Beta-glucans inclusion rate (ppm)							
Parameter	NC	BD	BD125	BD250	BD375	BD500	BD625	SEM	P-value
FI (g/bird)	1341.00	1091.00	1198.80	1107.20	1067.90	1277.60	1207.60	100.21	0.44
BWG (g/bird)	636.29	486.22	577.21	591.50	586.70	569.70	484.93	49.42	0.32
FCR	2.09	2.41	2.09	1.88	1.82	2.25	2.50	0.22	0.32

[ab] Treatment means within the same row with different superscript differed significantly ($P<0.05$). SEM- Standard error of mean, BWG-body weight gain, FCR-feed conversion ratio, p-value- probability level

Performance indices showed that varying the inclusion levels of yeast beta-glucans did not result in any significant improvement in feed intake, BWG and FCR across the six contaminated treatment diets. Generally, reports of beta-glucans' effects on performance in farm animals are conflicting. Zhang *et al*. (2008) reported increase in BWG of broiler chicken when yeast beta-glucans was included in the diet at 50 and 75ppm. Beta-glucans addition in swine has also been reported to improve performance (Hahn *et al*., 2006). Other researchers did not observe any improvement in BWG of chickens fed beta-glucans supplemented diets (Cox *et al*., 2010). Huff *et al*. (2006) reported that birds on beta-glucans diet challenged with *Escherichia coli* showed improvement in BWG while the unchallenged birds with beta-glucans had reduction in BWG. On the contrary, Cheng *et al*. (2004) and Chae *et al*. (2006) reported no significant effect of beta-glucans on growth performance and that beta-glucans do not negatively affect performance in both challenged and unchallenged birds. A similar result was also reported by Morales-Lopez *et al*. (2009) and these latter findings correlate with the performance results obtained in the current study.

Conclusion

The study showed that baker's yeast-derived beta-glucans did not elicit a clear improvement on growth performance of broiler chicken and did not exhibit negative effects either on growth when compared to the performance of chicks on the uncontaminated diet. Increase in gut viscosity which might also reduce nutrients uptake is the probable reason why beta-glucans was unable to have improvement on growth above that of chicks on uncontaminated diet. However, yeast beta-glucans was able to reduce the absorption of aflatoxins from the gastrointestinal tract, from 98.86% absorption in chicks on unmitigated diet to 46.13% and 20.69% in chicks fed contaminated diets containing 250 and 375 ppm beta-glucans, respectively. Based on this, the unadsorbed or the inevitably absorbed fraction of the ingested aflatoxins following yeast beta-glucans addition to the contaminated diet may be sufficient to elicit negative effects, depending on the initial concentration of aflatoxins in the feed. Therefore, additional complementary strategy to reducing the effect of the absorbed portion of the toxin will be appropriate to having a comprehensive mitigation approach towards counteracting the adverse effects of dietary aflatoxins in broiler chickens.

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