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Effects of feeding *Fusarium Verticillioides* culture material containing known levels of fumonisin B_1 on growth performance and egg quality traits of laying hens.

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Target Audience: Livestock farmers, Feed millers, Animal Nutritionists and Poultry products consumers

Abstract

The implications of fumonisin B_1, a secondary metabolite of Fusarium verticillioides(common contaminant of maize) on growth, pubertal development and egg quality traits of laying hens were studied in a 16-week experiment. Sixty Isa-Brown Point-of-lay(POL) birds with an average weight of 710.02g were divided into four groups with fifteen birds per group such that the weight per group range from 710.50-720.42g. Four nutritionally balanced test diets were prepared to contain 0.2, 5.2, 10.2 and 15.2 ppm of fumonisin B_1 constituting diets 1(control), 2, 3 and 4 respectively by substituting ground corn cultured with F.verticillioides for ground autoclaved, noncultured corn in graded proportions. Each group was assigned to one experimental diet in a completely randomised design .Feed intake and body weight gain were determined daily. The age at which 50% of the POL birds on each experimental diets began to lay was considered their pubertal age. Ten eggs were randomly selected from each treatment every week for a period of five weeks for the assessment of egg quality traits .Results showed that the daily feed intake, daily weight gain and feed conversion ratio of the laying hens were not adversely affected ($P>0.05$) by the dietary fumonisin B_1 concentrations. Statistically similar values($P>0.05$) were obtained for the pubertal age of the experimental birds across the treatments. The dietary mycotoxin also failed to exert any significant influence ($P>0.05$) on the external and internal egg quality traits investigated except the yolk colour of laying hens on diet 4 which was statistically superior ($P<0.05$) to those on diets 1,2 and 3, respectively. Although, egg cholesterol values tended to decrease while those of egg triglycerides apparently increased with increase in the levels of dietary fumonisin B_1, the parameters were not significantly influenced($P>0.05$) by the dietary fumonisin concentrations. These results imply that laying birds can tolerate fumonisinB_1 up to 15.2ppm in their diets without compromising the growth performance of the birds, nutritional and reproductive potentials of their eggs.

Keywords: Egg quality, Fumonisin B_1., Isa Brown point-of-lay, Pubertal development,

Description of Problem

Fumonisin is a secondary metabolite of the fungus *Fusarium verticillioides* (=*Fusarium moniliforme*). It is a toxic, fungal contaminant of maize intended for man and animal consumption and has been implicated by association as the cause of various human and animal diseases(11). Pure fumonisin B_1(FB_1) has been shown to induce equine leukoencephalomalacia (9, 12), Porcine pulmonary oedema(7) and to be hepatotoxic and carcinogenic in rats(6).It has also been shown to increase the risk of oesophageal cancer in humans consuming infected maize(22).

Ingestion of fumonisin contaminated diets by animals has been reported to have detrimental effects on their reproductive performance, feed intake and body weight particularly in species like horses, rabbits and swine which have been reported to be more susceptible(4,14,24). Available data on Poultry species like turkey poults (10), day-old broilers(26) and day-old Peking ducklings(2) revealed that fumonisin contaminated diets in the range of 0-190ppm fed to these poultry species had no significant effect on their feed intake, body weight gain and feed conversion.

However, mycotoxins in the diet have been implicated to have detrimental effects on egg production and quality. Aflatoxin has been reported to inhibit egg production at the point of commitment of an ovum to maturation and reduce the quantity of yolk deposited in ova already committed (8). Among other disturbances in poultry, Ochratoxin and T-2 toxins in diets have been reported to depress egg production, with thin, rubbery shells(8). Blood spots were also reported to increase significantly in the eggs of birds ingesting T-2 toxin.

With these reported effects of mycotoxins on egg qualities, coupled with current paucity of information on the effects of fumonisin on the performance of laying hens which largely depend on maize as a feed source, this study was therefore designed to explore the growth performance and egg quality traits of laying birds fed with graded levels of fumonisin in their diets with a view to elucidate more on its implication on laying hens.

Materials and Methods

Materials collection and preparation

The feeding trial was carried out in the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Nigeria. Clean maize grains intended for inoculation with the fungus *Fusarium verticillioides* were purchased from reputable feed mills and Bodija market in Ibadan. The grains were autoclaved and inoculated with the fungus according to the method of Nelson and Ross (13) in the mycotoxin laboratory at International Institute of Tropical Agriculture(2TA), Ibadan, Nigeria. The inoculated maize grains were air- dried for two weeks in a screen house, milled and quantified for fumonisin using the Neorgen's veratox fumonisin quantitative test kits (Neorgen Corp; USA)- a Competitive Direct Enzyme Linked Immunosorbent Assay(CD-ELISA) at 2TA, Ibadan, Nigeria.

The ground cultured maize was substituted for autoclaved, non cultured maize in graded proportions to formulate four experimental diets containing 0.2, 5.2, 10.2 and 15.2ppm FB_1, as determined by the Neorgen's veratox fumonisin quantitative test kits (Neorgen Corp; USA), constituting diets 1(Control), 2, 3 and 4 respectively (Table 1). All diets were isocaloric, isonitrogenous and supplemented with feed grade methionine and lysine (Table 1)

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

Ingredients	Dietary Treatments, g/100g			
	1	2	3	4
	0.2ppm	5.2ppm	10.2ppm	15.2ppm
Non-Inoculated Maize	45.00	43.26	41.52	39.78
Inoculated Maize[a]	-	1.74	3.48	5.22
Soy Bean Meal	14.00	14.00	14.00	14.00
Wheat Offal	16.00	16.00	16.00	16.00
Fish Meal	2.00	2.00	2.00	2.00
Palm Kernel Cake	13.20	13.20	13.20	13.20
Di calcium Phosphate	2.50	2.50	2.50	2.50
Oyster Shell	6.50	6.50	6.50	6.50
Salt (Nacl)	0.25	0.25	0.25	0.25
Premix[b]	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
Lysine	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00

[a] Inoculated with *Fusarium verticillioides*

[b] To provide per kg of diet: Vit. A (8,000i.u); Vit. D3 (2,000i.u); Vit .E(5 i.u); Vit. K(3.2mg);Choline chloride(3,000mg); Folic acid(0.5mg); Mn(56mg); I(1mg); Fe(20mg); Cu(10mg); Zn(50mg); Co(1.25mg);Riboflavin(4.2mg); Vit. B12(0.01mg); Pantothenic acid(5mg); Nicotinic acid(20mg); ppm: Equivalent of mg fumonisin/ kg diet.

Layer bird management and experimental design

A total of sixty Isa Brown Point-of-lay (POL) birds with an average weight of 710.02g were randomly divided into four groups comprising of 15 birds per group. The average group weights of the experimental birds ranged between 710.50g-720.42g at the beginning of the experiment. The experimental design was the completely randomised type with 15 POL birds per diet in 3 replications of 5 POL birds per replicate. Routine medications and vaccinations were administered. The birds were fed their respective diets *adlibitum* for 16 weeks during which records on feed intake, weight gain and egg production were taken.

Pubertal age of the layer birds

When the female fowl is sexually mature, it usually starts to lay eggs (17).The age at which 50% of the POL birds on each experimental diet began to lay eggs was taken as their age at puberty.

Egg collection and analysis

Ten eggs were randomly selected every week from each treatment. The external and internal qualities of these eggs were determined as described by (15). Egg cholesterol and triglycerides were determined using Randox Kits at the Animal Physiology Laboratory, University of Ibadan.

Statistical analysis

The various data collected on the different parameters were subjected to one-way analysis of variance(ANOVA) of Statistical Analysis System(20). Where significant differences were found, the means were compared using the Duncan procedure of the same soft ware.

Results and Discussion

Laying birds performance characteristics

The chemical composition of the experimental diets and the performance data of the laying birds on the diets are shown in Tables 2 and 3 respectively. The average feed intake of birds on diets 1 and 3(85.21g/day and 85.50g/day respectively) were highest but were not significantly ($p>0.05$) different from those on diets 2(81.82g/day) and 4(81.33g/day). The body weight gain and feed conversion ratio of the laying birds were positively correlated and followed the same trend(Table 3) with the average feed intake in spite of the increasing levels of dietary fumonisin and their expected hazard potentials in the body of the birds. Birds fed 15.2ppm(diet 4) dietary fumonisin showed no significant differences($p>0.05$) in final live weight, body weight gain, average egg weight and feed conversion ratio from the control birds fed 0.2ppm(diet 1) dietary fumonisin.

Table 2: Proximate Composition (g/100g) of the experimental diets.

Composition	Dietary Treatments g/100g			
	1	2	3	4
Dry Matter	88.32	88.34	88.31	88.33
Crude Protein	18.29	18.31	18.22	18.05
Crude Fibre	5.68	5.59	5.72	5.70
Ether Extract	6.85	6.90	6.69	6.83
Ash	11.60	11.54	11.65	11.59
Nitrogen Free Extract	57.58	57.66	57.72	57.83

Table 3: Growth Performance of laying birds fed graded levels of dietary fumonisin

Parameters	Dietary Treatments				
	1	2	3	4	SEM
Initial Live Weight (g)	720.42	712.34	714.59	710.50	-
Final Live Weight (g)	1515.84	1495.42	1508.34	1498.84	111.80
Ave. Feed Intake (g/day)	85.21	81.82	85.50	81.33	0.38
Ave. Body Weight Gain (g/day)	9.44	9.33	9.45	9.38	2.23
Ave. Egg Weight (g/bird)	60.25	60.45	55.30	59.94	2.18
Feed Conversion Ratio	1.41	1.35	1.55	1.36	0.58

Values shown on the table are means. SEM: Standard Error of Means

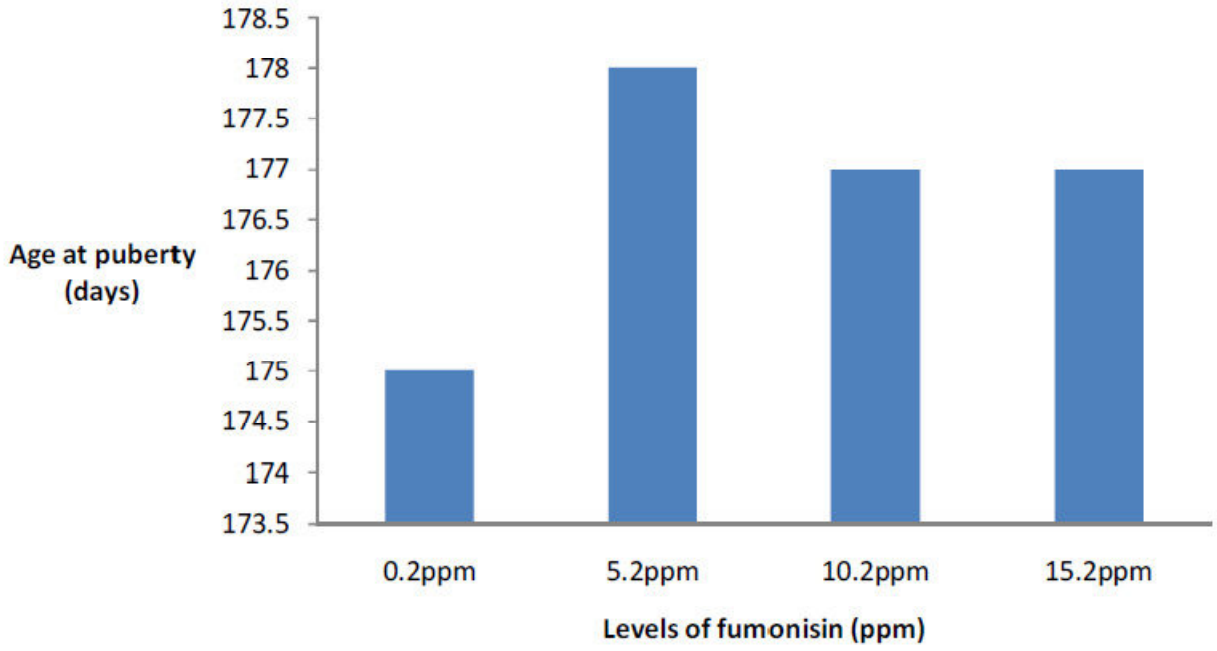

Figure 1: Age at Puberty of laying hens fed graded levels of dietary fumonisin.

The pubertal ages of the experimental birds are shown in Fig.1. Birds on control diet(0.2ppm) attained puberty(175th day) 3 days ahead of those on diet 2(178thday) and 2 days ahead of those on diets 3 and 4, respectively. Laying birds on diets 3 and 4 also became sexually matured (177thday) a day, ahead those on diet 2. The pubertal ages also correspond to the

5^{th}, 6^{th}, 5^{th} and 5^{th} week in lay for birds on diets 1, 2, 3 and 4 respectively.

Egg quality traits of experimental laying hens

The various inclusion levels of fumonisin in the diets of laying hens had no significant effect (P>0.05) on the external egg quality traits investigated (Table 4). The yolk weight, yolk width, shell weight and egg yolk cholesterol apparently decline with increase in dietary fumonisin levels but were not adversely (P>0.05) affected. Yolk colour of birds fed 15.2 ppm dietary fumonisin was statistically superior (P<0.05) to those fed 0.2, 5.2, and 10.2 ppm dietary fumonisin. Egg yolk cholesterol and Triglyceride were inversely related. Birds on diet 4(15.2 ppm) had a higher but statistically similar egg yolk triglyceride value with those on diets 1, 2 and 3.

It is evident from the results presented in Table 3 that the average feed intake, average body weight gain, average egg weight and feed conversion ratio of the laying birds were not significantly influenced by the dietary fumonisin levels. These results are consistent with those of (23) that there were no significant differences in feed intake and body weight gain of male rats fed fumonisin B_1 for 2 years when compared to rats on control diets. The apparent lack of statistical significance in the feed intake of the birds could be occasioned by the low levels of dietary fumonisin and the failure of the mycotoxin to induce "feed refusal effect" in the laying hens. This opinion is contrary to that reported by (5) that male fischer rats fed fumonisin treated diets for 21 days had a depressed feed intake as a result of feed refusal effect. Similar evidences of depressed feed consumption was reported by (3, 18 and 21) in their separate studies. Laying birds are known to have natural instinct to eat to satisfy their nutrient requirements for body growth and egg laying. In this study, the pattern of body weight gain, egg weight and feed conversion ratio of the birds were directly related to their feed consumption and unaffected by dietary fumonisin levels. The results suggest that the absorption and utilisation of dietary nutrients by the birds fed diets 2, 3 and 4 were not inhibited by the dietary fumonisin levels when compared with those on control diet. These results are similar to those of (26) for broiler chicks, (2) for ducklings and (19) for minks fed different levels of dietary fumonisin when compared with those on control diet. Conversely, these results are at variance with those of (3) that dietary fumonisin probably impedes nutrient absorption and utilisation.

Table 4: Egg quality characteristics of laying birds fed graded levels of dietary fumonisin.

Parameters	Dietary Treatments				
	1	2	3	4	SEM
Egg Weight (g)	60.25	60.45	55.30	59.94	2.18
Egg Length (cm)	5.79	5.76	5.56	5.74	0.08
Egg Width (cm)	4.36	4.38	4.27	4.38	0.07
Yolk Weight (g)	15.98	15.74	15.20	15.36	0.45
Yolk Width (cm)	4.08	4.04	3.97	3.98	0.06
Yolk Height (cm)	1.73	1.79	1.71	1.81	0.06
Yolk Index (%)	42.40	44.31	43.07	45.48	2.79
Yolk Colour	1.00^{b}	1.00^{b}	1.00^{b}	1.25^{a}	0.07
Albumen Height (cm)	0.85	0.85	0.84	0.81	0.05
Haugh Unit	92.01	92.01	92.78	89.93	0.93
Shell Weight (g)	5.20	5.16	4.62	5.07	0.17
Shell Thickness (mm)	0.24	0.24	0.21	0.22	0.10
Egg Yolk Cholesterol (mg/dl)	150.20	138.84	130.20	125.00	19.49
Egg Triglycerides(mg/dl)	442.11	365.79	453.95	492.11	54.42

a, b : Values differently superscripted are significantly($p<0.05$) different. SEM: Standard Error of Means

The apparent lack of statistical significance in the pubertal age(s) of the laying hens used in this study suggests that the dietary mycotoxin did not have detrimental effect on the physiology of egg production and by extension, the attainment of sexual maturity(puberty) in the laying birds. However, this result is at variance with the report of (8) and (1) that aflatoxin and T-2 toxin inhibit egg production at the point of commitment of ovum to maturation and have detrimental effect on date of puberty in bob white quail hens.

The fact that egg shell thickness and other external egg quality traits did not decrease significantly as the dietary fumonisin levels increased revealed that Calcium and Phosphorus in the diet were adequate and their absorption and utilisation were not impeded. These results further suggest that the quality of the eggs did not deteriorate. The report of (16) that a significant decrease in egg shell thickness is an indication of deterioration in egg quality further corroborates the results obtained in this study. The statistical superiority of the egg yolk colour of birds fed 15.2 ppm (diet 4) to others may be an indication of the nutritional relevance of fumonisin in the diets of laying hens. It is suspected that the biosynthesis of complex sphingolipids was not inhibited by the dietary fumonisin levels in the experimental birds , thereby resulting in the production of egg yolk cholesterol and triglyceride levels statistically similar to those of the control birds. Weibking *et al*., (26) reported that the cholesterol

values of broilers fed fumonisin treated diets were statistically similar to the controls.

Conclusion and Application

Since the growth and egg quality parameters of the birds investigated in this study were not adversely affected by the various inclusion levels of dietary fumonisin, it can be concluded that

1. Laying birds can tolerate dietary fumonisin up to 15.2 ppm.
2. The nutritional and reproductive potentials of eggs laid by such birds may be unimpaired.

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