Growth performance and egg quality traits of laying hens fed graded levels of dietary fumonisin B₁

¹JacobTaiwo Ogunlade, ²Emmanuel Olubisi Ewuola, ³Francis Ayodeji Gbore, ⁴Simeon Olutoye Olawumi,⁵ Oluwole Moses David and ⁶Gabriel Nwachukwu Egbunike.

^{1,4}Department of Animal Production and Health Sciences, Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti, Nigeria.

^{2,6}Department of Animal Science, University of Ibadan, Nigeria.

³Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba Akoko, Nigeria. ⁵Department of Microbiology, Ekiti State University, Ado- Ekiti, Nigeria.

Abstract: The implications of fumonisin $B_1(FB_1)$ on growth, pubertal development and egg quality of laying hens were studied in a 16-week experiment. 120 Isa-Brown Point-of-lay(POL) birds were divided into four groups. Four diets were prepared to contain 0.2, 5.2, 10.2 and 15.2 ppm of FB₁ constituting diets 1(control), 2, 3 and 4 respectively. Each group was assigned to one diet in a completely randomised design .The age at which 50% of the birds on each diet began to lay was considered their pubertal age. Thirty eggs were selected per treatment every week for five weeks to assess egg quality. Results showed that daily feed intake, daily weight gain and feed conversion ratio of the birds were not significantly affected (p>0.05) . Statistically similar values(p>0.05) were obtained for the pubertal ages of the birds. FB₁ did not exert any significant influence (p>0.05) on the egg quality traits investigated except the yolk colour of birds on diet 4 which was statistically superior (p<0.05) to those on other diets. Egg cholesterol and triglycerides were not significantly influenced(p>0.0) by FB₁ levels. These imply that laying birds can tolerate dietary FB₁ up to 15.2ppm without compromising their growth, nutritional and reproductive potentials of their eggs.

Key words: Egg quality, Fumonisin B₁, Isa Brown point-of-lay, Pubertal development,

I. Introduction

Functional Functional Function Functio

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[7, 8, 9]. Available data on Poultry species like turkey poults [10], day-old broilers[11] and day-old Peking ducklings[12] 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 [13]. Among other disturbances in poultry, Ochratoxin and T-2 toxins in diets have been reported to depress egg production, with thin, rubbery shells[13]. 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.

II. Materials and Methods

2.1Materials 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[14] in the mycotoxin laboratory at International Institute of Tropical Agriculture(IITA), 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 IITA, 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)

2.2Layer Bird Husbandry and Experimental Design

A total of One hundred and twenty Isa Brown Point-of-lay(POL) birds with an average weight of 710.02g were randomly divided into four groups comprising of 30 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 30 POL birds per diet in 3 replications of 10 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.

2.3Pubertal Age of the Layer birds

When the female fowl is sexually mature, it usually starts to lay eggs[15]. The age at which 50% of the POL birds on each experimental diet began to lay eggs was taken as their age at puberty.

2.4Egg collection and Analysis

Thirty eggs were randomly selected every week from each treatment. The external and internal qualities of these eggs were determined as described by Olawumi and Ogunlade[16]. Egg cholesterol and triglycerides were determined using Randox Kits at the Animal Physiology Laboratory, University of Ibadan.

III. Statistical Analysis

The various data collected on the different parameters were subjected to one-way analysis of variance(ANOVA) of Statistical Analysis System[17]. Where significant differences were found, the means were compared using the Duncan procedure of the same soft ware.

Table 1: Percentage composition of the experimental diets(g/100g)					
Ingredients	Tre	atments			
	Diet 1	Diet 2	Diet 3	Diet 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	

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

^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.

Table 2 : Proximate Composition(g/100g) of the experimental diets.						
Composition	Treatments					
	Diet 1	Diet 2	Diet 3	Diet 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		

IV. Results

Table 3: Growth Performance of la	aying	birds fed	graded levels of dietar	y fumonisin

Parameters	Treatments				
	Diet 1	Diet 2	Diet 3	Diet 4 S	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
Average Feed Intake(g/day)	85.21	81.82	85.50	81.33	0.38
Average Body Weight					
Gain(g/day)	9.44	9.33	9.45	9.38	2.23
Average 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.

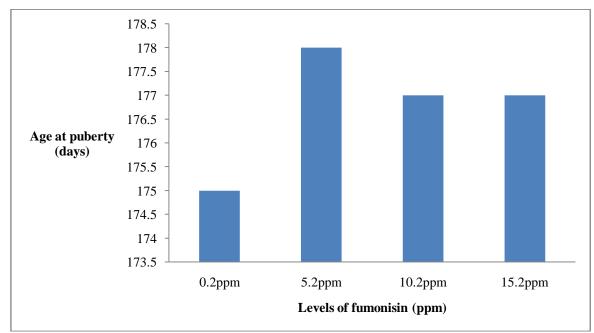
SEM: Standard Error of Means

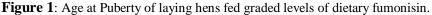
Results

V.

4.1Laying 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.





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

Parameters	Treatments				
	Diet 1	Diet 2	Diet 3	Diet 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 0.79	
Yolk Colour	1.00^{b}	1.00^{b}	1.00^{b}	$1.25^{\rm a}$ 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	

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

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

values are means. SEIVI. Standard Error of Means

4.2Egg 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.

VI. Discussion

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 US NTP[18] that there were no significant differences in feed intake and body weight gain of male rats fed fumonisin B₁ 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 Gelderblom *et al.*, [19] 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 Ewuola *et al.*, [20], Powell *et al.*, [21] and Sydenham *et al.*, [22] 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 Weibking *et al.*,[11] for broiler chicks, Bernudez *et al.*,[12] for ducklings and Restum *et al.*,[23] for minks fed different levels of dietary fumonisin when compared with those on control diet. Conversely, these results are at variance with those of Ewuola *et al.*,[20] that dietary fumonisin probably impedes nutrient absorption and utilisation.

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 Jewers[13] and Beasley [24] 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 Olorede and Longe [25] 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.*,[11] reported that the cholesterol values of broilers fed fumonisin treated diets were statistically similar to the controls.

VII. Conclusion.

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 B_1 , it can be concluded that laying birds can tolerate dietary fumonisin up to 15.2 ppm and that the nutritional and reproductive potentials of eggs laid by such birds may be unimpaired.

Acknowledgements

The authors wish to express their gratitude to Dr. R. Bandyopadhyay and Mr. O. Ayinde of the Mycotoxin Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for their technical assistance.

References

- [1] W. F. O. Marasas, *Fusarium moniliforme* a mycotoxicological miasma. *Mycotoxins and phycotoxin.* edited by P.S Steyn and R. Vleggaar (Amsterdam:Elsevier) 1986, 19-20.
- [2] T.S. Kellerman, W.F.O. Marasas, P.G. Thiel, W.C.A. Gelderblom, M. Cawood, and J.A.W. Coetzer . Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B₁, *Onderstepoort. J. Vet. Res.* 57, 1990, 269-275.
- [3] W.F.O. Marasas, T.S. Kellerman, W.C.A. Gelderblom, J.A.W. Coetzer, P.G. Thiel, and J.J. Van Derlugt. Leukoencephalomalacia in a horse induced by Fumonisin B₁ isolated from *Fusarium moniliforme*, *Onderstepoort Journal of Veterinary Research*, 55, 1988, 197-203.
- [4] L.R. Harrison, B.M. Colvin, J.T. Greene, L.E. Newman and J.R. Jr Cole, Pulmonary oedema and hydrothorax in swine produced by fumonisin B₁ a toxic metabolite of *Fusarium moniliforme*, J. Vet. Diagn. Invest. 2, 1990, 217-221.
- [5] W.C.A. Gelderblom, N.P.J. Kriek, W.F.O. Marasas, and P.G. Thiel, Toxicity and Carcinogenicity of the Fusarium moniliforme metabolite, fumonisin B₁ in rats, Carcinogenesis, 12, 1991, 1247-1251.
- [6] P.G. Thiel, W.F.O. Marasas, E.W. Sydenham, G.S. Shephard, and W.C.A. Gelderbom, The implications of naturally occurring levels of fumonisin in corn for human and animal health, *Myctopathologia*, 117,1997,3-9.
- [7] F.A. Gbore, E.O. Ewuola, J.T. Ogunlade, K.O. Idahor, A.O. Salako, and G.N. Egbunike, Spermatogenesis, gonadal sperm reserves and fertility of rabbits fed micro doses of fumonisin, *Nig. J. Anim. Prod.34*(2), 2007, 316-322.
- [8] J.T. Ogunlade, E.O. Ewuola, F.A. Gbore, R. Bandyopadhyay, J. Niezen, and G.N. Egbunike, Testicular and Epididymal Sperm Reserves of Rabbits Fed Fumonisin contaminated diets, W. Applied Sci. J. 1(1), 2006, 35-38.
- [9] US. F.D.A. Background paper in support of fumonisin levels in Animal feed. US food and Drug Administration Centre for Veterinary Medicine, June 6, 2000.
- [10] D.R. Ledoux, A.J. Bermudez, and G.E. Rottinghaus, Effects of feeding *Fusarium moniliforme* culture material, containing known levels of fumonisin B₁ in the young turkey poult, *Poult. Sci. 75(12)*, 1996,1472-1478.
- [11] T.S. Weibking, D.R. Leudox, A.J. Bermudez, J.R. Turk, G.E. Rottinghaus, E. Wang, and A.H. Merill Jr., Effects of feeding *Fusarium moniliforme* culture material, containing known levels of fumonisin B₁ on the young broiler chick, *Poult. Sci.* 72(3), 1993, 456-466.
- [12] A.J. Bermudez, D.R. Ledoux, and G.E. Rottinghaus, Effects of *Fusarium moniliforme* culture material containing known levels of fumonisin B₁ in ducklings, *Avian Dis.*, 39(4), 1995,879-886.
- [13] K. Jewers, Mycotoxins and their effects on poultry production. Engormix. Com. <u>http://www.engormix.com/mycotoxins</u> and their effect_ e_ articles _96_MYC, 2004
- [14] P.E. Nelson, and P.F. Ross, Fumonisin production by Fusarium species on solid substrates. Abstr.104,106th A.O.A.C Ann. Meet., Cincinnati, OH.,1992, Aug.31-Sept. 2.
- [15] J.A. Oluyemi, and F.A. Roberts, Poultry production in warm wet climates. Spectrum Books Ltd.(publ.), Ibadan, 2000, Pp.10
- [16] S.O. Olawumi, and J.T. Ogunlade, Phenotypic correlations between some external and internal egg quality traits in the exotic Isa Brown layer Breeders, Asian Journal of Poultry Science. 2(1), 2008, 30-35.
- [17] SAS Institute Inc., SAS/STAT User's Guide. Version 8 for windows. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina, U.S.A., 1999.
- [18] US NTP., NTP technical report on the toxicology and carcinogenesis studies of fumonisin B₁(CAS No.116355-83-0) in F344/N rats and B6C₃F₁ mice (feed studies). Research Triangle Park, North Carolina, US Department of Health and Human Services National Toxicology Program(NTP TR 496; NIH publication No. 99-3955), 1999.
- [19] W.C.A. Gelderblom, M.E. Cawood, S.D. Snyman, and W.F.O. Marasas, Fumonisin B₁ dosimetry in relation to cancer initiation in rat liver. *Carcinogenesis*, 15, 1994, 209-214.
- [20] E.O, Ewuola, F.A. Gbore, J.T. Ogunlade, R. Bandyopadhyay, J. Niezen and G.N. Egbunike, Physiological Response of Rabbit bucks to dietary fumonisin: Performance, Haematology and Serum Biochemistry. *Mycopathologia 165*, 2008, 99-104.
- [21] D.C. Powell, S.J. Bursian, C.R. Bush, J.A. Render, G.E. Rottinghaus, and R.J. Aulerich, Effects of dietary exposure to fumonisins from *F. moniliforme* culture material(M1325) on the reproductive performance of female mink. *Arch. Environ. Contam. Toxicol.*, 31, 1996, 286-292.

- [22] E.W. Sydenham, G.S. Shephard, W.C.A. Gelderblom, P.G. Thiel, and W.F.O. Marasas, Fumonisins: their implication for human and animal health. In: Scudamore K ed. Proceedings of the UK workshop on occurrence and significance of mycotoxins. Slough, United Kingdom, Ministry of Agriculture, Fisheries and Food, Central Science Laboratory, 1993, Pp. 42-48.
- [23] J.C. Restum, S.J. Burson, M. Millerick, H.A. Merill, Jr., E. Wang, G.E. Rottinghaus, and R.J. Aulerich, Chronic toxicity of fumonisins from F.moniliforme culture material (M-1325) fed to mink. Arch. Environ. Contam. Toxicol, 29, 1995, 545-550.
- [24] V. Beasley, Effect of fungal mycotoxins on tissues reproductive performance in bobwhite quail. In: Veterinary toxicology. Beasley, V.(Ed.).International Veterinary Information Service,Itacha,N.Y.1999: A2628;0899.
- [25] B.R. Olorede, and O.G. Longe, Effect of replacing Palm Kernel Cake with Shea butter Cake on egg quality characteristics, Haematology and Serum chemistry of laying laying hens. *Nigerian Journal of Animal Production 27(1)*, 2000,19-23.