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Egg Qualities and Embryonic Development of Eggs of Laying Hens Fed with Graded Levels of fumonisin B_1 With or Without Vitamin C

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ABSTRACT

The detoxifying effect of Vitamin C in the graded levels of Fumonisin B_1 fed to birds was conducted with One hundred and five (105) point-of-lay Isa brown breed at 18 weeks and twenty-one (21) cocks at 25 weeks to access egg qualities and embryonic development of their eggs. The birds (layers) were assigned to Seven (7) dietary treatments of 15 birds each at three (3) birds per replicate. The treatments were classified according to inclusion level of Fumonisin B_1 and Vitamin C. Treatment A served as the control, B (10 mg/kg of FB_1), C (20 mg/kg of FB_1), D (30 mg/kg of FB_1), E (10 mg/kg of FB_1 + Vitamin C), F (20 mg/kg of FB_1 + Vitamin C), and G (30mg/kg of FB_1 + Vitamin C). The birds were artificially fertilised with semen collected from intact cocks. All the eggs collected from day three (3) after artificial insemination for seven (7) days were incubated to assess the egg fertility and monitor the development of resulting embryos at 7 and 14 days. A significant difference was observed in shell weight ($p \leq 0.05$) while other external qualities of eggs examined were not significantly ($p \geq 0.05$) influenced by the level of fumonisin B_1. Yolk height and yolk diameter had significance ($p \leq 0.05$) while other internal qualities were not significantly ($p \geq 0.05$) affected. No significant effect of FB_1 was observed for the embryonic development just as there was no difference between the selected embryos of the treated group and the control. Meanwhile, the result shows that Vitamin C can effectively ameliorate the toxic effect of the FB_1 on the egg qualities (external and internal) and embryonic development of eggs.

Introduction

Fungi growth has been a significant problem in the feed industry. Many things have been done right from the field to prevent the occurrence and threat to feed quality. Mycotoxigenic *Fusarium verticillioides* is present in virtually all corn samples worldwide (Marasas *et al.*, 2001) which are the primary energy component of livestock feeds producing Mycotoxin in which can affect the health and performance of livestock. Mycotoxin in feeds causes reduced feed quality and reduced animal efficiency either through feed refusal, poor conversion of nutrients (Gbore *et al.*, 2010), diminished body weight gain (Ewuola *et al.*, 2008; Gbore 2009a) or problems such as interference with reproductive capacities (Gbore and Egbunike, 2008; Gbore, 2009b; Ewuola and Egbunike, 2010). At present, one of the most promising and practical approaches to detoxifying Fusarium contaminated grain on a large scale is the use of adsorbents. However, several absorbents have been shown to impair nutrient utilisation (Kubena *et al.*, 1993) and mineral absorption (Chestnut *et al.*, 1992) which may result in reduced growth and egg production in poultry. Adebayo et al., (2018a) reported that the dietary fumonisin B_1 did not have an inflammatory effect on laying hens and the immune status of the birds were not affected. Also, fumonisin B_1 was reported to caused poor feed conversion rate (Adebayo et al., 2018b), Earlier studies in poultry have shown that exogenous ascorbic and supplemented in feed or drinking or by injection improved performance of chicken during heat stress (Pardue and Thaxton 1982 and Pardue *et al.*, 1984).

Considerable research has been directed at finding methods to prevent toxicity of mycotoxins. Some of the approaches included detoxification and inactivation methods which include the use of binders and sequentially agents added to feed as an approach to reduce the toxicity of Mycotoxin and reducing their intestinal absorption (Chestnut *et al*., 1992). Therefore, the potential role of Vitamin C supplementation in preventing overreaction of heat and stressful nutritional stimulation help other animals to cope with such challenges (Jones *et al*., 1996). This study was therefore designed to explore the egg qualities (external and internal) and the embryonic development of eggs of laying hens fed with the graded level of fumonisin B_1 with or without Vitamin C in the diet. The present study is intended at assessing the detoxifying effect of vitamin C inclusion in the graded level fumonisin B_1 fed to birds on their egg qualities (external and internal) and embryonic development.

Materials and methods

Fumonisin Production

Autoclaved maize grains were cultured with a toxigenic strain of *F. verticillioides* (MRC 286) inoculum obtained from the Plant Pathology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria to produce fumonisin according to Nelson *et al*. (1994).

Experimental site and operations

The experiment was carried out in the Poultry unit of the Teaching and Research Farm, Federal University of Technology, Akure, Nigeria.

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The vegetation of the area is that of rainforest characterised by two peaks of rainfall and humidity during the rains. The mean rainfall is about 1500 mm and the rain last from March to November, while mean annual relative humidity is over 75%.

Experimental birds and diets

One hundred and five (105) point-of-lay of Isa brown breed at 18 weeks and twenty-one (21) cocks at 25 weeks were procured from a reputable farm. The birds (layers) were allocated to 7 dietary treatments of 15 birds each at three (3) birds per replicate. The treatments were classified according to the inclusion level of Fumonisin B_1, and Vitamin C. Treatment A served as the control, treatment B (10 mg/kg of Fumonisin B_1), treatment C (20 mg/kg of Fumonisin B_1), treatment D (30 mg/kg of Fumonisin B_1), treatment E (10 mg/kg of Fumonisin B_1 + Vitamin C), treatment F (20 mg/kg of Fumonisin B_1 + Vitamin C), and treatment G (30mg/kg of Fumonisin B_1 + Vitamin C)

Fertility of laying hens

The remaining treated hens were artificially inseminated with semen collected from intact cocks. All the eggs collected from day three (3) after artificial insemination for seven days were incubated to assess the egg fertility and monitor the development of resulting embryos at 7 and 14 days.

Egg collection

Eggs laid on the 21st, 28th, 35th, 42nd, 49th, 56th and 63rd day were collected and taken to the laboratory for egg quality determination. The eggs were weighed before being carefully broken, the contents removed and the shells air-dried for two days before taken the weight. Egg weight, yolk weight and shell weight were measured with a sensitive scale calibrated in grammes. The albumen weight was calculated by subtracting the sum of the weights of the shell and the yolk from the total egg weight. Shell thickness was measured with micrometre screw gauge. The mean of the measurements taken from these regions (broad, narrow and equatorial) was taken to ensure accuracy. Percentage shell weights were measured by finding the ratio of the shell weight to the egg weight as shown:

$$\% \text{ Shell weight} = \frac{\text{Shell weight} \times 100}{\text{Egg weight}}$$

Shell surface area (SSA) was calculated by the formula:

$$SSA = W^{0.667} \times 4.67$$

Where W = Average egg weight

0.667 and 4.67 are constants

Hen-day production (HDP) in percentage was calculated by adding all the eggs per replicates on a weekly basis using the formula below:

$$\frac{Ne}{Nb \times 7} \times \frac{100}{1}$$

Where Ne = Number of eggs laid per replicate per week

Nb = Number of birds per replicate

Seven = Number of days per week

Yolk index and Haugh unit were determined as follows,

$$\text{Yolk index} = \frac{\text{Yolk height}}{\text{Yolk width}}$$

$$HU = 100\log (H + 7.57 - 1.7W^{0.37})$$

Where H = Observed albumen height in mm

W = Observed weight of eggs in grammes

Yolk weight percentage, the ratio of the yolk weight to the egg weight, was calculated by the formula:

$$\% \text{ Yolk weight} = \frac{\text{Yolk weight}}{\text{Egg weight}} \times \frac{100}{1}$$

Statistical Analysis

The obtained data were subjected to analysis of variance of a 2x4 factorial experiment in a completely randomised design (CRD) using SAS (2008) package and significant difference between means were tested using Duncan Multiple Range Test of the same software**.**

Results

Table 2 shows the external qualities of the eggs laid by the hens, treated with fumonisin B_1 diets supplemented with or without Vitamin C, apart from the shell weight ($p \leq 0.05$), the other external qualities of the eggs examined were not significantly ($p \geq 0.05$) influenced by the levels of fumonisin B_1. The highest value for the shell weight (5.88 g) was observed in the eggs collected from the hens fed 20 mg fumonisin B_1 which was insignificantly different from both

Table 1. Gross Composition of the experimental diet for Layers mash.

Fumonisin B_1 (mg/kg)	**Quantity (%)**						
	0.02	**10**	**20**	**30**	**10**	**20**	**30**
Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Non-inoculated Maize	55.00	53.01	51.02	49.03	53.01	51.02	49.03
Inoculated maize	0.00	1.99	3.98	5.97	1.99	3.98	5.97
Soya bean meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Groundnut cake	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Wheat offal	5.49	5.49	5.49	5.49	5.49	5.49	5.49
Palm kernel cake	5.66	5.66	5.66	5.66	5.66	5.66	5.66
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish meal	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	8.50	8.50	8.50	8.50	8.50	8.50	8.50
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin C	0.00	0.00	0.00	0.00	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis							
Metabolisable Energy (kcal/kg)	2687.28	2687.28	2687.28	2687.28	2687.28	2687.28	2687.28
Crude protein (%)	16.74	16.74	16.74	16.74	16.74	16.74	16.74
Calcium (%)	3.49	3.49	3.49	3.49	3.49	3.49	3.49
Available phosphorus (%)	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Methionine	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Lysine	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Crude fibre	3.51	3.51	3.51	3.51	3.51	3.51	3.51

10 and 30 mg levels of fumonisin B_1 inclusion. Also, the highest values ($p \geq 0.05$) of egg weight (53.50 g) and egg length (5.34 mm) were observed in 20 mg fumonisin B_1 while that of egg width (4.20 mm) and shell diameter (0.46 mm) was noticed in the control and 30 mg fumonisin respectively.

The dietary treatment of fumonisin B_1 with or without Vitamin C, likewise, had an effect on the mean shell weight with its significant ($p \leq 0.05$) higher value (5.90 g) found in fumonisin with Vitamin C. The same was observed for egg weight and egg length, though not significant. Egg width (4.18 mm) and shell diameter (0.43 mm) were insignificantly ($p \geq 0.05$) higher in fumonisin B_1 without Vitamin C.

The interaction of the increasing levels of fumonisin B_1 with or without Vitamin C had no significant ($p \geq 0.05$) effect on all the egg qualities likewise, levels of fumonisin B_1 with Vitamin C had non-significant increasing effects on egg weight and egg length. Also, the interaction of the increasing levels of fumonisin B_1 without Vitamin C had a non-significant increasing effect on egg length while the effect of these interactions did not follow a particular pattern in the remaining external egg parameter.

Table 3 reveals the internal qualities of eggs produced by the hens fed dietary treatment of fumonisin B_1 with or without Vitamin C. The results showed that external qualities are not synonymous to internal qualities of an egg. The yolk height, yolk diameter and Haugh unit were significantly ($p \leq 0.05$) different due to dietary treatment with varying levels of FB_1. The highest value for yolk height (1.51 mm) was observed in 20 mg fumonisin while it was at the control for both yolk diameter (3.90 mm) and Haugh unit (83.03). The highest values of 4.18, 13.80 g, 32.68 g and 6.60 mm, though insignificant, were observed in control for yolk colour, yolk weight, albumen weight and albumen height respectively. For the albumen length that was likewise not significantly ($p \leq 0.05$) influenced, its highest value (8.04 mm) was observed in 30 mg fumonisin.

Table 2. External egg qualities of laying hens fed dietary fumonisin B_1 with or without Vitamin C.

Diets	Level of Fumonisin(mg)	Vitamin C treatment	Egg Weight	Egg Length	Egg Width	Shell Weight	Shell Diameter
Control	0		53.34	5.30	4.20	5.38	0.34
10FB	10	NoVitamin C	52.36	5.28	4.16	5.48	0.35
20FB	20	NoVitamin C	53.95	5.33	4.19	5.89	0.35
30FB	30	NoVitamin C	53.18	5.34	4.18	5.83	0.59
10FB	10	Vitamin C	54.48	5.38	4.21	5.93	0.35
20FB	20	Vitamin C	53.02	5.35	4.12	5.87	0.35
30FB	30	Vitamin C	52.71	5.32	4.16	5.89	0.34
SEM			3.67	0.14	0.10	0.38	0.03
Mean separation							
Level of Fumonisin effect							
0			53.34	5.30	4.20	5.38[b]	0.34
10			53.34	5.33	4.18	5.69[a]	0.35
20			53.50	5.34	4.16	5.88[a]	0.35
30			52.94	5.33	4.17	5.86[a]	0.46
Vitamin C treatment							
Vitamin C			53.35	5.35	4.16	5.90	0.34
NoVitamin C			53.17	5.31	4.18	5.73	0.43
Statistical significance							
Treatment			0.97	0.43	0.62	0.01	0.45
Level			0.95	0.89	0.74	0.01	0.52
Treatment* Level			0.10	0.18	0.07	1.00	0.10

ab: Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

Table 3. Internal egg quality of laying hens fed dietary fumonisin B_1 with or without Vitamin C.

Diets	Level of Fumonisin (mg)	Vitamin C Treatment	Yolk colour	Yolk Weight (g)	Yolk Height (mm)	Yolk diameter (mm)	Albumen Weight (g)	Albumen Length (mm)	Albumen Height (mm)	Haugh Unit
Control	0		4.18	13.80	1.40	3.90	32.68	7.76	6.60	83.03
10FB	10	NoVitaminC	4.00	13.01	1.44	3.56	31.92	7.62	6.46	82.36
20FB	20	NoVitamin C	4.07	13.78	1.56	3.92	32.09	7.91	6.46	81.81
30FB	30	NoVitamin C	3.85	13.43	1.48	3.81	32.79	8.16	6.20	80.31
10FB	10	Vitamin C	3.92	13.59	1.43	3.93	33.16	7.90	6.18	79.78
20FB	20	Vitamin C	4.08	13.11	1.45	3.80	32.16	8.05	6.35	81.38
30FB	30	Vitamin C	4.21	13.34	1.46	3.81	32.19	7.92	6.58	83.02
±SEM			0.56	1.02	0.11	0.17	2.75	0.55	0.49	3.19
Mean separation										
Level of Fumonisin effect										
0			4.18	13.80	1.40[b]	3.90[a]	32.68	7.76	6.60	83.03[a]
10			3.96	13.28	1.44[ab]	3.73[a]	32.49	7.75	6.33	81.17[b]
20			4.07	13.46	1.51[a]	3.86[a]	32.12	7.97	6.40	81.61[b]
30			4.04	13.39	1.47[ab]	3.81[ab]	32.48	8.04	6.40	81.72[b]
Vitamin C treatment										
Vitamin C			4.08	13.34	1.45[ab]	3.84	32.48	7.96	6.38	81.48
NoVitamin C			3.98	13.40	1.50[a]	3.76	32.25	7.89	6.38	81.52
Statistical significance										
Treatment			0.49	0.42	0.03	0.02	0.87	0.58	0.37	0.33
Level			0.72	0.55	0.04	0.01	0.93	0.20	0.51	0.45
Treatment* Level			0.20	0.07	0.70	<0.0001	0.24	0.14	0.04	0.01

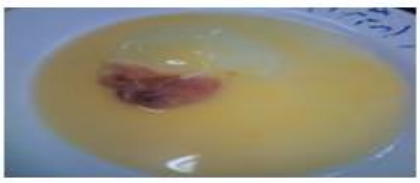
ab: Means within the same column with different superscripts differ significantly ($p \leq 0.05$)

The dietary treatment of fumonisin B_1 with or without Vitamin C also had significant ($p \leq 0.05$) effect on both the yolk height and yolk diameter. Its effect on the other qualities was not significant ($p \geq 0.05$). Yolk colour, yolk diameter albumen weight and albumen length had the higher values (4.08, 3.84 mm, 32.48 g and 7.96 mm respectively) in fumonisin B_1 with Vitamin C while yolk weight, yolk height and Haugh unit had higher mean values (13.40 g, 1.50 mm and 81.52 respectively) observed in fumonisin B_1 without Vitamin C. For both treatments, albumen height has the same insignificant ($p \geq 0.05$) value of 6.38 mm.

The interaction of the increasing dosages of fumonisin B_1 with or without Vitamin C had significant ($p \leq 0.05$) effects in yolk diameter, albumen height and Haugh unit. The interaction of the increasing dosages of fumonisin B_1 with Vitamin C was observed to have an increasing effect on albumen height and Haugh unit; while without Vitamin C, it had a decreasing impact on both the former and latter qualities. The yolk diameter was observed to be influenced by the interaction of increasing levels of fumonisin B_1 with and without Vitamin C, but no particular trend was followed. Fumonisin B_1 levels increased both yolk colour and yolk height ($p > 0.05$) with Vitamin C while both albumen weight and albumen length ($p \geq 0.05$) were increased by fumonisin B_1 without Vitamin C

Plate 1 and 2 show the pictorial presentations of the 7th and 14th-day embryonic development of the eggs laid by the hens treated with fumonisin B_1 respectively.

Control

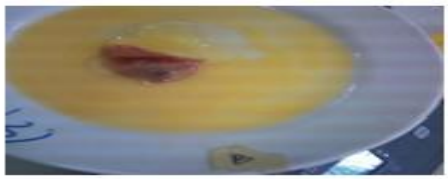


10 FB_1 + No Vitamin C

20 FB_1 + No Vitamin C

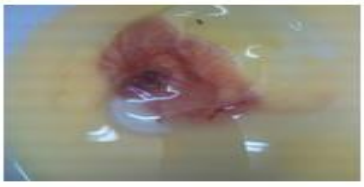
30 FB_1 + No Vitamin C

Plate 1a. Embryonic development at 7th days.

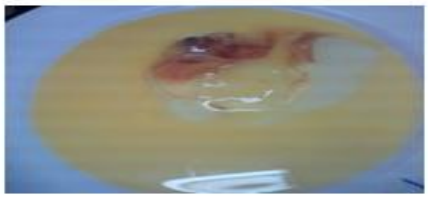


Control

10 FB_1 + Vitamin C

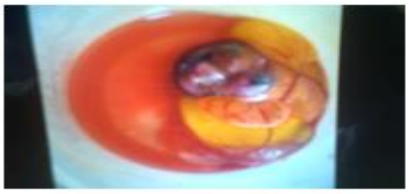


20 FB_1 + Vitamin C



30 FB_1 + Vitamin C

Plate 1b. Embryonic development at 7th days.



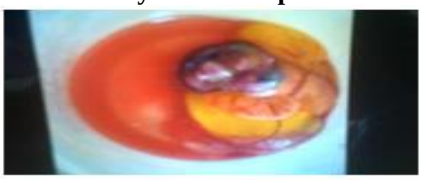
Control

10 FB_1 + No Vitamin C

20 FB_1 + No Vitamin C

30 FB_1 + No Vitamin C

Plate 2a. Embryonic development at 14th day.



Control

10 FB_1 + Vitamin C

20 FB_1 + Vitamin C

30 FB_1 +Vitamin C

Plate 2b. Embryonic development at 14th day.

It can be observed from the pictures that the development of the embryos of the selected eggs treated with fumonisin B_1 with or without Vitamin C was not conspicuously different from that of the control. The development on the 14th day was more pronounced when compared with that of the 7th day. The head regions of the embryos were observed to be distinctly developed on the 14th day.

Discussion

External egg qualities

The presence of Vitamin C in the diet-induced increased shell weight, egg weight and egg length. Also, dietary fumonisin B_1 had a significant influence only on the shell weight while its effect on the other external qualities was not remarkable. This result was in support of Siloto *et al.* (2010)

who indicated a possible additive and adverse effect of mycotoxin on the external qualities especially egg weight. In contrast, Kubena *et al.* (1999) found higher egg weight in layers fed 100 and 200 mg FB_1/kg, while Butkeraitis (2004) observed reduced egg weight in quails fed 50 and 250 mg FB_1/kg feed.

Internal egg qualities

The presence of Vitamin C in the diet gave an improvement in the yolk height, and yolk diameter as the different levels of FB_1 had a reducing effect on these qualities. This further substantiated the claim that Vitamin C has an ameliorative property. Just like Oliveira *et al.* (2000) who observed the lower value of Haugh unit of the eggs laid by laying hens contaminated with 300 and 500 µg aflatoxin/kg feed when compared to the non-contaminated control, the same influence was observed for this quality in this study. The improvement observed in albumen height due to the dietary FB_1 and Vitamin C caused a substantial improvement in the Haugh unit. This indicated that albumen height could be used to predict the Haugh unit.

Embryonic development

Fumonisin B_1 is poorly absorbed, but rapidly distributed and eliminated in many animal species including laying hens, swine, cow, rat, and mouse and non-human primates (Prelusky *et al*., 1996a, b; Norred *et al*., 1996; 1998; EHC, 2000). No significant effect of FB_1 was observed in this study for the embryonic development just as there was no difference between the selected embryos of the treated group and the control. This was in support of the report that there are no data demonstrating that fumonisin B_1 consumption results in transfer to chicken eggs (Vudathala *et al*., 1994; Prelusky *et al*., 1996a) or that it crosses the placenta in rats (Voss *et al*., 1996; Collins *et al*., 1998a, b), mice (Reddy *et al*., 1996) or rabbits (Laborde *et al*., 1997). Meanwhile, Javed *et al*., (1993) reported that injection of purified FB_1 into fertile chicken eggs resulted in a time- and dose-dependent embryopathic and embryocidal effects. This was not observed in this study as the FB_1 was not injected into the fertile eggs.

Conclusion

This study shows that fumonisin B_1 has a toxic effect on the yolk diameter, albumen height and Haugh unit resulting in eggs with lower quality. Meanwhile, the Vitamin C effectively ameliorated the toxic effect of the fumonisinB_1. It is therefore essential to include Vitamin C in the diet of laying hens to reduce the adverse effects of fumonisin B_1 on the qualities of eggs laid and embryonic development.

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