Available online at www.elixirpublishers.com (Elixir International Journal)

Agriculture

Elixir Agriculture 121 (2018) 51621-51626

ISSN: 2229-712X

Haematology and Serum Biochemistry of Laying Birds Fed With Graded Levels of Fumonisin B_1With or Without Vitamin C

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ARTICLE INFO

Article history:
Received: 5 July 2018;
Received in revised form:
20 August 2018;
Accepted: 29 August 2018;

Keywords
Fumonisin B_1,
Haematology,
Serum,
Vitamin C.

ABSTRACT

An experiment 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 assess the haematology and serum biochemistry of laying birds fed with graded levels of Fumonisin B_1 (FB_1) with or without vitamin C. 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). Significant differences were observed in PCV, Hb, WBC and Eos for levels of FB1 inclusion. Glucose and AST, showed significant differences ($P<0.05$) in levels of inclusion of FB1 in the diets. Meanwhile, the result shows that Vitamin C can effectively ameliorated the toxic effect of the FB_1.

Introduction

The economic consequences of mycotoxin contamination are profound, and exposure of humans and livestock to mycotoxin-contaminated food is particularly a serious problem in the tropics (Reddy and Raghavender, 2008). Mycotoxins are virtually ubiquitous in livestock diets, and are mainly produced by the four genera of fungi i.e. *Fusarium*, *Claviceps*, *Aspergillus* and *Penicillium*, which, according to Petzinger and Weidenbach (2002), grow on almost every kind of nourishing medium. *Fusarium verticillioides* (Sacc) Nirenberg (*F. moniliforme* Sheld.) is associated with dietary staples such as corn intended for human and animal consumption throughout the world, producing mycotoxins such as fumonisins, moniliformin and trichothecenes. Under the prevalent environment condition in the tropics the conta0ination of various commodities with *Fusarium* fungi and their mycotoxins is unavoidable (Aventaggiato *et al*., 2005). Fusarium B_1 belong to the recently discovered toxins (fumonisin) which are produced by *Fusarium verticilloides* (older synonym is *F. moniliforme*) and *F. proliferatum*,that commonly contaminate maize. But it has been claimed also that F. napiforme, F. anthophilum, F. dlamini and F. nygamai are able to produce FB_1 (EHC, 2000, NTP, 1999). In addition, Fumonisin have been found at high level in wheat, asparagus, tea and cowpea (EFSA, 2005). It is very difficult to obtain uncontaminated maize, even if the contamination level is not significant. Fumonisin have been detected in cereals at very low concentration as 0.02 mg/kg (EFSA, 2005). *Fusarium* mycotoxins 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). Considerable research has been directed at finding methods to prevent toxicity of mycotoxins. Some of the approaches included detoxification and inactivation (Avantaggiato *et al.*, 2005). Detoxification and inactivation methods include the use of binders or sequestering agents added to feed as an approach to reduce toxicity of mycotoxins by reducing reactivity of bound mycotoxins and reducing their intestinal absorption (Chestnut *et al.*, 1992). Detoxifying agents are supposed to detoxify the contaminated feedstuff during passage through the digestive tract by adsorbing and/or degrading the mycotoxins under the pH-, temperature- and moisture-conditions of the digestive tract. Therefore, increasing attention has been focused on the potential role of vitamin C supplementation in preventing over reaction of heat and nutritional stressful stimulation to help chicken and other animals to cope with such challenges. (Jones *et al.*, 1996). This study was therefore designed to explore the growth and laying performance of laying birds fed with graded level of fumonisin with or without Vitamin C in their diet. The present study aimed at assessing the detoxifying effect of Vitamin C inclusion in the graded level of fumonisin fed to bird on their growing and laying performance.

Materials and Method

Fumonisin Production

Autoclaved maize grains was 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,

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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

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. The vegetation of the area is that of rain forest, characterized 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 purchased from a reputable farm. The birds (layers) were assigned to 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 has 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).

Blood collection and analysis

On the last day of the experiment, three birds were randomly selected from each treatment, fasted over- night and slaughtered for blood analysis. Blood samples were collected for haematological and serum biochemical responses. Blood samples were collected into labelled bottles, one set of which contained Ethylene diaminetetraacetic acid (EDTA), an anti-coagulant while the others without EDTA for serum biochemistry. The blood without anti-coagulant was allowed to stand in test tube rack in the laboratory in a slanting position. The serum separated from each blood sample was then decanted after centrifugation. The sera were later analysed for serum biochemical indices.

The blood samples in the EDTA bottles were used for haematological analyses. Blood samples were analysed for packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC), Mean corpuscular volume, (MCV), Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total protein, globulin and albumin as described by Tietz (1995), the serum creatinine and urea nitrogen was estimated by deproteinisation and Urease-Berthelot colorimetric methods respectively, using a commercial kit (Randox Laboratories Ltd., U.K.). Also, the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods respectively using commercial test kits (Quimica Clinica Applicada, S.A.), while the serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits.

Statistical Analysis

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

Results and Discussion

Haematology of laying hens

Table 2 reveals the result of effect of fumonisin B_1 with or without Vitamin C supplementation on the haematological status of laying hens. The dietary FB_1 had a significant ($p \leq 0.05$) effect only in the Eos. with 10 mg/kg producing the lowest value (1.00 %). For other parameters examined, no significant ($p \geq 0.05$) effect of dietary FB_1 was noticed. The interaction of dietary FB_1 with or without Vitamin C produced no significant ($p \geq 0.05$) variations in the haematological indices with the exemption of WBC and MCHC that were significantly ($p \leq 0.05$) difference.

Table 2. Haematology of laying hens fed dietary fumonisin B_1 with or without Vitamin C.

Diets	Level of Fumonisin (mg)	Vitamin C treatment	PCV (%)	Hb (g/dl)	WBC (10^3/mm^3)	Het (%)	Lym (%)	Eos. (10^3/mm^3)	Plat (%)	RBC (10^6/mm^3)	Bas (%)	Mono (%)	MCV (μ^3)	MCH (ρg)	MCHC (%)
Control	0		32.67	10.89	11.80	31.00	47.33	2.00	5.70	2.58	1.33	1.00	126.97	42.32	33.55
10FB	10	No Vitamin C	28.25	9.42	12.25	27.25	51.25	1.00	9.25	2.48	1.50	1.75	114.64	38.21	34.00
20FB	20	No Vitamin C	27.33	9.11	12.06	30.67	48.67	1.00	6.00	2.33	1.33	1.00	117.18	39.06	33.38
30FB	30	No Vitamin C	27.67	9.22	13.60	29.00	50.33	1.33	8.00	2.25	1.33	1.00	123.20	41.07	34.00
10FB	10	Vitamin C	29.00	9.67	12.50	28.60	51.00	1.00	7.50	2.32	1.50	1.00	124.97	41.66	33.78
20FB	20	Vitamin C	29.00	9.67	12.90	30.33	48.00	2.00	8.00	2.32	1.00	1.33	125.63	41.88	34.33
30FB	30	Vitamin C	29.00	9.67	12.50	28.00	50.67	2.00	10.67	2.31	1.00	1.00	125.74	41.91	33.98
± SEM			3.15	1.05	0.80	3.98	3.61	0.25	3.63	0.25	0.48	0.60	10.99	3.66	0.36
Mean separation															
Level of Fumonisin effect 0															
0			32.67^{a}	10.89^{a}	11.80^{b}	31.00	47.33	2.00^{a}	5.70	2.58	1.33	1.00	126.97	42.32	33.55
10			28.50^{b}	9.50^{b}	12.33^{ab}	37.50	51.17	1.00^{c}	8.67	2.43	1.50	1.60	118.08	39.36	33.89
20			28.17^{b}	9.39^{b}	12.48^{ab}	30.50	48.33	1.40^{bc}	7.00	2.33	1.17	1.20	121.41	40.47	33.86
30			28.33^{b}	9.44^{b}	13.50^{a}	38.50	50.50	1.60^{ab}	9.33	2.28	1.17	1.00	124.47	41.49	33.99
Vitamin C treatment															
Vitamin C			29.44	9.81	12.77	37.56	51.00	1.57	8.78	2.36	1.11	1.38	125.09	41.70	34.03
NoVitamin C			27.22	9.07	12.67	30.11	49.00	1.14	7.89	2.33	1.44	1.14	117.55	39.18	33.79
Statistical significance															
Treatment			0.05	0.05	0.22	0.30	0.28	0.001	0.46	0.35	0.37	0.70	0.27	0.27	0.1394
Level			0.22	0.22	0.20	0.49	0.36	0.003	0.47	0.39	0.60	0.47	0.64	0.66	0.4190
Treatment*Level			1.00	1.00	0.05	0.27	0.19	0.33	0.76	1.00	1.00	0.23	1.00	1.00	0.0223

Packed Cell Volume; Hb = Haemoglobin; WBC = White blood cells; Het = Heterophils; Lym = Lymphocytes; Eos. = Eosinophils; Plat = Platelets;
RBC = Red blood cells; Bas = Basophils; Mono = Monocytes; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin;MCHC = Mean Corpuscular Haemoglobin Concentration

The influence of this interaction for the two parameters (i.e. WBC and MCHC) did not follow a particular trend but it must be emphasized that all the values obtained for WBC were higher than the control (11.80 x 10^3/mm^3). The lowest values of MCHC were observed in the group fed 10 mg FB_1 without Vitamin C (33.38%) which was still lower than the control (33.55%). In addition, the supplementation with Vitamin C produced significant ($p \leq 0.05$) changes in the values of PCV, Hb and Eos. It was observed that the values of PCV and Hb were 29.44% and 9.81g/dl with Vitamin C supplementation and this tended towards the values of the control (32.67% and 10.89g/dl respectively) whereas, 27.22% and 9.07g/dl were the values for the group of laying hens without Vitamin C. For the Eos, its value with Vitamin C supplementation was 1.57% and without Vitamin C, 1.14%. However, all other parameters were insignificantly different ($p \geq 0.05$) for the dosage levels of dietary FB_1 and Vitamin C supplementation.

Serum biochemistry of laying hens fed dietary fumonisin B_1 with or without vitamin C

The serum biochemistry of laying hens fed different levels of fumonisin B_1 with or without Vitamin C supplemented diet is presented in Table 3. The interaction of dietary supplementation of Vitamin C and fumonisin B_1 revealed a significant ($p<0.05$) variation in few of the serum parameters with AST and ALP having a significant variation among others in interaction between the treatments and levels of fumonisin B_1 inclusion. It was generally discovered that samples with Vitamin C have a slight reduction in mean values in some of the parameters such as urea, glucose, chol, ALT and globulin compared with those without Vitamin C.

The results showed a significant ($p \leq 0.05$) difference in the levels of fumonisin fed to the laying hens in the amount of AST. The serum AST was significantly higher in level in the different dosages of fumonisin B_1 than the control. This trend was also observed in glucose and albumin with no significant ($p \geq 0.05$) difference. There was no significant ($p \geq 0.05$) variation in the dietary Vitamin C treatments except in Alb ($p \leq 0.05$) whose value of Vitamin C (3.99g/dl) was numerically higher than that of no Vitamin C (3.92 g/dl). This pattern of numerical higher value due to Vitamin C supplementation, although not significantly ($p \geq 0.05$) influenced, was noticed in glucose (153.22 mg/dl), AST (97.22iu/l), and Alb: glo (1.63). The values of other parameters (like urea, cholesterol, ALT, ALP, total protein and globulin) were seen to be numerically higher in the animals whose diet were not supplemented with vitamin C.

Discussion

Haematology

The red blood cell (RBC) components of the blood of the experimental laying hens were not affected by the dietary levels of FB_1. The mean values obtained for the RBC or erythrocytes of the hens were reduced, although with no dietary fumonisin influence, but within the reported range of 1.58-3.80x10^6/mm^3 and 2.5-3.5x10^6 µl for normal female chicken by Mitruka and Rawnsley (1981) and Jain (1993) respectively. The packed cell volume (PCV) values of the laying hens were influenced by the fumonisin administration causing a reduction which tended to be normalized by the Vitamin C supplementation. However, the values of the PCV obtained were all within the range (22-35%) stated by Jain (1993) for domestic chickens. Lindsay (1997) and Maxwell *et al.* (1990) reported that reduction in PCV and RBC values indicates anaemia. Therefore, the dietary fumonisin B_1 level even with no Vitamin C supplementation did not induce anaemic condition in the experimental hens. Also when supplemented with Vitamin C, there was an improvement in the status of the PCV and RBC. The non-significant influence of the dietary FB_1 levels with or without Vitamin C supplementation on the mean corpuscular volume (MCV) of the hens suggested that their RBCs were normal. According to Jain (1993), MCV below 102.0 μ^3 indicates microcytosis, whereas a value of over 129.0 μ^3 indicates macrocytosis. Haemaglobin (Hb), an iron-containing conjugated protein, has been described (Tietz, 1995) to have physiological function of transporting oxygen and carbon dioxide. Although the haemoglobin values were significantly reduced with increased dosage of FB_1, the Vitamin C supplementation

Table 3. Serum biochemistry of laying hens fed dietary fumonisin B_1 with or without Vitamin C.

Diets	Level of Fumonisin(mg)	Vitamin C Treatment	Urea (mg/dl	Glucose (mg/dl)	Chol. (mg/dl)	AST (iu/l)	ALT (iu/l)	ALP (iu/l)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb:glo
Control	0		6.00	80.33	37.00	90.00	12.33	41.00	6.11	3.31	2.80	1.19
10FB	10	No Vitamin C	6.25	210.25	67.75	98.50	14.00	33.33	6.67	4.08	2.59	1.62
20FB	20	No Vitamin C	10.00	149.67	74.33	124.67	15.00	66.70	6.47	3.94	2.53	1.79
30FB	30	No Vitamin C	10.00	155.67	82.00	89.33	25.67	42.50	6.56	3.80	2.76	1.37
10FB	10	Vitamin C	7.00	172.50	55.50	86.50	14.00	56.30	6.45	3.94	2.51	1.57
20FB	20	Vitamin C	6.33	88.00	67.67	84.33	12.67	47.13	6.57	4.05	2.53	1.61
30FB	30	Vitamin C	4.33	126.67	62.67	119.33	17.67	56.33	6.37	3.90	2.47	1.59
± SEM			2.60	64.19	30.31	19.69	6.98	2.48	0.55	0.39	0.50	0.36
Mean separation												
Level of Fumonisin effect												
0			6.00	80.33^b	37.00	90.00^b	12.33	41.00	6.11	3.31^b	2.80	1.19
10			6.50	197.67^a	63.67	92.50^{ab}	14.00	44.50	6.59	4.03^a	2.56	1.60
20			8.17	118.83^{ab}	71.00	104.50^a	13.83	56.91	6.52	4.00^a	2.52	1.70
30			7.17	141.17^{ab}	72.33	104.33^a	21.67	49.42	6.46	3.85^a	2.61	1.48
Vitamin C treatment												
Vitamin C			6.11	153.22	68.22	97.22	4.56	42.63	6.46	3.99	2.47	1.63
No Vitamin C			8.44	151.89	69.78	96.11	8.44	48.91	6.59	3.92	2.67	1.56
Statistical significance												
Treatment			0.11	0.23	0.27	0.14	0.34	0.82	0.44	0.04	0.53	0.21
Level			0.64	0.08	0.42	0.04	0.16	0.92	0.65	0.08	0.87	0.27
Treatment*Level			0.06	0.21	1.00	0.03	1.00	0.03	1.00	1.00	1.00	1.00

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

Chol. = cholesterol; AST =aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; Alb:Glo = albumin globulin ratio

tended to stabilize this parameter. The values of haemoglobin obtained for the experimental hens fed different levels of fumonisin B_1 with or without Vitamin C supplementation were within the reference range of 7.0-13.0 g/dl and 7.4-12.2 g/dl for normal female chicken as reported by Tietz (1995) and Mitruka and Rawnsley (1981) respectively. Furthermore, mean corpuscular haemoglobin concentration (MCHC) which is the average haemoglobin of each red cell, was influenced by the dietary treatments but not to the detriment of the hens, as the values obtained were within the range for normal chicken. The result therefore suggested that the experimental hens did not suffer a depressed respiratory capability and gas exchange capacity of the RBCs. The influence of dietary FB_1 with or without Vitamin C on RBC, PCV and Hb in this study demonstrated that FB_1 is a non-haematotoxic mycotoxin. These results are in agreement with those of Zomborsky-Kovacs *et al.* (2002), Ogunlade *et al.* (2004) and Ewuola *et al.* (2008) that considered fumonisin as non-haematotoxic mycotoxin.

Moreover, the white blood cell (WBC) or leucocytes play a central role in the inflammatory response and host defence to infection. They participate in the effective cleaning of gram-positive and gram-negative bacteria (Ainsworth *et al.*, 1996; Holst *et al.*, 1996; Laichalk *et al.*, 1996) and they are also the cells of the immune system. The values of the WBC counts were improved significantly by the supplementation of Vitamin C. Despite the reducing effect of the dietary fumonisin, all the counts were within the range values of 9.20-28.6 x $10^3/mm^3$ (Mitruka and Rawnsley, 1981) for normal female chickens. Also, eosinophils have two distinct functions in the immune system. First, they destroy invading germs like viruses, bacteria or parasites, and also create an inflammatory response. The eosinophil counts obtained from the study was significantly reduced by dietary FB_1 but the count was improved when supplemented with Vitamin C. The effects of these dietary treatments did not alter the eosinophil counts from the range values, 0.67-2.07 x $10^3/mm^3$ according to Mitruka and Rawnsley (1981), for normal chickens. The result, therefore, showed that the dietary fumonisin did not have any inflammatory effect on the hens and the immunity status of the birds was not affected.

The remaining parameters were not affected by the dietary treatments as all the values were within the range for domestic chickens. However, Rotter *et al.* (1996) and Espada *et al.* (1997) had earlier reported changes in selected haematological parameters in pigs and broilers fed fumonisin contaminated diets respectively and these did not agreed with this present study. Also, alterations in some haematological parameters have been reported in pigs (Gbore *et al.*, 2010).

Serum Biochemistry

Fumonisin B_1 inclusion dosages caused different changes in the levels of few of the serum biochemical examined. Cholesterol, alkaline phosphatate, total protein and albumin were dietary FB_1 concentration dependent. Total protein and albumin decreased with increase in dietary FB_1 levels while cholesterol and alkaline phosphatase increased with increase in dietary FB_1 among the levels. At variance to Ogunlade and Egbunike (2013), albumin values across the treatments were significantly increased by the dietary FB_1levels in comparison to the control. The values, with the exemption of the control, were above the normal physiological range of 2.10-3.45 g/dl reported by Mitruka and Rawnsley (1981). This could be as a result of dehydration or shock as reported by Mitruka and Rawnsley (1981). Likewise, the levels of globulin were seen to increase insignificantly and this caused increased levels of albumin:globulin that were above the physiological range of 0.45-0.96 g/dl and 0.58-1.30 respectively. This might be due to stress caused by the fumonisin challenge according to Mitruka and Rawnsley (1981).

The pattern of influence of the dietary FB_1 with Vitamin C levels on AST and ALP was inconsistent as the levels were either too low below or too high above the physiological range values (88-208 iu/l and 24.5-44.4 iu/l) for normal chickens. This suggested that the laying hens might be suffering from liver and biliary system disease.

This study shows that, fumonisin had a remarkable effect on the haematology and serum having no inflammatory effect on the hens and the immune system of the bird were not affected. Therefore vitamin C shows the ameliorative effect on the toxicity of the Fumonisin B_1 fed to laying birds.

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