# INFLUENCE OF DIETARY CULTURE MATERIAL CONTAINING AFLATOXIN AND T-2 TOXIN ON GROWTH RATE AND HAEMATOLOGY IN TURKEY POULTS (*Meliagridesgallopavo*) -BELTSVILLE SMALL WHITE VARIETY

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Abstract: Experimental mycotoxicoses were induced singly and in combination in 48 turkey poults (Beltsville small white) for a period of 0 to 13 weeks by feeding diets containing 100 ppb AF and 1 ppm T-2 toxin.Comparison of weekly mean body weights revealed significant (P<0.05) differences from first week onwards between the control and mycotoxin treated groups. Among the toxin treated groups, the AF group revealed significant (P<0.05) difference during the second week only, T-2 group revealed significant (P<0.05) differences during the third week to sixth week, while AF-T-2 group revealed significant (P<0.05) differences from first week till 13<sup>th</sup> week except 8<sup>th</sup> and 9<sup>th</sup> week. There was a significant (P<0.05) decrease in the body weight gain in toxin fed birds when compared to control birds. Significant (P<0.05) differences in feed conversion were observed between the control and toxin treated groups. Significant (P<0.05) decrease in feed conversion efficiency ratio was observed in the toxin treated groups. Blood samples were collected from each group at the end of 49<sup>th</sup> and 91<sup>st</sup> days of trial to study haematological parameters. The PCV, Hb and TEC values decreased significantly (P<0.05) within toxin fed birds when compared to the control. Comparison of means for erythrocyte indices revealed no significant differences between the control and mycotoxin treated groups except MCV which revealed significant (P<0.05) increase between AF and AF-T-2 toxin group showing a macrocytic normochromic blood picture.

**Keywords:** Turkey poults, aflatoxin, T-2 toxin, growth rate, haematology.

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## **INTRODUCTION**

Contamination of food and feed by mycotoxins (toxic metabolites of fungi) in the form of multiple mycotoxicoses is the current problem faced by the poultry farmers. Regardless of decades of extensive research, mould infection still remains a challenging problem (Munkvold 2003).Aflatoxin (AF) and T-2 toxin are the most frequently encountered mycotoxins. AF is a potent hepatotoxin with dihydrofuran-coumarin moiety and is of importance in producing the biological effects and is produced by *Aspergillusflavus*and *A. parasiticus*. T-2 toxin is a 3 hydroxy 4, 15 diacetoxy 8 (3-methylbutryloxy), 12, 13 epoxy trichothec-9-ene metabolite. It is a potent irritant, inflammatory (dermotoxic, alimentary toxic, hepatotoxic and growth inhibitory agent) and radiomimetic agent produced by *Fusarium*species. The AF,by binding to both RNA and DNA blocks transcription whereas, T-2 toxin blocks initiation of translation. Thestudies on aflatoxicosis in turkey poultswere limited owing to the potential sensitivity of the species, the same on T-2 was scant and there werenone on AF-T-2 combined toxicity for a period of 91days. Hence, the present study was conducted.

### MATERIALS AND METHODS

AF was produced on rice (Shotwell*et al.*, 1966) by using A. *Parasiticus*NRRL 2999 strain. The T-2 toxin was producedon corn grits (Burmeister, 1971) by using *F. sporotrichoides*MTCC 1894 strain (Microbial Type Culture Collection, Chandigarh,India). The mycotoxin content in cultured material was analysed at Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai,Tamilnadu, India. Knownamounts of AF and T-2 toxin containing powdered substrates were incorporated into turkeybrooder mashboth singly and in combination to yield 100 ppb AF and 1 ppm T-2 toxin. Forty-eight newly hatched unsexed turkey poultsobtained from standard hatcheries were wing banded, weighed and housed in battery brooders with *ad libidum*supply of feedand water. They were randomly distributed into four groups of twelve chicks each. The control and toxin mixeddiets were fed to different groups for 91 days from the day of hatch.

## Growth rate study

Body weights (g) were recorded at weekly intervals. Average weekly feed consumption (g) and feed conversion were arrived as follows.

Feed consumption =  $\frac{\text{Total feed consumed during the week } (g)}{\text{Number of birds fed during the week}}$ 

Feed conversion = 
$$\frac{\text{Average feed consumption per bird during the week (g)}}{\text{Average weight gain per birdduring the week (g)}}$$

Samples of blood were collected from six birds by intracardiac puncture in Heller and Paul anticoagulant mixture on 28<sup>th</sup> day of experiment. Haematological studies were conducted to determine the Hb by acid haematin method and PCV by microhaematocrit method (Coles, 1986).

## RESULTS

The mean ( $\pm$  SE) body weight and relative body weight of turkey poults fed AF and T-2 singly and in combination are presented in Tables 1, (Figure 1) and 2respectively. Comparison of weekly means revealed significant (P<0.05) differences from first week onwards between the control and mycotoxin treated groups. Among the toxin treated groups, the AF group revealed significant (P<0.05) differences during the second week only, T-2 group revealed significant (P<0.05) differences during the third week to sixth week, while AF-T-2 group revealed significant (P<0.05) differences from first week till 13<sup>th</sup> week except 8<sup>th</sup> and 9<sup>th</sup> week.

### Feed consumption and conversion

The mean ( $\pm$  SE) weekly feed consumption and feed conversion turkey poults fed AF and T-2 singly and in combination are presented in Tables 3,(Figure 2)and 4 respectively. Significant (P<0.05) differences in feed conversion were observed between the control and toxin treated groups. Significant (P<0.05) differences in feed conversion was observed among the toxin treated groups between (AF, T-2) and AF-T-2. Comparison of overall means revealed (P<0.05) increase in feed conversion ratio between the control and toxin treated groups while feed conversion ratio revealed significant (P<0.05) increase among the toxin treated groups between (AF, T-2) and AF-T-2 groups.

## Haematology

Mean ( $\pm$  SE) hematological values and erythrocytic indices of turkey poults fed AF and T-2 singly and in combination are presented in Tables 5 and 6 respectively and Fig. 3-5. The PCV values revealed significant (P<0.05) differences between the control and toxin fed birds. No significant differences were observed between the control & AF and T-2 & AF-T-2 groups. The Hb values revealed significant (P<0.05) differences between the control & AF and T-2 & AF-T-2 groups. AF-T-2 groups. Among the toxin treated groups significant (P<0.05) differences were observed between the T-2 & AF-T-2 groups. The TEC values revealed significant (P<0.05) differences between the control significant (P<0.05) differences were observed between the T-2 & AF-T-2 groups. The TEC values revealed significant (P<0.05) differences between the control and groups.

differed significantly (P<0.05) from each other. Comparison of means for erythrocyte indices revealed no significant differences between the control and mycotoxin treated groups except MCV which revealed significant (P<0.05) increase between AF and AF-T-2 toxin group.

#### DISCUSSION

## **Growth performance**

Feeding 100 ppb AF and 1 ppm T-2 toxin individually or in combination for 13 weeks significantly reduced the growth rate in turkey poults. The body weight gain showed significant reduction in all toxin treated birds from first week onwards. The relative body weight of AF was 71 per cent at the end of the trial. The T-2 toxin group also showed similar relative body weight (70%). The effect was shown in T-2 toxin group which was fed 10 times more than AF. The reduction in weight gain due to experimental aflatoxicosis has been reported from 200 ppb onwards (Hamilton *et al.*, 1972) - 250 ppb; (Arafa*et al.*, 1981) - 0.7 mg/kg AF; (Giambrone*et al.*, 1985a) - 400 ppb; (Kubena*et al.*, 1995) 75 mg AF/kg showed reduced body weight gain by 39 per cent; (Giacomini *et al.*, 2006) 1000 ppb AF showed reduced body weight gain by 38 per cent.

The T-2 toxin treated birds showed significant reduction in weight gain from first week onwards at 1 ppm level with significant reduction during third week to seventh week and maintained from eighth week onwards, compared with AF and AF-T-2. Depression in body weight gains of poults observed in the 3 ppm T-2 treatment group is consistent with previous studies in which turkey poults were fed diets containing 2 and 10 ppm (Richard *et al.*, 1978), 5 ppm (Kubena*et al.*, 1995), 4 ppm (Ogunbo*et al.*, 2007) and 3ppm (BabuPrasath, 2008) of T-2 toxin for 28, 21, 21 and 28 days respectively. However, Sklan*et al.* (2003) and BabuPrasath (2008) did not observe reduction in body weight gain of turkey poults fed 1 ppm T-2 toxin for 32 and 28 days respectively. Kamalavenkatesh (2003) and Krishnamoorthy (2004) reported significant reduction in weight gain in broilers fed T-2 toxin from 0.5ppm and 1ppm for 0 to 28 days.

The AF-T-2 fed group showed lesser weight gain than the individual AF and T-2 groups from first week, till 13<sup>th</sup> week except during the 8<sup>th</sup> and 9<sup>th</sup> week. Similar observations were reported by Elisângela Aparecida Guaiume (2005) in turkey poultsfed diets containing 2 mg T-2 toxin/kg diet singly or in combination with 0.15 mg/kg AFB1 from first week onwards in a 21 day trial with significant reduction in body weight gain.The combined toxicity group birds gained 37 per cent less weight than the control and 7 to 8 per cent less than the AF and T-2 groups. This indicated a less than additive effect of AF and T-2 in combination in

affecting the growth rate. Similar observations were made by Huff *et al.* (1988) who fed 2.5  $\mu$ g AF/g and 4.0  $\mu$ g T-2 toxin/g in broilers from 1 to 3 weeks of age; Raju and Devegowda (2000) 0.3 mg AF/kg and 3 mg T-2 toxin/kg to broiler chickens from 1 to 35 days of age and Madheswaran *et al.* (2005) AF (3 ppm) and T-2 toxin (4 ppm) from 0 to 35 days of age in Japanese quails. Reduced weight gain induced by AF, T-2 toxin or in combination could be attributed to the reduced feed consumption as observed in this study and inhibition of protein synthesis in AF and T-2 toxicosis (Krogh, 1987; Pier, 1992). This was also indicated by hypoproteinaemia encountered in the toxin fed groups. The pathological effects of toxins on the organs of digestive system as evident from this study could have interfered with the digestion and absorption of feed, thus contributing to the lowered weight gain.

#### Feed consumption and conversion

Feeding 100 ppb AF and 1 ppm T-2 toxin individually or in combination for 13 weeks significantly reduced the feed conversion efficiency in turkey poults. Feeding 100 ppb AF for 13 weeks significantly reduced the feed consumption and increased feed conversion ratio in turkey poults. Similar findings were recorded by Giambrone *et al.* (1985b) who fed crude AF containing 100, 200, 400, or 800 ppb of AFB1 to turkey poults and observed that crude AF greater than or equal to 400 ppb was found to be highly toxic to turkeys with a significant decrease in the feed conversion during 5 weeks.

On the contrary, earlier works revealed no significant change in feed conversion in turkey poults fed a diet containing 50 or 150 ppb AF for 11 or 13 weeks or when fed these diets were fed for 11 weeks and then followed by the control diet for 1 or 2 weeks Richard *et al.* (1986). Rauber (2007) reported no significant changes in feed consumption in poults fed up to 100 ppb of AF at 42 d of age. However, significant reduction in feed consumption was reported in broiler chicks fed 100, 200 and 300 ppb AF up to six weeks of age and lowered feed efficiency observed at 300 ppb level (Gopinath*et al.* 2001). Other reports include significant reduction in feed consumption and no change in feed efficiency in broiler chicks fed 1 ppm AF for 28 days (Kumar and Balachandran, 2005), significant reduction in feed conversion efficiency in broiler chicks fed 1 ppm AFB<sub>1</sub> from 15 to 42 days of age (Mekala *et al.*, 2006) and feeding AF (0.5 ppm) from 0 to 6 weeks to broiler chicken (Theophilus, 2006).

Feeding 1 ppm T-2 toxin for 13 weeks reduced the feed consumption and feed conversion efficiency in turkey poults. These findings are in accordance with Richard *et al.* (1978) who reported reduction in feed conversion efficiency in Broad Breasted White turkey poults fed 2

and 10 ppm of T-2 toxin for four weeks and Ogunbo *et al.* (2007) who observed significant reduction in feed consumption in turkey poults fed 4 ppm of T-2 toxin for 21 days. However, Babu Prasath, (2008) observed no significant reduction in feed consumption and feed conversion in turkey poults fed 1 and 3 ppm T-2 toxin.

Feeding 100 ppb AF and 1 ppm T-2 in combination for 13 weeks significantly reduced the feed conversion efficiency in turkey poults, which was significantly higher than the individual toxin fed group. These findings are in accordance with Elisângela Aparecida Guaiume (2005) who reported that turkey poultsfed diets containing 2 mg T-2 toxin /kg diet in combination with 0.15 mg/kg AFB1 revealed significant decrease in the feed consumption from second week onwards in a 21 day trial. Similar findings were recorded in broiler chickens by Raju and Devegowda (2000) who reported that feeding 0.3 mg AF/kg and 3 mg T-2 toxin/kg from 1 to 35 days of age resulted in significant reduction in feed intake and feed conversion ratio.

Reduced weight gain induced by AF and T-2 toxin could be attributed to inappetance, reduced feed consumption, absorption and assimilation as observed in this study and inhibition of protein synthesis in AF and T-2 toxicoses (Krogh, 1987 and Pier, 1992). This was also indicated by hypoproteinaemia encountered in the mycotoxin fed groups. The pathological effects of these mycotoxins on the organs of digestive system as evident from this study could have interfered with the digestion and absorption of feed, thus contributing to lowered weight gain.

### Haematology

Feeding 100 ppb AF and 1 ppm T-2 individually or in combination for 13 weeks significantly decreased PCV, Hb and TEC in turkey poults. There was a significant reduction in PCV values in T-2 and AF-T-2 groups when compared with the control and AF fed groups. The TEC values revealed a significant reduction in mycotoxin treated groups compared with the control group. The toxin treated groups also showed a significant reduction among them indicating anaemia. The erythrocyte indices revealed significant increase in MCV in T-2 and AF-T-2 toxin group. AF caused hematopoietic suppression and anaemia and was observed as decrease in the TEC, PCV and Hb (Reddy, 1981; Huff *et al.*, 1986; Mohiuddin*et al.*, 1986).

Significant reduction in PCV, Hb and TEC values were observed in 1 ppm T-2 toxin treated birds indicating anaemia. Similar observations were also made in turkey poults fed diet containing *Fusarium*mycotoxins for four weeks (Chowdhury *et al.*, 2005) and feeding T-2 toxin(1 and 3 ppm) from 0-28 days in turkey poults (Babu Prasath, 2008). Marginally

reduced feed consumption and alimentary tract lesions affecting digestion and absorption of nutrients observed in this study might have contributed to anaemia. Besides, it was postulated that the anaemia observed in T-2 toxicosis could be due to its direct cytotoxicity to erythropoietic marrow or inhibition of uptake of iron into the erythropoietic cells (Doi*et al.*, 2006).

Significant reduction in PCV, Hb and TEC values were observed in combined 100 ppb AF and 1 ppm T-2 toxin treated group for 13 weeks indicating anaemia. However, Elisângela Aparecida Guaiume (2005) observed no significant difference in haematocrit values in turkey poultsfed diets containing 2 mg T-2 toxin/kg diet in combination with 0.15 mg/kg AFB1 in a 21 day trial. Similar observations were observed in Japanese quail fed 4 ppm T-2 toxin from 0 to 35 days of age (Madheswaran *et al.*, 2005). Such changes might be due to cytotoxicity to erythropoietic marrow of T-2 toxicosis (Doi *et al.*, 2006). Aflatoxin is known to produce hemolyticanemia by decreasing the circulating mature erythrocytes and lysis of erythrocytes would result in above-normal levels of cellular debris in circulation (Tung *et al.*, 1975). As a consequence of lysis of erythrocytes the spleen appeared congested because of an unusually high concentration of inorganic iron and debris from the circulation (Wyatt, 1991) which might lead to anemia. Among the toxin treated groups, MCV revealed significant increase in AF-T-2 toxin indicating macrocytic anaemia.

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# Mean (± SE) weekly body weights (g) of turkey poults fed aflatoxin and T-2 toxin singly and in combination

	1	1													
	Hatch		Age in weeks												
Groups	weight	1	2	3	4	5	6	7	8	9	10	11	12	13	
	(n=12)	(n=12)	(n=12)	(n=12)	(n=12)	(n=12)	(n=12)	(n=12)	( <b>n=6</b> )	( <b>n=6</b> )	( <b>n=6</b> )	( <b>n=6</b> )	( <b>n=6</b> )	( <b>n=6</b> )	
Control	47.05	84.91 <sup>a</sup>	152.58 <sup>a</sup>	243.51 <sup>a</sup>	355.53 <sup>a</sup>	486.96 <sup>a</sup>	637.01 <sup>a</sup>	798.66 <sup>a</sup>	981.94 <sup>a</sup>	1175.05 <sup>a</sup>	1390.58 <sup>a</sup>	1628.52 <sup>a</sup>	1884.31 <sup>a</sup>	2161.97 <sup>a</sup>	
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	
	0.93	1.66	2.85	5.20	5.50	6.24	7.11	9.19	11.18	15.28	21.29	29.09	39.59	53.38	
Aflatoxin (100 ppb)	47.04	88 59 <sup>b</sup> +	141.41 <sup>c</sup>	228.14 <sup>b</sup>	314.69 <sup>b</sup>	417.96 <sup>b</sup>	530.41 <sup>b</sup>	656.50 <sup>b</sup>	782.97 <sup>b</sup>	918.14 <sup>b</sup>	1051.87 <sup>b</sup>	1199.22 <sup>b</sup>	1358.25 <sup>b</sup>	1530.32 <sup>b</sup>	
(100 ppb)	± 0.89	2.12	± 2.47	± 3.27	± 5.45	± 7.93	± 11.72	± 16.25	± 20.50	22.97	± 24.76	± 26.94	± 27.66	± 28.54	
T-2 toxin	47.05	84.04 <sup>b</sup>	144.90 <sup>b</sup>	201.74 <sup>c</sup>	288.78 <sup>c</sup>	393.62 <sup>c</sup>	504.30 <sup>c</sup>	622.26 <sup>bc</sup>	756.75 <sup>bc</sup>	894.31 <sup>bc</sup>	1041.26 <sup>b</sup>	1194.97 <sup>b</sup>	1347.80 <sup>b</sup>	1509.46 <sup>b</sup>	
(1 ppm)	± 0.88	± 2.63	± 3.14	± 4.54	± 6.48	± 7.32	± 8.19	± 10.15	± 13.92	± 14.93	± 18.93	± 23.72	± 27.70	± 32.80	
Aflatoxin (100 ppb) <sub>+</sub> T-2 toxin (1 ppm)	47.08 ± 0.91	77.00 <sup>c</sup> ± 2.87	112.77 <sup>d</sup> ± 3.69	$162.78^{d}$ $\pm$ 3.77	236.49 <sup>d</sup> ± 6.84	345.57 <sup>d</sup> ± 9.69	469.19 <sup>d</sup> ± 11.32	599.07 <sup>c</sup> ± 12.25	715.79° ± 11.52	$835.10^{\circ}$ ± 14.02	956.59 <sup>c</sup> ± 18.15	$1083.82^{\circ}$ ± 22.33	$1220.06^{\circ}$ $\pm$ 25.87	$1358.83^{\circ}$ $\pm$ 30.05	

Means with different superscripts within a column differ significantly (P<0.05)

Mean (± SE) relative weekly body weights (%) of turkey poults fed aflatoxinand T-2 toxin singly and in combination

Groups	Hatch	Age in weeks												
	weight (n=12)	1 (n=12)	2 (n=12)	3 (n=12)	4 (n=12)	5 (n=12)	6 (n=12)	7 (n=12)	8 (n= 6)	9 (n= 6)	10 (n= 6)	11 (n= 6)	12 (n= 6)	13 (n= 6)
Control	47.05	100	100	100	100	100	100	100	100	100	100	100	100	100
Aflatoxin (100 ppb)	47.04	104	93	94	89	86	84	82	80	78	76	74	72	71
T-2 toxin (1 ppm)	47.05	99	95	83	81	81	79	78	77	76	75	73	72	70
Aflatoxin (100 ppb) <sub>+</sub> T-2 toxin (1 ppm)	47.08	91	74	67	67	71	74	75	73	71	69	67	65	63

# Mean (± SE) Feed consumption (g) of turkey poults fed aflatoxin and T-2 toxin singly and in combination

	Age in weeks													Original faced
Groups	1 (n=12)	2 (n=12)	3 (n=12)	4 (n=12)	5 (n=12)	6 (n=12)	7 (n=12)	8 (n= 6)	9 (n= 6)	10 (n= 6)	11 (n= 6)	12 (n= 6)	13 (n= 6)	consumption
Control	86.00	171.00	289.00	351.00	415.00	502.00	556.00	622.00	661.00	731.00	827.00	877.00	941.00	7029.00
Aflatoxin (100 ppb)	97.00	184.00	274.00	298.00	374.00	432.00	518.00	605.00	642.00	681.00	743.00	811.00	854.00	6513.00
T-2 toxin (1 ppm)	87.00	157.00	193.00	291.00	393.00	452.00	512.00	611.00	640.00	741.00	798.00	806.00	845.00	6526.00
Aflatoxin (100 ppb) <sub>+</sub> T-2 toxin (1 ppm)	81.00	127.00	183.00	281.00	402.00	497.00	541.00	578.00	612.00	684.00	768.00	828.00	861.00	6443.00

Means with different superscripts within a column differ significantly (P<0.05)

# Mean (± SE) Feed conversion ratio of turkey poults fed aflatoxin and T-2 toxin singly and in combination

	Age in weeks													
Groups	1 (n=12)	2 (n=12)	3 (n=12)	4 (n=12)	5 (n=12)	6 (n=12)	7 (n=12)	8 (n= 6)	9 (n= 6)	10 (n= 6)	11 (n= 6)	12 (n= 6)	13 (n= 6)	Overall means
Control	2.29	2.55	3.22	3.14	3.17	3.35	3.45	3.50	3.44	3.42	3.52	3.50	3.49	$3.23^{\circ} \pm 0.10$
Aflatoxin (100 ppb)	2.36	3.50	3.17	3.48	3.66	3.92	4.20	5.16	4.80	5.11	5.06	5.11	4.97	$4.19^{b} \pm 0.25$
T-2 toxin (1 ppm)	2.41	2.58	3.42	3.40	3.77	4.11	4.37	4.54	4.68	5.10	5.25	5.32	5.28	$4.17^{b} \pm 0.28$
Aflatoxin (100 ppb) <sub>+</sub> T-2 toxin (1 ppm)	2.84	3.60	3.69	3.90	3.72	4.04	4.18	4.85	5.17	5.72	6.11	6.14	6.30	$4.64^{a} \pm 1.15$

Means with different superscripts within a column differ significantly (P<0.05)

Mean (±SE) haematological values of turkey poults fed aflatoxin and T-2 toxin singly and in combination

Groups	PCV (%) (n=6)	Hb (g/dL) (n=6)	TEC (m/cmm) (n=6)
Control	39.67 <sup>a</sup> ±0.66	13.30 <sup>a</sup> ±0.15	2.48 <sup>a</sup> ±0.06
Aflatoxin (100 ppb)	$38.50^{a} \pm 0.69$	13.03 <sup>a</sup> ±0.17	2.27 <sup>b</sup> ±0.08
T-2 toxin (1 ppm)	35.25 <sup>b</sup> ±0.55	11.75 <sup>b</sup> ±0.14	2.03 <sup>c</sup> ±0.07
Aflatoxin (100 ppb) <sub>+</sub> T-2 toxin (1 ppm)	$34.17^{b} \pm 0.82$	11.18 <sup>c</sup> ±0.21	$1.88^{d} \pm 0.06$

Overall means with different superscripts within a column differ significantly (P<0.05)

# TABLE 6

### Mean (±SE) Erythrocyte indices of turkey poults fed aflatoxin and T-2 toxin singly and in combination

Groups	MCV (fL) (n=6)	MCHC (g/dL) (n=6)	MCH (pg) (n=6)
Control	$160.42^{\circ}\pm2.41$	33.58 ±0.33	53.87 <sup>b</sup> ±0.98
Aflatoxin (100 ppb)	171.44 <sup>b</sup> ±4.36	33.92±0.59	58.23 <sup>a</sup> ±2.00
T-2 toxin (1 ppm)	175.19 <sup>ab</sup> ±5.32	33.39±0.46	58.46 <sup>a</sup> ±1.84
Aflatoxin (100 ppb) <sub>+</sub> T-2 toxin (1 ppm)	183.97 <sup>a</sup> ±5.72	$32.78 \pm 0.38$	$60.19^{a} \pm 1.70$

Overall means with different superscripts within a column differ significantly (P<0.05).











