Effect Of Aflatoxin B1, Zearalenone And Ochratoxin A On Some Hormones Related To Fertility In Male Rats

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ABSTRACT

Three hundreds samples of feeds and sera of cattle and sheep (one hundred samples of each) were collected from farms at Minufiya, El-Behira and Assiute governorates in which animals (cattle and sheep) suffered from loss of weight gain, low productivity and disturbance in fertility. These samples were evaluated for mycotoxins contamination. Aflatoxins were detected in 30% of feed samples with the mean amount of 3.4 ± 0.1 ppm and ochratoxins in 20% with the mean values of 2.2 ± 0.02 ppm . Whereas, T-2 toxins and zearalenone were gained from 20% and 16% of samples with the mean levels of 36.0 ± 1.0 and 22 ± 0.3 ppm, respectively. But fuminosin B1 (FB1) toxin was found in 2% of samples at mean levels of 70 ± 0.01 ppm. The detection of mycotoxins in sera of diseased cattle and sheep showed that the most prevalent mycotoxins in cattle sera was aflatoxin B1 which detected in 40% of cases with the mean level of (5.4 ± 0.1), followed by ochratoxin A in 33% of cases with the mean level of (8.2 ± 0.1), T2 in 17% with the mean level of (26 ± 0.2) and zearalenon in (10%) with mean level of (19 ± 0.2). The lowest incidence was detected in cases 0f FB1 which obtained from 2% of cattle cases with the mean levels of (55 ± 0.6). Also , the pattern of incidence of mycotoxins in sheep sera were nearly similar to those in cattle with the exception that the FB1 not detected at all in sheep. The mycotoxins, aflatoxins, ochratoxins and zearalenone were given to male albino rats in the doses of 0.5, 1.0 and 2.5 ppm in feeds(respectively), for up to 6 months of age to investigate their effects on the growth rates and hormones regulating fertility (FSH, LH, Testosterone, T3 and T4). The results indicated the obvious adverse effects of mycotoxins on the secretion of these hormones and productivity of animals. The environmental pollutions particularly feed contamination was suggested to be the main source of the problem. Hence, regulatory measures must be undertaken to prevent such contaminants to reach the feed of animals. The significance of our results were fully discussed.

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Key Words: Mycotoxins, Aflatoxin, Ochratoxin A, Zearalenone, Fungi, Hormones, luteinizing H., follicle stimulating H. testosterone, thyroxin H.

Abbreviations: AF.: aflatoxin, T2: member of trichothecine toxins ,FSH follicle stimulating hormone, LH: luetinizing hormnone, T3& T4: tri-iodo-thyronine (total T3) and total thyroxin (total T4) hormones

1. INTRODUCTION

Up to date the progressive increase in world population require a parallel rise in the production of food. The majority of these food particularly that of animal origin may carry the dangerous factors for human and animal health. The fungal metabolites namely mycotoxins represent the most significant contaminants of food and feed **(Aly, 1993; Debey *et al*., 1995; Magnoli *et al*., 1999 and Hassan, 2003).** Various members of myoctoxins were detected in animal sera, feed and food and produced severe dangerous changes in active organs (**Hassan *et al*., 2004 and 2007and 2008 ; Hassan and Abd El-Dayem, 2004;).** The mycotoxins in feed consumed by animal and their serum cause disturbances in the hormonal profile related to fertility including follicle stimulating hormone (FSH), luetinizing hormone (LH), testosterone (TES.), thyroxin 3&4 (T3, T4) and can cause abnormal fetal development in farm animals which affect the normal function of reproductive organs and elsewhere the productivity of animals **(Xie *et al*., 1991; England *et al*., 1998; Tiemannand Vanselow, 2003; Hassan *et al*., 2003 and Ragheb and Srour, 2005).** Aflatoxins disturb the thymus gland functions which produced a significant reduction in feed intake and low body weight gain (**Fiorito *et al*., 1991 and Mocchogiani *et al*., 1998).**

However, the mycotoxins may be affected the semen quality which caused an increased in incidence of abnormal spermatozoa in animal and birds that administrated mycotoxins in diet **(Muthiah *et al*., 1997 and Yang et al., 2007 a & b),** who reported that treatment of mice with different doses of zearalenone caused increased numbers of abnormal spermatozoa and decreased amount of live spermatozoa with a significant reduction in body weight and relative epididymis weights. They also stated that after ingestion of contaminated cereals with fungi of fusarium and their toxins zearalenone, it may leads to animal fertility disturbances and other reproductive pathologies particularly suppressive effect on testosterone secretion. On the other hand, the mycotoxins reduced progesterone synthesis by inhibition the follicle stimulating hormone secretion (FSH) **(Tiemann and Vanselow, 2003).**

Therefore, the aim of the present work was to evaluate the effects of some mycotoxins on growth rate

and some the hormones related to fertility in male albino rats.

2. MATERIAL AND METHODS

2.1. MATERIAL:

2.1.1. Samples:

Serum and Feed samples: Three hundred samples of feeds and sera of cattle and sheep (one hundred samples of each) were collected from the farms at minufiya, El-Behira and Assiute governorates, in which animals (cattle and sheep) suffered from loss of weight gain, low productivity and disturbance in fertility. The feed samples were collected from store houses of these farms and the feeders of animals.

2.1.2. Strain for mycotoxins production: The mycotoxins were produced using toxigenic fungal strain as *A. flavus* (for aflatoxins), *A. ochraceus* (for ochratoxin A) and *Fusarium graminearum or F. roseum* (for zearalenone). These strains were isolated from animal feeds in laboratory of mycology of animal health research institute, Dokki.

2.1.3. Mycotoxins standard solution for TLC : Mycotoxins standard of Aflatoxins B_1, B_2, G_1, G_2, Ochratoxin A, Zearalenone , T2 and Fumonisin B1 were purchased from (Sigma Chemical Company, St. Louis U.S.A).

2.1.4. Laboratory animals:

One hundred apparently healthy male albino rats weighted (100-120 g) were housed under hygienic conventional conditions in stainless steel cages. Prior to experiment, rats fed on healthy basal diet free from any cause of disease. Drinking water was supplied in glass bottles, cleaned three times a week.

2.1.5. Biochemical reagents and kits for measurement the levels of hormones were purchased from Sigma chemical Company, USA.

2.2. Methods:

2.2.1. Detection of mycotoxins in serum and feeds:

Measurement of mycotoxins in serum and feeds was applied according to the fluorometeric method reported by **Hansen (1993).** The recommended amount of samples subjected for extraction of toxins by addition of methanol and water and passed over immunoaffinity column(each toxin have specific column).The obtained extract was measured by fluorometer or T.L.C.

2.2.2.Production of mycotoxins (aflatoxin, ochratoxin and zearalenone): The mycotoxins were produced using toxigenic fungal strain as recommended by **Smith (1997**) for aflatoxins**, by D'Mello *et al.*, (1997**) for zearalenone and by **Marquardt and Frohlich (1992)** for ochratoxins.

The isolates of *A. flavus*, *A. ochraceus* and *Fusarium graminearum or F. roseum*) which were isolated from feed samples and used for mycotoxins (aflatoxins, ochratoxin A and zearalenone) production, respectively. A flasks, each containing 100 gm of finely ground corn and 40-50 ml of distilled water was mixed and autoclaved at 121°C for one hour. The flask was shaken to prevent cooking of yellow corn. It was inoculated with corresponding fungus for required mycotoxins and incubated for 4 weeks at 25-28°C. In case of zearalenone production, the flasks were transferred to 8-10°C for additional 2 weeks. After end of incubation period , the corn was removed from flasks, dried, finely ground and 50 g of each was subjected to toxin extraction as recommended by **(Wyllie and Morehous, 1978 and Hansen (1993)).**

2.2.3. Experimental design:

A. The male albino rats were divided into 4 groups (25 of each). The first group kept as a control given healthy feed. Whereas, the second group given 0.5 ppm of aflatoxin in feed. However, the third group dosed with 1 ppm of ochratoxin in feed. The 4th group was treated by addition of 2.5 ppm of zearalenone in feed. The experimental work was continuoused up to 6 months.

B. Blood samples: During the experimental period (6 months), blood samples were collected every month from retro-orbital venous plexus **(Halperin *et al.*, 1951**) and serum was separated for determination the levels of LH, FSH, T3 and T4 hormones . At the beginning of 4th month of male age, blood samples were collected every 2 weeks till age of 6 month and serum samples were separated for estimation the level of testosterone hormone.

C. Body weight gain and growth rate were recorded every month from the beginning of the experiment till the end (1 to 6 month of age).

2.2.4. Determination of Luteinizing hormone (LH) and Follicular stimulating hormone (FSH) were performed according to the methods recommended by Santener *et al.* (1981).

2.2.5. Determination of serum testosterone hormone was performed according to the method described after Wilson and Foster (1992).

2.2.6. Estimation of serum total tri-odo-thyronine (total T3) and total thyroxine (total T4) hormones were performed as described by **Kornilvakayk *et al.* (1996).**

2.2.7. Statistical analysis: Data obtained were statistically analyzed using analysis of variance and comparing between groups were performed u-sing ANOVA test and Least Significant Difference (LSD) at $P < 0.05$ according to **Petrie and Waston (1999) and computerized using SPSS 11 (2002).**

3- RESULTS

In table 1, the mycotoxins were detected in feed and serum, samples collected from various farms of cattle and sheep at minufiya, El-Behira and Assiute governorates, in which animals were suffered from general low production, infertility and decreased growth rate. Aflatoxins were detected in 30% of the feed samples with the mean amount of 3.4 ± 0.1 ppm and ochratoxin in 20% with the mean level of 2.2 ± 0.2 ppm. However, it was revealed that the fusarium toxins were obtained at

lower rates (20% for T-2, 16% for zearalenone and 2% for fumonisin B1) with the mean amount of (36 ± 1.0 ppm, 22 ± 0.3 and 70 ± 0.00 ppm), respectively.

The sera of diseased cattle and sheep were examined for detection of mycotoxins and the results revealed that in case of cattle the most prevalent mycotoxins was aflatoxin B1 which detected in 40% of cases with the mean level of (5.4 ± 0.1), followed by ochratoxin A in 33% of cases with the mean level of (8.2 ± 0.1), T2 in 17% with the mean level of (26 ± 0.2) and zearalenone in (10%) with mean level of (19 ± 0.2). The lowest incidence was detected in cases of FB1 which obtained from 2% of cattle cases with the mean levels of (55 ± 0.6) (table, 2).

Also, the pattern of incidence of mycotoxins in sheep sera were nearly similar to those in cattle with the exception that the FB1 not detected at all in sheep(table 3).

However, the weight gain and growth rates were significantly decreased ($P<0.05$) due to administration of mycotoxins during the period of the experiment up to 6 month (Table, 4).

Moreover, the mycotoxins (aflatoxin, ochratoxins and zearalenone) reduced the LH and FSH in male rats which are related to regulations of normal productivity of animal (Table,5).

Data displayed in table (6) revealed a significant change in levels of testosterone of male rats which were administered mycotoxins in diet.

As shown in Tables (7&8) mycotoxins caused significant decrease ($P<0.05$) in T3, T4 and T3 / T4 ratio compared to controls.

DISCUSSION

The mycotoxins primarily enter the body systems of mammals by ingestion of contaminated food and feed stuffs and absorbed from alimentary tract. The principle point of entry of mycotoxins exposure for human and animals will be a direct consumption of contaminated food and feed **(D'Mello, 1997).**

The presences of mycotoxins in our study in feeds and sera of animals in Egypt was previously reviewed by **Hassan *et al.* (2002); Hassan (2003); Hassan *et al.* (2004) and Ragheb and Srour (2005),** who detected different mycotoxins in feeds and sera of animal including aflatoxin B1, zearalenone, T-2 and ochratoxin. They suggested that these toxins were responsible for the diseases of investigated animal cases and the rates of these toxins measures the severity of infection. The main effect of these toxins is the inhibition of protein synthesis throughout binding with DNA and RNA perhaps as a result of interference with nitrogen metabolism produced immunosuppresion and reduced antibody formation **(Zaghloul and Shehata, 1991; Hassan *et al.* 1997, 1998 and 2004).** Most of mycotoxins resulted in economic losses in animal wealth through their dangerous and carcinogenic effect in organs of animals particularly in liver, kidney and reduced productivity, reproductive insufficiency and male infertility**, (Wu *et al.*, 1991; Smith, 1997; Wang *et al.*, 2000 and Hassan *et al.*, 2004 and Ragheb and Srour, 2005).**

Therefore, the influence of mycotoxins on some hormones which are related to the regulation of normal productivity and fertility of male were investigated in present work. Similar to the obtained results of decreased weight gain, it was reported that the mycotoxins produced a variety of adverse health effects in farm animals, as inhibition of protein synthesis, reduction of feed intake which reflected in low weight gain **(Mocchegiani *et al.*, 1998; Hassan *et al.*, 2002; Hassan *et al.*, 2004 and 2008 and Ragheb and Srour, 2005).**

It is interesting to report here that the mycotoxins may be affected the semen quality which resulted increased incidence of abnormal spermatozoa in animal and birds administrated mycotoxins in diet (**Yang et al., 2007 a & b)**. Using of ANOVA test and comparison between groups that administrated mycotoxins resulted in detection a significant differences in levels of LH and FSH of male rats compared to controls. These results were previously reported by **Mitton *et al.* (1975); Smith (1982); Allen *et al.* (1983); Xie *et al.* (1991); England *et al.* (1998) and Hassan *et al.* (2004) and Ragheb and Srour (2005).**

The changes in levels of testosterone of male rats which were administered mycotoxins in diet could be attributed to low semen quality of breeders animal which affected by mycotoxins due to increased incidence of abnormal spermatozoa as a result of consumption of aflatoxin and zearalenone contaminated-diet (**Muthiah *et al.*, 1997and Yang et al., 2007 a & b)**. Also, the mycotoxins produced by several fungi in different food products or grains, cheese and meat, resulted in an drop in testosterone level by (66.6%) in male rats treated for 60 days **(Selmanoglu and Kockaya, 2004)**. The testis of treated rats with mycotoxins particularly patulin (a Penicillium and Aspergillus toxin) and zearalenone produced edema, fibrosis and local leydig cell hyperplasia in interstitial tissue and de-organization of semenifrous tubule epithelium and higher relative weight of seminal vesicle than those of control . These changes in testicular tissue resulted in insufficient of sperms production and reduced testosterone level lead to infertility of rats at different degrees and decrease the chance of normal reproductive activity **(Selmanoglu and Kockaya, 2004 and Yang et al., 2007 a & b)**..

The changes in thyroid functions hormones may be due to lymphoid cell infiltration and enlargement of interstitial tissue between follicle and degenerated colloid secretion in thyroid gland **(Selmanoglu and Kockaya, 2004).**

In the present work, the T4/T3 ratios showed lower results than the controls. This was detected in the hypo-function of thyroid gland, which was reflected in decreased feed intake, low body weigh gain and reduced productivity of animals ((**Fiorito *et al.*, 1991; Mocchogiani *et al.*, 1998 and Selmanoglu and Kockaya, 2004).** Also, many functional disturbances associated with hypothyroidism are due to a reduction in basal metabolic rate which resulted in decreased body weight gain without an associated change in appetite (**Botts *et al.*, 1991**). The hypothyroidism was reported to be responsible for abnormalities in reproduction of

breeding animal **(Wanda and Colin, 1998**). It could be results in reduction in testicular development and fertility of animals depending on the severity of the hormone deficiency **(Bell and Freeman, 1971).**

The public concern expressed about mycotoxins is not restricted to the effects of that mycotoxins contaminated food or feed on growth and health of animals and poultry but also about possible transmission of toxic residues in meat, milk, and eggs resulting in a potential hazard of human health **(Smith and Handerson, 1991).**

Conclusion

The results reported the significant influence of mycotoxins for some endocrine function of reproductive organs and thyroid gland which were reflected on the low productivity and high losses in animal wealth. The main source of these changes is attributed to the environmental pollution of food and feeds by fungi and their toxins. Therefore, every hygienic care must be undertaken during all steps of feed and food production and other factors related to the environment of animal to prevent such pollution. Hence the productivity of animal and human health become under control.

Table (1): Prevalence of mycotoxins in animal rations collected from farms in which animal suffered from loss of weight gain, low productivity and disturbance in fertility (ppm).

Mycotoxins	+ve samples	-ve samples	% of positive	Levels of mycotoxins (ppm)		
				Min	Max	Mean ± S.E.
Aflatoxin	15	35	30	1.5	1.5	3.4 ± 0.1
Ochratoxin A	10	40	20	1.0	3.2	2.2 ± 0.2
T2	15	40	20	2.0	50	36 ± 0.1
Zearalenone	8	42	16	10	30	22 ± 0.3
Fumonisin B1	1	49	2	70	70	70 ± 0.0

Table (2): Prevalence of mycotoxins in serum of diseased cattle.

Mycotoxins	+ve samples	-ve samples	% of positive	Levels of mycotoxins (ppm)		
				Min	Max	Mean ± S.E.
Aflatoxin B1	40	60	40	1.5	9.5	5.4 ± 0.1
Ochratoxin A	33	67	33	1.0	12.2	8.2 ± 0.1
T2	17	83	17	1.0	20	26 ± 0.2
Zearalenone	10	90	10	4	22	19 ± 0.2
Fumonisin B1	2	98	2	17	38	55 ± 0.6

Table (3): Prevalence of mycotoxins in serum of diseased sheep .

Mycotoxins	+ve samples	-ve samples	% of positive	Levels of mycotoxins (ppm)		
				Min	Max	Mean ± S.E.
Aflatoxin B1	35	65	35	2.5	11.5	8.6 ± 0.4
Ochratoxin A	25	75	25	3.0	14.1	10.5 ± 0.2
T2	4	96	4	0.8	17.0	12.1 ± 0.1
Zearalenone	8	92	8	2.2	19.0	8.9 ± 0.4
Fumonisin B1	0	100	0	0.0	0.0	0.0

Table (4): Influence of feeding a diet-containing mycotoxins (aflatoxin, ochratoxin and zearalenone) on weight gains (g) and relative weight ratio of male albino rats compared to controls (mean ± S.E.).

Treatment / Age (month)		Control (0.0)	Aflatoxin (0.5 ppm)	Ochratoxin (1.0 ppm)	Zearlaenone (2.5 ppm)	F-value
Weight gains (g)	1-2	1.04 ± 0.01^{A}	1.16 ± 0.02^{aB}	1.36 ± 0.018^{abC}	1.50 ± 0.027^{abc}	86.529#
	2-3	1.93 ± 0.04^{A}	1.78 ± 0.017^{aB}	1.43 ± 0.014^{abC}	1.93 ± 0.047^{bc}	50.799#
	3-4	1.43 ± 0.011^{A}	1.14 ± 0.015^{aB}	1.13 ± 0.022^{abC}	1.29 ± 0.045^{abc}	407.925#
	4-5	0.75 ± 0.024^{A}	0.04 ± 0.010^{aB}	0.02 ± 0.001^{aC}	0.51 ± 0.017^{abc}	517.167#
	5-6	0.57 ± 0.013^{A}	0.13 ± 0.011^{aB}	0.14 ± 0.013^{abC}	0.31 ± 0.017^{abc}	389.048#
Relative growth rates	1-2	46.03 ± 0.86^{A}	42.6 ± 1.67^{aB}	60.9 ± 1.24^{aC}	80.7 ± 1.31^{abc}	33.191#
	2-3	60.9 ± 1.05^{A}	52.2 ± 0.71^{aB}	41.4 ± 0.96^{abC}	59.5 ± 1.01^{bc}	89.339#
	3-4	4.04 ± 0.19^{A}	1.33 ± 0.07^{aB}	11.24 ± 0.52^{ab}	12.0 ± 0.25^{ab}	289.250#
	4-5	23.3 ± 0.46^{A}	1.32 ± 0.04^{aB}	0.63 ± 0.07^{ab}	14.5 ± 0.33^{ab}	447.945#
	5-6	0.79 ± 0.06^{A}	10.4 ± 0.71^{aB}	5.54 ± 0.37^{abC}	3.89 ± 0.19^{a}	92.997#

Significant at $P < 0.05$ using ANOVA test
Aa, Bb, Cc Significantly difference between two comparison groups in the same raw against capital litter at $P < 0.05$ using LSD.

Table (5): Influence of feeding a diet-containing mycotoxins (aflatoxin, ochratoxin and zearalenone) on serum leutinizing hormone levels (LH and FSH), (miu/ml) of male rats in comparison to controls values (mean ± S.E.).

Estimated Hormones	Treatment / Age (month)	Control (0.0)	Aflatoxin (0.5 ppm)	Ochratoxin (1.0 ppm)	Zearlaenone (2.5 ppm)	F-value
LH (miu/ml)	2	0.59 ± 0.02^{A}	0.59 ± 0.04^{B}	0.59 ± 0.04^{C}	0.39 ± 0.02^{abc}	7.278#
	3	0.69 ± 0.05^{A}	0.30 ± 0.01^{aB}	0.28 ± 0.02^{ab}	0.29 ± 0.02	38.210#
	4	0.90 ± 0.07^{A}	0.34 ± 0.02^{aB}	0.45 ± 0.04^{ab}	0.39 ± 0.02^{ab}	29.802#
	5	0.99 ± 0.05^{A}	0.19 ± 0.02^{aB}	0.15 ± 0.02^{ab}	0.20 ± 0.02^{ab}	128.403#
	6	0.91 ± 0.06^{A}	0.45 ± 0.05^{a}	0.60 ± 0.06^{aC}	0.55 ± 0.05^{ac}	10.761#
FSH (miu/ml)	2	6.27 ± 0.29^{A}	3.96 ± 0.50^{aB}	3.64 ± 0.32^{ab}	3.43 ± 0.31^{ab}	8.704#
	3	6.58 ± 0.49^{A}	4.02 ± 0.32^{aB}	3.22 ± 0.25^{ab}	3.23 ± 0.26^{ab}	6.452#
	4	6.06 ± 0.46^{A}	4.13 ± 0.19^{aB}	3.56 ± 0.30^{ab}	3.40 ± 0.32^{ab}	7.140#
	5	6.31 ± 0.46^{A}	4.67 ± 0.25^{aB}	3.55 ± 0.32^{ab}	3.47 ± 0.25^{ab}	6.511#
	6	6.91 ± 0.06^{A}	4.48 ± 0.26^{aB}	3.42 ± 0.29^{ab}	3.57 ± 0.25^{ab}	86.787#

Significant at $P < 0.05$ using ANOVA test
Aa, Bb, Cc Significantly difference between two comparison groups in the same raw against capital litter at $P < 0.05$ using LSD.

Table (6): Influence of feeding a diet-containing mycotoxins (aflatoxin, ochratoxin and zearalenone) on serum testosterone levels (ng/ml) of male rats compared to controls (mean ± S.E.).

Treatment / Age (month)	Control (0.0)	Aflatoxin (0.5 ppm)	Ochratoxin (1.0 ppm)	Zearlaenone (2.5 ppm)	F-value
4	7.46 ± 0.61^{A}	7.00 ± 0.64^{aB}	6.72 ± 0.55^{abC}	3.94 ± 0.31^{abc}	8.397#
4.5	15.16 ± 1.29^{A}	7.50 ± 0.64^{aB}	8.40 ± 0.77^{abC}	3.14 ± 0.39^{abc}	34.609#
5	13.98 ± 1.2^{A}	3.48 ± 0.46^{aB}	7.29 ± 0.79^{abC}	4.12 ± 0.55^{abc}	14.198#
5.5	15.12 ± 0.95^{A}	6.00 ± 0.64^{aB}	7.84 ± 0.46^{abC}	4.20 ± 0.44^{abc}	12.687#
6	12.22 ± 0.82^{A}	2.38 ± 0.46^{aB}	1.68 ± 0.23^{abC}	1.14 ± 0.18^{abc}	30.513#

Significant at $P < 0.05$ using ANOVA test
Aa, Bb, Cc Significant difference between two comparison groups in the same raw against capital litter at $P < 0.05$ using LSD.

Table (7): Influence of feeding a diet-containing mycotoxins (aflatoxin, ochratoxin and zearalenone) on serum triiodothyronine T3 (ng/ml) and thyroxine (T4) "ug/ml" of male albino rats compared to controls (mean ± S.E.).

	Treatment / Age (month)	Control (0.0)	Aflatoxin (0.5 ppm)	Ochratoxin (1.0 ppm)	Zearlaenone (2.5 ppm)	F-value
T3	2	224.0 ± 22.4^{A}	190.6 ± 22.5^{aB}	202.6 ± 11.4^{aC}	184.6 ± 6.52^{abc}	6.074#
	3	226.6 ± 19.3^{A}	184.6 ± 5.9^{aB}	189.4 ± 7.8^{aC}	164.6 ± 5.9^{abc}	7.048#
	4	219.6 ± 14.5^{A}	179.6 ± 6.52^{aB}	181.4 ± 7.87^{aC}	160.8 ± 5.82^{acb}	7.748#
	5	214.4 ± 4.89^{A}	140.8 ± 5.8^{aB}	143.6 ± 4.53^{aC}	136.6 ± 5.94^{abc}	9.584#
	6	231.4 ± 4.89^{A}	154.6 ± 6.52^{aB}	159.6 ± 6.52^{aC}	139.6 ± 8.87^{abc}	8.065#
T4	2	4.84 ± 0.27^{A}	3.84 ± 0.31^{a}	3.94 ± 0.30^{a}	3.64 ± 0.28^{a}	6.171#
	3	4.96 ± 0.43^{A}	4.04 ± 0.31^{a}	3.82 ± 0.27^{a}	4.06 ± 0.96^{a}	5.247#
	4	4.76 ± 0.50^{A}	4.48 ± 0.46^{a}	4.46 ± 0.49^{a}	4.36 ± 0.44^{a}	4.085#
	5	4.84 ± 0.56^{A}	4.14 ± 0.52^{a}	4.18 ± 0.46^{a}	4.22 ± 0.45^{a}	5.034#
	6	5.16 ± 0.50^{A}	4.34 ± 0.39^{a}	4.24 ± 0.31^{a}	4.36 ± 0.43^{a}	6.022#

Significant at $P < 0.05$ using ANOVA test
Aa, Bb, Cc Significant difference between two comparison groups in the same raw against capital litter at $P < 0.05$ using LSD.

Table (8) Influence of feeding a diet-containing mycotoxins (aflatoxin, ochratoxin and zearalenone) on serum T4/T3 ratio of male albino rats compared to controls (mean ± S.E.).

Treatment / Age (month)	Control (0.0)	Aflatoxin (0.5 ppm)	Ochratoxin (1.0 ppm)	Zearlaenone (2.5 ppm)	F-value
2	2.16 ± 0.26	2.01 ± 0.14	1.94 ± 0.19	1.97 ± 1.02	1.121
3	2.19 ± 0.30^{A}	2.19 ± 0.18^{B}	2.01 ± 0.21^{abC}	2.47 ± 1.74^{abc}	5.014#
4	2.16 ± 0.29^{A}	2.49 ± 0.16^{a}	2.45 ± 0.17^{a}	2.71 ± 2.11^{a}	6.123#
5	2.26 ± 0.25	2.94 ± 0.23^{aB}	2.91 ± 0.14^{a}C	3.08 ± 1.83^{a}bc	6.874#
6	2.23 ± 0.17^{A}	2.81 ± 0.26^{aB}	2.65 ± 0.11^{aC}	3.12 ± 2.08^{abc}	8.874#

Significant at P < 0.05 using ANOVA test
Aa, Bb, Cc Significantly difference between two comparison groups in the same raw against capital litter ay P < 0.05 using LSD.

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