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Original Research

Individual and synergic influence of aflatoxin B1 and ochratoxin A on the productive, hematological and genetic parameters of the male broiler breeds

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ABSTRACT:

This experiment was designed to study the individual and synergic effect of fungal toxins (aflatoxin B1 and ochratoxin A) on the productive, hematological and genetic parameters of the male broiler breeds ISA (Institute Selection Animal). A factorial experimental setup (2×2) was maintained where, aflatoxin B1 were at the concentration of 0 and 3µg/g diet and ochratoxin A were at the concentration of 0 and 5µg/g diet. The birds were reared from 1 day to 3 weeks old. Results showed that the individual effect of fungal toxins aflatoxin B1 and ochratoxin A, have significantly reduced (P≤0.05) the body weight, the blood serum traits (proteins, albumin and cholesterol), and significantly (P≤0.05) increased mortality and relative weight of internal organs (liver, kidney, spleen, pancreas, gizzard, heart and bursa). It also reduced alanine amino transferase enzymes (ALT) with an increase of uric acid concentration. Fungal toxins had a significant effect (P≤0.05) on cell division and different types of chromosomal aberrations. Fungal toxins synergic influence was more harmful on the traits studied.

Keywords:

Synergic, Aflatoxin B1, Ochratoxin A, Hematological, Male broiler breeds.

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INTRODUCTION

Although there are more than 100 varieties of fungus grow on stored feed which produce almost toxic substances. Twenty of these toxins were associated with the occurrence of humans diseases. Fungal toxins were a fungi metabolic byproduct which cause abnormal biological changes that are harmful to humans, animals, plants or microorganisms (Zain, 2011).

Despite large number of fungal toxins that infect poultry. The most dangerous poisons are aflatoxin and ochratoxin, Aflatoxin is produced from *Aspergillus flavus* and *Penicillium puberulum* fungi (Ahmed and Papenbrock, 2015). The toxin aflatoxin B1 is the most toxic because it combines with the nucleus and mitochondria (Jun *et al.,* 2015), causing liver cancer tumors, reinforcement of effective genetic mutations hormonal changes and increased susceptibility of various diseases (Williams *et al.,* 2004). Aflatoxin B1 effects on poultry, increases the mortality (Rawal *et al.,* 2010). The effective rate of aflatoxin B1 in poultry diet is 1.5ppm (Magnoli *et al.,* 2011), ochratoxin type A, a secondary metabolite of *Penicillium viridicatum* or *Aspergillus ochraceus* (Wang *et al.,* 2016), causing damage to the kidneys and livers and affect the effectiveness of reproduction in birds (Abedi and Talebi, 2015). The effective rate of ochratoxin A toxin in poultry diet is 2.0ppm (Patil *et al.,* 2014). In this study we evaluate the effect of these two toxins and their effect on the reproduction, hematological and genetic performance of the ISA (Institute Selection Animal) male broiler breeder.

MATERIALS AND METHODS

Animals and dietary treatments

This study was conducted in the poultry fields of the department of animal production at the Ministry of Science and Technology from March 2017 to May 2017. Rearing period lasted for fifteen days, the chemical and hematological analysis were completed thereafter. 120 male broiler breeds were used in this experiment and were distributed to four treatments, each treatment containing 30 chicks with three replicates for each treatment. The treatments first group fed on the diet and not the toxin (control diet) and the second group was fed on the diet supplemented with aflatoxin B1 (3µg/g), which was isolated from *Aspergillus flavus* (Table 1). The third treatment was fed on the diet supplemented with ochratoxin A 5µg/g isolated from *Aspergillus ochraceus*, and the fourth treatment was fed on the diet supplemented with the three micrograms of aflatoxin B1 and 5 micrograms of ochratoxin A per gram of diet. The ochratoxin the Isolation and addition of aflatoxin B1 in the diets of male broiler breeds were done by the method given by Sadiq *et al.* (2003), while the ochratoxin A by the method proposed by Huff *et al*. (1988).

Sampling and measurements

The chicks were weekly weighed from one to three week and at the end of 3 weeks, blood sample was taken from three birds of each replicate by piercing the heart. Blood samples were divided into two parts. The first part is used to estimate the hemoglobin, count of red blood cells and Packed Cell Volume (PCV%), the

Table 1. Basal diet and their composition*

S. No	Items	(%)
1	Yellow corn, ground	54.9
2	Soybean meal (44% CP)	27.5
3	Protein concentrates	12.5
4	Plant oil	3.5
5	Di-calcium phosphate	0.8
6	Limestone	0.4
7	Sodium chloride	0.3
8	DL- Methionine	0.1
9	Total	100
	Calculated analysis (%)	
1	Metabolism energy (kilo calorie per kg. Diet)	3109
2	Crude protein (%)	23.07
3	Calcium (%)	1.1
4	Available phosphorus (%)	0.5
5	Lysine (%)	1.33
6	Methionine (%)	0.45
7	Methionine +Cystine (%)	0.92

* Al-Ghadhir company for animals and poultry diets

Table 2. Individual and synergic influence of aflatoxin B1 and ochratoxin A on the body weight, mortality and feed in ISA male broiler breeds

S. No	Aflatoxin B1 µg/g diet	Ochratoxin A µg/g diet	Body weight			Mortality	Feed conversion
			1 week	2 week	3 week		
1	0	0	2.15±117.6	5.45±235.8^{a}	9.2±456.0^{a}	0.003±5.0^{a}	0.01±1.60
2	3.0	0	2.4±110.0	6.12±174.6^{b}	11.3±342.8^{b}	0.002±7.5^{b}	0.04±1.98
3	0	5.0	2.2±112.0	4.9±207.0^{b}	8.9±364.8^{b}	0.002± 7.5^{b}	0.06±1.9^{b}
4	3.0	5.0	2.1±109.0^{a}	6.0±167.0^{c}	11.5±230.0^{c*}	0.001±10.0^{c}	0.5±2.3^{b}

Different alphabets vertically indicated the existence of significant differences at 0.05 between the averages. Significant interaction between aflatoxin B1 and ochratoxin A at the possibility of 0.01.

second part was centrifuged by a speed of 3000rpm, to estimated total protein, albumin, cholesterol and uric acid and aspartate aminotransferase, using appropriate solutions prepared at Ministry of Science and Technology Laboratories. The birds were slaughtered and their internal organs were weighed (liver, kidney, spleen, pancreas, gizzard, heart and bursa) according to Al-Fayadh and Naji (2012). Chromosomes were prepared to measure the division coefficient and chromosomal aberrations (Allen *et al.,* 1977).

Statistical analysis

This research used one way complete random sampling (SAS, 2001). Data were analyzed by two way analysis of variance (ANOVA). If the treatment significantly affected the chicken, Duncan multiple range were applied (Duncan, 1955). Differences among treatments were considered as significant at (P≤0.05).

RESULTS AND DISCUSSION

Table 2 shows that the fungal toxins of aflatoxin B1 and ochratoxin A caused a significant decrease (P≤0.05) in the body weight at the age of 2 and 3 weeks. A significant influence (P≤0.05) was noted at the presence of these two types of toxins. It has led to a greater reduction in the body weight when compared with the use of each of them separately. The overall interaction was significant**,** aflatoxin B1 has reduced the body weight by 25%, while ochratoxin A reduced by 20%, however, when using two types of toxins, the decline reached 50%**.** It is also noted from Table 2. that the effects of aflatoxin B1 and ochratoxin A caused a significant increase (P≤0.05) in the mortality, feed conversion and has significantly decreased (P≤0.05) with both the types of fungal toxins.

Low body weight, deterioration of food conversion and increased mortality may be due to the low efficiency of digestive enzymes (Yunus *et al.,* 2011). as well as the disruption of the process of the transfer of fatty substances from the liver to members of the body (Smith *et al.,* 1993). In addition to an interaction between fungal toxins and environmental stresses (Wyatt *et al.,* 1975), disease attacks such as Coccidiosis, Newcastle and Merck (Wyatt and Hamilton, 1975), the low representation of protein and fat absorption would also a reason for the defect (Al-Hilali *et al.,* 2002).

Table 3 shows that the most affected part due to the fungal toxin is liver because it is a treatment center for toxins and the nearby compounds absorbed from the

Table 3. Individual and synergic influence of aflatoxin B1 and ochratoxin A on the relative weight of internal organs in ISA male Broiler breeds

S. No	Aflatoxin B1 µg/g diet	Ochratoxin A µg/g diet	Liver (%)	Kidney (%)	Spleen (%)	Pancreas (%)	Gizzard (%)	Heart (%)	Bursa (%)
1	0	0	0.10±3.70^{c}	0.01±0.60^{c}	0.01±0.10^{d}	0.05±0.40^{c}	0.02±2.50^{b}	0.01±0.63^{b}	0.03±0.29^{a}
2	3.0	0	0.14±4.50^{b}	0.02±0.89^{b}	0.01±0.15^{c}	0.03±0.50^{b}	0.03±3.40^{a}	0.02±0.74^{b}	0.02±0.36^{a}
3	0	5.0	0.15±4.10^{b}	0.04±0.86^{b}	0.03±0.20^{b}	0.20±0.47^{b}	0.04±2.60^{b}	0.01±0.72^{b}	0.03±0.35^{a}
4	3.0	5.0	0.19±5.30^{a}	0.03±1.36^{a*}	0.03±0.25^{a}	0.02±0.63^{a*}	9.08±3.70^{a}	0.03±0.80^{a}	0.04±0.42^{a*}

Different alphabets vertically indicated the existence of significant differences at 0.05 between the averages. Significant interaction between aflatoxin B1 and ochratoxin A at the possibility of 0.01.

Table 4. Individual and synergic influence of aflatoxin B1 and ochratoxin A on the hematological parameters in ISA male broiler breeds

S. No	Aflatoxin B1 µg/g diet	Ochratoxin A µg/g diet	RBC (10^8 /mm^3)	PCV (%)	Hb (g/dL)
1	0	0	0.5±4.50[a]	0.4±34.60[a]	0.2± 11.50[a]
2	3.0	0	0.5±4.00[b]	0.6±32.20[b]	0.4± 10.73[a]
3	0	5.0	0.8±4.20[b]	0.5±32.30[b]	0.4± 10.76[a]
4	3.0	5.0	0.9±3.95[b*]	0.4±32.10[b]	0.6± 9.00[b]

Different alphabets vertically indicated the existence of significant differences at 0.05 between the averages. Significant interaction between aflatoxin B1 and ochratoxin A at the possibility of 0.01.

Table 5. Individual and synergic influence of aflatoxin B1 and ochratoxin A on the biochemistry of blood serum of ISA male broiler breeds

S. No	Aflatoxin B1 µg/g diet	Ochratoxin A µg/g diet	Total protein g/dL	Albumin g/dL	Cholesterol mg/dL	Uric acid mg/dL	(ALT) mg/dL
1	0	0	0.1±2.60[a]	0.03±1.17[a]	8.0±152.00[a]	5.0±127.00[a]	0.40±7.40[b]
2	3.0	0	0.2±1.02[d]	0.01±0.42[d]	11.0±53.00[b]	3.5±80.00[c]	0.40±5.30[b]
3	0	5.0	0.1±1.78[b]	0.04±0.76[b]	7.5±90.00[c]	3.0±124.00[a]	40. ±15.68[a]
4	3.0	5.0	0.3±1.33[c]	0.05±0.57[c]	11.0±69.00[c]	6.0±106.00[b]	0.40±12.12[a]

Different alphabets vertically indicated the existence of significant differences at 0.05 between the averages. Significant interaction between aflatoxin B1 and ochratoxin A at the possibility of (0.01).

intestines, which leads to a decrease in the efficiency of the transmission of fatty substances from the liver to the body (Smith *et al.,* 1993). Also Table 3 shows that both aflatoxin B1 and ochratoxin A significantly increased (P≤0.05) in the relative weight of the kidney and gizzard, this increase was significant (P≤0.05) in the case of the combined effect of both toxins. The relative weight of spleen and pancreas were significantly (P≤0.05) increased when using individual and synergic of toxins. Bursa was significantly altered (P≤0.05) in the treatments containing toxins compared to the control treatment. The change in most of the internal organs was due to the presence of aflatoxin B1 and ochratoxin A and their presence together may be due to a disorder of digestion and dysfunction of digestion enzymes (trypsin, amylase and lipase) (Al-Hilali *et al.,* 2002)

Table 4 indicates that the fungal toxins (aflatoxin B1 and ochratoxin A) caused a significant decrease (P≤0.05) in the number of red blood cells and PCV, whereas the interaction between fungal toxins decreased significantly (P≤0.05) in the hemoglobin of broiler blood. In general, the studied parameters of the blood have changed significantly. The significant decrease in blood characteristics may be due to the impaired intestinal susceptibility to iron absorption (Al-Daraji *et al.,* 2005a), reducing the number of red blood cells and thus hemoglobin (Al-Daraji *et al.,* 2006)

Table 5 indicated that the toxins were more effective on albumin, protein, cholesterol and uric acid and transport amino group enzymes. This effect may be

Table 6. Individual and synergic influence of aflatoxin B1 and ochratoxin A on the division coefficient and chromosomal aberration of ISA male broiler breeds

S. No	Aflatoxin B1 µg/g diet	Ochratoxin A µg/g diet	Division coefficient (dividing cell number/1000 divided cells)	Chromatid breakage	Chromosome breakage	Telocentic chromosomes	Non centric chromosomes
1	0	0	0.1710.04 [a]	0.2[d]	0.5[c]	0.1[c]	0.9[c]
2	3.0	0	0.50±5.70[b]	8.3[c]	2.4[b]	14.6[b]	5.2[b]
3	0	5.0	0.75±4.00[b]	6.8[b]	10.6[b]	3.9[b]	5.1[b]
4	3.0	5.0	0.35±5.30[b]	14.9[a]	18.0[a*]	22.0[a*]	8.2[a*]

Different alphabets vertically indicated the existence of significant differences at 0.05 between the averages. Significant interaction between aflatoxin B1 and ochratoxin A at the possibility of 0.01.

a result of the inhibition of RNA and DNA synthesis resulting in the protein degradation, increased uric disease and low cholesterol (Al-Daraji *et al.,* 2005b).

Table 6 shows a significant decrease (P≤0.05) in the chromosome division coefficient of birds of the experiment fed on toxin-contaminated diets compared to the control treatment with a mean of 10.04, 5.7, 4.0 and 5.3 for control and treatment containing aflatoxin B1 and ochratoxin A respectively**.** This indicates that the presence of fungal toxins leads to a decrease in the chromosomal division coefficient, also observed different types of chromosomal anomalies which are observed and this chromosomal abnormality has been increased in the food-contaminated treatments of fungal toxins**.** It is clear that the presence of fungal toxins in the bush affect the production performance, phylogenetic, cellular and genetics and increase this effect on polluters together**.**

CONCLUSION

It was noted that the individual effect of aflatoxin B1 and ochratoxin A isolates reduced the body weight, serum proteins, albumin and cholesterol, increased mortality and internal organs weight (heart, liver, kidneys, and gizzard), reduced the effectiveness of phosphatase enzymes and the amino acid transport enzymes with increased uric acid concentrations. The cellular effects of aflatoxin B1 and ochratoxin A have a significant effect on cellular division and different types of chromosomal aberration. The combined effect of fungal toxins have a significant impact on birds health.

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