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EFFECT OF BREAD YEAST AND ANTI-MYCOTOXIN, BENTONITE ON THE PHYSIOLOGICAL TRAITS OF LAYING HENS SUBJECTED TO AFLATOXIN

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Abstract

The study was conducted at a raising poultry hall in Ameriya Fallujah. The hall divided into 51 cages (1×2m) to investigate the interactive effect of bread yeast with the anti-mycotoxin, Bentonite, on limiting the mycotoxin influence on the physiological blood traits. The study included 75 laying hens, type (Luhman Brown) aged 38 weeks. The experiment consisted of 5 treatments replicated three times and 5 hens were included in each replicate. The first treatment was the control, with no any addition, while 3 mg aflatoxin/kg was added to the second, third, fourth, and fifth treatments. To the third treatment, 3g Bentonite were added and to the fourth treatment, 5g bread yeast was added, whereas 3 g Bentonite and 5g bread yeast was added to the fifth treatment. Results referred that both the bread yeast and Bentonite led to the body stability where the traits: size of the compacted blood cells, red blood cell, hemoglobin, white blood cells, and the ratio of white lymphocytes to heterogeneous cells as well as the blood biochemical traits included cholesterol, triglycerides, high density lipoproteins (HDL), and low density lipoproteins (LDL), glucose, protein, and albumin were not affected by the first treatment recording normal values compared to the second treatment. The globulin was not affected by the experimental treatments. Aflatoxin without additive had an effective role in the second treatment resulted in significant variances between it and other treatments contained additive nutrients. While the other experiment treatments were significantly superior in the compacted blood cells, red blood cell, and the ratio of white lymphocytes to heterogeneous cells hemoglobin, the second treatment was significantly superior in the white blood cells. A significant superiority was also observed in the fat picture of the second treatment compared to the other treatment, in contrary, for the chemical blood traits, the experimental treatments were significantly superior to the second treatment.

Key words : Aflatoxin, adsorbent, laying hen.

Introduction

Mycotoxins are one of the most important problems that the poultry industry sector in Iraq suffers from for their prevalence and the role in causing several metabolic and physiological problems; furthermore, getting rid of mycotoxins and discarding them out the poultry's body is a complicated matter (Gul *et al.,* 2016). Aflatoxin leads to a physiological disorder in poultry including a significant decrease in egg production, a decrease in the weight of the growing chicks and high percentage of perished birds, depending on the concentration of the toxins in the feed (Ru Jia *et al.,* 2016). These symptoms are accompanied by a significant disorder in the liver enzyme activity and some biochemical characteristics including the total proteins, cholesterol, triglycerides, high density lipoproteins (HDL), and low density lipoproteins (LDL) in addition to some physiological blood characteristics, such as white blood cells, lymphocytes to heterogeneous cells ratio and the size of the compacted red blood cells in case of the direct effect (Aljugaifi, 2015). Studies have trended to effective methods to minimize the mycotoxin harms in the poultry's body relying on adding some materials reducing the toxin activity or linking to the toxins discarding them out of the body (Kazem and Mahdi, 2015). Anti-toxins, including Bentonite, were also used which adsorb to the toxins or inhibit their action (Yenice

et al., 2015) and do not allow the body cells to absorb them. There are two types of aflatoxin infections, first that sharp one where the poultry bird is subjected to high levels of toxins within a short time causing a major harm that may end with death, second in which the poultry bird is undergone to low levels of mycotoxins for a long time that significantly affects the body cells ending with the bird mortality accompanying damages in body organs especially the liver, liver ducts and intestinal villi (Prasai *et al.,* 2018). So the study was conducted.

Having the previous research reviewed and using the mycotoxin concentration 3mg.kg feed^{-1}, the study was conducted to investigate the physiological performance of 5 laying hens (Lohman) aged 38 weeks for 10 weeks.

Materials and Methods

Mycotoxin

The isolation taken from the fungus *Aspergillus flavus* was used that was supplied by the Unit of Shared Diseases / College of Veterinary Medicine/ University of Baghdad and the Ministry of Science and Technology/ Branch of Food Safety. The method of Shotwell et al (1966) modified by West *et al.*, (1973) and AL-Warshan (2006) was followed using rice as a media for producing aflatoxin B1. The aflatoxin levels in the sample extracts were measured using the Enzyme-Linked Immuno Sorbent (ELISA). The extracts were prepared by grounding 5g of rice then, 25 ml of methanol 70% was added with stirring for three minutes. The extract of each sample was filtered with filter paper type Whatman No.1. The aflatoxin was calculated according to the procedure recommended by the company that supplied the aflatoxin kit.

The field experiment

The study experiment was conducted at halls of raising poultry (sized 60 x 12 m) in Ameriya Fallujah province/ Anbar govern. The hall contained 15 cages of 1×2 m in size. The lighting duration was 16 hours light + 8 hours of darkness. The hens were fed on feed containing 2700 calories/ kg of metabolizable energy and 15.5 % protein according to the nourishment system of 125 g/ hen using 5 kg- hanging cylindrical feeders. Water was provided throughout the day using inverted founts with a capacity of 5 liters. The study involved 75 laying hens aged 38 weeks distributed among 5 treatments replicated three times where each experimental unit consisted of 5 hens. The five treatments included:

1. First treatment: Control (with nothing added)
2. Second treatment: Adding 3mg aflatoxin/kg
3. Third treatment: adding 3mg aflatoxin/kg + 3g Bentonite/ kg
4. Fourth treatment: adding 3mg aflatoxin/kg + 5g bred yeast/ kg.
5. Fifth treatment: adding 3mg aflatoxin/ kg + 3g Bentonite/ kg + 5g bred yeast/ kg.

Medical syringe of 5 cc was used for drawing blood samples from the wing ulnar vein and collected in two test tubes first was free of the anti- clotting EDTA for performing the biochemical blood tests including, cholesterol, triglycerides, total protein, albumin, globulin, high-density lipoproteins, low-density lipoproteins, and glucose while the second test tube contained anti - clotting EDTA to be used for the full blood picture tests, including the size of the compacted blood cells, hemoglobin, white blood cells, and the ratio of white lymphocytes to heterogeneous cells. The study was carried out according to the completely randomized design (CRD) and the data were collected and analyzed using version 9.1 of the software SAS (SAS 2004). The significance of differences among means was tested relying upon Duncan's Multiple Range Test at the two significance levels 0.05 and 0.01.

Results and Discussion

Table 1 shows the significant superiority of the treatments T_5, T_4, T_3, and T_1 compared to the treatment T_2 in the traits compacted blood cells, hemoglobin, and red blood cells. On the other hand, the treatment T_2 was superior to the other treatments in the number the white blood cells and the ratio of lymphocytes to heterogeneous cells. The reduction in the number of the red blood cells of T_2 may be due to the toxin effect of aflatoxin on the intestinal wall that reduced the iron absorbed through the intestine that reduced the hemoglobin and red blood cells, or due to the effect of aflatoxin on the hematopoietic cells in the bone marrow that consequently reflects negatively on all the former indicators (Aljugaifi, 2015). All treatment used in the experiment enhanced the number of the red blood cells due to their role in preventing the negative effect of aflatoxin where the yeast played an important role in reducing the potential effort on the hen body caused by aflatoxin that may be due to the bread yeast content of nutrients including the polysaccharide in the yeast wall reducing the aflatoxin effect. An increase in the number of the white blood cells as well as the ratio of ratio of lymphocytes to heterogeneous cells were observed in table 1 as a results of T_2, which may be due to subjecting the hens for a long- time stress of aflatoxin that resulted in the T_2 superiority (AL-Warshan, 2006).

GUL *et al.*, (2016) referred to the role of Bentonite in decreasing the aflatoxin activity through adsorbing the

Table 1: Effect of experiment coefficients on cellular blood traits.

Capacity	Transactions					Average quality	SEM*	Level of significance
	T_1	T_2	T_3	T_4	T_5			
Size blood call Stacked	34.6a	28.6B	33.3a	34.6a	32.3a	32.7	1.87	0.01
Hemoglobin	10.5a	8.56B	10.2a	10.5a	6.73Ab	9.90	0.651	0.05
White blood cells	4900b	10333A	5066b	4700b	5366b	6073	733	0.01
Red blood cells	2.9a	2.20B	3.1a	3.11a	3.09a	2.88	0.512	0.05
Proportion heterogeneous lymphoma	1.79b	6.97A	2.08b	1.82b	1.48b	2.83	0.738	0.01

a, b, c: different characters within a row indicate significant differences between the coefficients at a significant level ($P \leq 0.01$) and ($P \leq 0.05$). Transactions: T_1: control, T_2: 3 milligram of aflatoxin poison, T_3: Yeast 5 g + 3 milligram of aflatoxin poison, T_4: Bentonite 3 g +3 milligram of aflatoxin poison, T_5: Yeast 5 g + Bentonite 3 g + 3 milligram of aflatoxin.

Table 2: Effect of experimental coefficients in the image of fat in the plasma of white chicken.

Capacity	Transactions					Average quality	SEM*	Level of significance
	T_1	T_2	T_3	T_4	T_5			
Cholesterol	271b	300a	219dc	243c	210d	248.6	20.0	0.05
Triglyceride	750b	1012a	671bc	655bc	599c	737.4	68.1	0.05
HDL	22.3bc	28.2a	23b	18.6c	19c	22.22	2.97	0.05
VLDL	248.8b	271.8a	196cd	224.4c	191d	226.38	13.7	0.01
LDL	150b	202.4a	134.2bc	131c	119.8c	147.48	17.03	0.01

a, b, c: different characters within a row indicate significant differences between the coefficients at a significant level ($P \leq 0.01$) and ($P \leq 0.05$). Transactions: T_1: control, T_2: 3 milligram of aflatoxin poison, T_3: Yeast 5 g + 3 milligram of aflatoxin poison, T_4: Bentonite 3 g +3 milligram of aflatoxin poison, T_5: Yeast 5 g + Bentonite 3 g + 3 milligram of aflatoxin poison.

Table 3: The effect of experiment parameters on the biochemical blood traits of blood plasma in laying hens.

Capacity	Transactions					Average quality	SEM*	Level of significance
	T_1	T_2	T_3	T_4	T_5			
Glucose	247a	145b	220a	206a	206a	3.73	26.7	0.05
Protein	4.76a	3.23b	5.46a	5.20a	5.60a	2.146	0.596	0.05
Albumin	3.00a	1.30b	3.00a	3.43a	3.53a	1.584	0.570	0.05
Globulin	1.76	1.93	2.46	1.77	2.07	3.73	0.362	N.S.**

**N .S. means that there are no significant differences between the coefficients.a, b, c: different characters within a row indicate significant differences between the coefficients at a significant level ($P \leq 0.01$) and ($P \leq 0.05$). Transactions: T_1: control, T_2: 3 milligram of aflatoxin poison, T_3: Yeast 5 g + 3 milligram of aflatoxin poison, T_4: Bentonite 3 g +3 milligram of aflatoxin poison, T_5: Yeast 5 g + Bentonite 3 g + 3 milligram of aflatoxin poison.

toxin and preventing it from being absorbed through the body cells, instead it would be discarded out the body through the feces. It was noticed in table 1 where the Bentonite effect was significant when it was mixed the yeast, i.e. the interactional role between them affected the studied traits significantly.

Table 2 showed significant superiority of T_2 to the other treatments in the cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, and very low density lipoproteins. Mycotoxins are stressful and the stress in birds causes cortisone to be secreted by the adrenal cortex, which has negative feedback on thyroid activity, causing an increase in cholesterol and triglycerides (Aljugaifi, 2015). This result was inconsistent with what was reported by Shabani *et al.*, (2010) that aflatoxin decreases the cholesterol and triglycerides percent in blood. This difference may be due to the percentage of toxins in the fodder or to the measurement methods in the different studies. Results also illustrated the ability of the bread yeast to decrease the cholesterol level, especially bad cholesterol LDL as well as decrease the triglycerides. The superiority of the biological treatments may be due to the role of the biological additives in decreasing the aflatoxin effect through adsorbing the toxins inside the digestive duct and prevent their absorption through the intestines; moreover, yeast inhibits the cholesterol synthesis through linking the cholesterol to the yeast cell walls (AL-Warshan, 2006).

Table 3 demonstrates the superiority of the experiment treatments T_5, T_4, T_3, and T_1 compared to T_2 in the biochemical blood traits including the glucose, protein, and albumin, while no significant difference was observed among treatments in globulin. Al Hadithi (2005) referred that aflatoxin B1 decreased the blood glucose

may be due to that toxin caused an increase in the metabolism processes and physiological stress in birds since these processes are an energy required and as glucose is the best and easier compound used for releasing energy, it was consumed and reduced in the blood. Prasai *et al*., (2018) reported that blood sugar was decreased when the birds found the toxin contaminated feed unpalatable as it is known that low feed consumption results in low blood glucose besides, the aflatoxin action on receptors present in the bird intestines reduces the absorption of the nutrients that are necessary for supplying the body cells (Jia *et al.,* 2016). The roles of Bentonite and yeast, as well as the interaction between them, resulted in aflatoxin unaffected protein maintaining the normal aflatoxin concentration in the birds body in addition to that Bentonite may contain some compounds or elements showing this result because of the different clay metals existed in the chemical structure (AL-Warshan, 2006). Yeast also contains some metals playing a role in preventing the cell against oxidation by aflatoxins. Adding bread yeast to feeds contaminated by mycotoxins increased the blood albumin as a result of containing antioxidants, mycotoxin adsorbents, and nutrients such as proteins and amino acids in addition to the polysaccharides in the yeast wall and as a result of the microorganism activity that heals the digestive system and reduces stress the birds subjecting to, which in total may increase the activity of body systems especially the liver and increase the protein production.

Conclusions

It is observed from the experiment results that yeast and Bentonite, as well as the interaction between them, have a role in reducing the aflatoxin effect on the blood traits in general that encourages introducing them into feed content to limit the mycotoxin effect.

References

Aljugaifi, W.I.K. (2015). Effect of the addition of different types of anti-mycotoxins on contaminated ration with aflatoxin B1 in productive performance and some physiological traits in broiler breeders. Thesis. Al- Anbar University College of Agriculture.

AL-Warshan, S.H.S. (2006). A comparison Between Probiotics with Two Adsorbents In Decreasing the Adverse Effect of Aflatoxin B_1 on Enhancement Broiler Performance. Thesis. the university of Baghdad In partial fulfillment.

Duncan, D.B. (1955). Multiple Rang and Multiple F-test. *Biometrics*, **11:** 4-42.

Gul, H., S. Khan, Z. Shah, S. Ahmad, M. Israr and M. Hussain (2017). Effects of local sodium bentonite as aflatoxin binder and its effects on production performance of laying hens. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, **23(1):** 31-37.

Kazem Karimi and Mahdi Zhandi (2015). The effect of β-mannanase and β-glucanase on small intestine morphology in male broilers fed diets containing various levels of metabolizable energy, *Journal of Applied Animal Research*, **43:3:** 324-329, DOI: 10.1080/09712119.2014.978770.

Jia, R., Q. Ma, Y. Fan, C. Ji, J. Zhang, T. Liu and L. Zhao (2016). The toxic effects of combined aflatoxins and zearalenone in naturally contaminated diets on laying performance, egg quality and mycotoxins residues in eggs of layers and the protective effect of Bacillus subtilis biodegradation product. *Food and Chemical Toxicology*, **90:** 142-150.

Jia, R., Q. Ma, Y. Fan, C. Ji, J. Zhang, T. Liu and L. Zhao (2016). The toxic effects of combined aflatoxins and zearalenone in naturally contaminated diets on laying performance, egg quality and mycotoxins residues in eggs of layers and the protective effect of *Bacillus subtilis* biodegradation product. *Food and Chemical Toxicology*.

Oadi Najim al Hadithi (2005). Serological and detoxification studies by use physical and biological agent on T2_toxin.ph.D. Thesis- Agriculture Science-University Of Baghdad.

Prasai, T.P., K.B. Walsh, D.J. Midmore and S.P. Bhattarai (2018). Effect of biochar, zeolite and bentonite feed supplements on egg yield and excreta attributes. *Animal Production Science*, **58(9):** 1632-1641.

SAS, (2004). SAS User's guide: statistical system, Inc. Cary, NC. USA.

Shabani, A., B. Dastar, M. Khomeiri, B. Shabanpour and S. Hassani (2010). Response of Broiler Chickens to Different Levels of Nanozeolite During Experimental Aflatoxicosis. *Journal of Biological Sciences*, **10(4):** 362-367.

Shotwell, O.L., C.W. Hesseltine, R.D. Stubblefield and W.G. Sorenson (1966). Production of aflatoxin on rice. *Appl. Microbiol*., **14(3):** 425 – 428.

West, S., R.D. Wyatt and P.B. Hamilton (1973). Improved yield of aflatoxin by incremental increases of temperature. *Appl. Microbio.*, **25:** 1018 – 1019.

Yenice, E., C. Mizrak, N. Ceylan, T. Yildiz, M. Gültekin and Z. Atik (2015). Effects of dietary sodium bentonite and mannan oligosaccharide supplementation on performance, egg quality, blood and digestion characteristics of laying hens fed aflatoxin contaminated diet. Kafkas Univ Vet Fak Derg, **2:** 211-218.