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Some dietary additives reducing effect of Aflatoxin B1 in feeds and their impact on the physiological performance of broiler

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Abstract :

The objective of this study was to study effect of adding different additives (Zeolite, *Saccharomyces cerevisiae*, *Milk thistle*) to diets for enhance physiological performance of broiler fed diets contaminated with aflatoxin B_1 2 mg/kg feed. Two Hundred-seventy chicks, one-day-old (Unsexed), were distributed in a completely randomized design that were divided to nine treatments with three replicates (10 chick/replicate). All diets were formulated to meet same requirements. Broiler were fed with water and feed *ad libitum*. Results indicated that *Milk thistle* powder + aflatoxin B1 contaminated feed recorded best values in red blood cell counts, while addition treatments individually or in combination recorded best values in white blood cell counts and heterophil/lymphocyte (H/L) ratio. No significant differences between treatments in packed cell volume and hemoglobin. The results of biochemical tests of blood serum showed significant differences between treatments in level of protein, albumin, and globulin. No significant differences recorded between treatments in glucose, uric acid and creatinine. A significant decrease ($P \leq 0.05$) was recorded for additives treatments in serum cholesterol, triglycerides, and low-density lipoprotein. No significant differences recorded between treatments in high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL).

Keywords: Zeolite, *Saccharomyces cerevisiae*, *Milk Thistle*, Aflatoxin B_1, Physiological Performance, Broiler.

بعض الإضافات الغذائية التي تقلل من تأثير الأفلاتوكسين B1 في الأعلاف وتأثيرها على الأداء الفسيولوجي لفروج اللحم

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مستخلص

كان الهدف من هذه الدراسة هو دراسة تأثير إضافة إضافات مختلفة (الزيوليت ، خميرة الخبز ، مسحوق نبات الكلغان) إلى علائق فروج اللحم الملوثة بسموم الأفلاتوكسين B1 بمعدل 2 ملغم / كغم علف. تم توزيع 270 فرخ (غير مجنس) بعمر يوم واحد في تصميم عشوائي كامل مقسم إلى تسعة معاملات وبثلاثة مكررات (10 افراخ / مكرر). تم تصميم جميع العلائق لتلبي نفس المتطلبات. تم تغذية الطيور بالماء والعلف . أشارت النتائج إلى أن إضافة مسحوق نبات الكلغان الى العلف الملوث بالأفلاتوكسين B1 سجل أفضل القيم في عدد كريات الدم الحمراء ، بينما سجلت المعاملات الإضافية بشكل انفرادي أو تازري أفضل القيم في عدد كريات الدم البيضاء ونسبة خلايا الهيتيروفيل / الليمفاوية (H / L). لم تسجل فروق معنوية ($P \leq 0.05$) بين المعاملات في حجم خلايا الدم المرصوصة والهيموغلوبين. أظهرت نتائج الاختبارات الكيموحيوية لمصل الدم فروقًا معنوية بين المعاملات في مستوى البروتين الكلي ، الألبومين والكلوبيولين. لم تسجل أي فروق معنوية بين المعاملات في الجلوكوز والحمض البوليك والكرياتينين. تم تسجيل انخفاض كبير ($P \leq 0.05$) في معاملات الإضافات في قيم الكوليسترول ، الكليسيريدات الثلاثية ، والبروتينات الدهنية منخفضة الكثافة في مصل الدم. لم تسجل أي فروق معنوية بين المعاملات التجريبية في قيم البروتينات الدهنية عالية الكثافة (HDL) والبروتينات الدهنية منخفضة الكثافة جدًا (VLDL).

الكلمات المفتاحية : الزيولايت ، خميرة الخبز ، نبات الكلغان ، الافلاتوكسين B1 ، الأداء الفسلجي ، فروج اللحم .

Introduction

Fungal toxins are considered highly hazardous due to lack of immune response and their physiological effects on bird cells. They act as immune suppressants, mutagens, and carcinogens (Althagafi *et al.*, 2019). Furthermore, exposure of digestive tract to fungal toxins can lead to damage to lining membranes of digestive system, which in turn affects digestion and absorption of nutrients from intestines (Chunpeng *et al.,* 2019). Aflatoxin B_1 is one of most widespread and dangerous fungal toxins. It is a secondary metabolite produced primarily by *Aspergillus flavus* and *parasiticus fungi* under certain conditions, including suitable moisture and high temperature (Rashid *et al*., 2008). Aflatoxin toxins have been associated with various diseases such as Aflatoxicosis in poultry (AFSSA, 2009). The presence of fungal toxin residues in poultry meat, eggs, and other products resulting from feeding on contaminated diets poses a clear threat to human health (Upadhaya *et al*., 2010).

Zeolite is considered one of safe and non-toxic silicate minerals. It is widely used in food industry, as it has a high adsorption capacity and ion exchange ability due to its tetrahedral structures, where each tetrahedron contains four oxygen atoms with negative charges that bind to mycotoxins and prevent their absorption. This has encouraged its use in poultry industry. Studies have shown that use of Zeolite in poultry farms has positively influenced productive performance and carcass quality (Dashtestani *et al*., 2021). Zeolite also has an open channel in its crystal structure, which allows it to absorb water and gases like ammonia (Vasconcelos *et al*., 2023).

Saccharomyces cerevisiae, is one of most widely marketed additives, considered an effective adsorbent rich in crude protein (40-45)% and a variety of B vitamins such as biotin, niacin, pantothenic acid, and thiamine, in addition to its high biological value (Paalme *et al*., 2014). The components of yeast cell wall work to improve growth, increase body weight, feed intake, stimulate mucosal lining of small intestine, raise birds' immunity levels, and increase protection against mycotoxins produced by pathogenic microorganisms by binding mycotoxins to cell wall and preventing their absorption by digestive system (Kyoung *et al*., 2023).

Milk thistle (*Silybum marianum*) contains many active compounds with numerous medicinal and biological properties. In addition, milk thistle has an activity equivalent to or greater than vitamin E in scavenging free radicals generated by mycotoxin contamination and work to increase glutathione production in liver (Serçe *et al*., 2016). Milk thistle also has properties that protect and regenerate liver cells from damage caused by mycotoxin contamination and plays a role in stimulating liver cells to increase RNA synthesis necessary for albumin production (Jsasim and Al-Jurany, 2023). Therefore, aim of this study was to investigate effect of adding Zeolite, *Saccharomyces cerevisiae*, *Milk thistle* and its role in reducing contamination of feed with aflatoxin B_1 and its impact on physiological performance of broiler chickens.

Materials and methods

This experiment was conducted at poultry field belong to Department of Animal Production / College of Agriculture - University of Anbar. The experiment was conducted from 30/9/2023 to 10/11/2023. Two Hundred-Seventy unsexed with an initial weight 42 gm. were randomly distributed to nine treatments with 3 replicates (10 chicks/ replicate). Temperature was controlled and gradually decreased from 35°C on first day to 22°C at 21 days of age. Diets were iso-caloric and iso-nitrogenous (NRC, 1994). Broilers were fed in three phases feeding program starter, grower and a finisher diets, ingredients and chemical composition of diets are presented in Table (1). Zeolite, *Saccharomyces cerevisiae*, *Milk thistle* has been purchased from local market from Baghdad. Treatments were as follow: T1: control diet without any additives, T2: diet contaminated with 2 mg/kg aflatoxin B_1, T3: diet contaminated with aflatoxin B_1 plus 4 g/kg natural Zeolite, T4: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae*, T5: diet contaminated with aflatoxin B_1 plus 10 g/kg *Milk Thistle* powder, T5: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 5 g/kg *Saccharomyces cerevisiae* , T7: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 10 g/kg *Milk Thistle* powder, T8: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae* and 10 g/kg *Milk Thistle* powder and T9: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite, 5 g/kg *Saccha-*

romyces cerevisiae, and 10 g/kg *Milk Thistle* powder. On day 42 of experiment, six birds represented treatment were randomly selected, allowed to fast for 12 hours, slaughtered. Chemical analyses of plasma were carried out for quantitative determination of blood parameters (RBC, WBC, PCV, Hb, H/L ratio, Glucose, Protein, Albumin, Globulin, Triglycerides, Cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol), were determined using commercial kits, following same steps as described by manufactures. Results were analyzed with Complete Randomized Design (CRD) to investigate effect of treatments differ in features studied as well multivariate Duncan test was used (Duncan, 1955) to Examine differences between averages in Average level 0.05 using Statistical analysis System (SAS).

Production of aflatoxin toxin B1:

Isolation of *Aspergillus flavus* was obtained from College of Veterinary Medicine, Diseases Branch, University of Baghdad, and. The method of Shotwell *et al.,* (1966) and modified by West *et al*., (1973) was used. The fungus was activated by cultivation medium Potato Dextrose Agar (PDA), and then strain was grown on grains of corn as a primary development medium, while main development medium was rice.

Estimating amount of aflatoxin B1 poison in contaminated rice:

Aflatoxin toxins were measured in extract of each sample in two ways: The first method was by using (Enzyme-Linked Immune Sorbent (ELISA) according to method of West *et al*., (1973) where 5 grams of ground rice powder were weighed and then 25 ml of methanol 70:30% distilled water was added to it with stirring sample to ensure mixing with extraction solution for a period of three minutes. Then sample was filtered with (Whitman No.1) filter paper to obtain extract of each sample and amount of aflatoxin B1 toxin was measured according to method recommended by company that supplied aflatoxin measuring kit. The second method is by high Performance Liquid Chromatography (HPLC) Adopting method by Seitz and Mohr (1977).

Table 1: Ingredient and Chemical composition calculated of experimental diets.

Ingredients	Starter 1-14 day	Grower 15-28	Finisher 29-42 day
Yellow corn	55.5	60.4	64.8
Soybean *	35	30	25
Protein concentrate **	5	5	5
Oil	2.2	2.67	3.6
Dicalcium phosphate	0.7	0.5	0.3
Limestone	1.2	1.1	1
DL-methionine	0.2	0.15	0.12
Lysine	0.1	0.08	0.08
Salt	0.1	0.1	0.1
Total	100	100	100
Chemical composition, Calculated ***			
Crude protein	23.52	21.53	19.51
ME, kcal/kg	3017	3101	3210
Methionine + Cystine	1.09	0.99	0.91
Calcium	0.97	0.88	0.78
phosphorus	0.48	0.43	0.39
Available phosphorus	0.39	0.35	0.30

* Soybean meal 48% crude protein

** Protein concentrate contains: 40% CP, 5% Ca,3.7% Methionine, 4.12% Methionine and Cystine, 3.85% Lysine , 4.68% AP, Metabolizable Energy 2107 Kcal , 2.50 mg Sodium, 1.70 mg threonine, 0.42mg Tryptophan, 4.20 mg choline and each 1 kg of this concentrate contain: 100000 IU vitamin A, 33000; IU vitamin D3, 100 mg; vitamin E, 2.55 mg; vitamin K3, 25 mg; vitamin B1, 10 mg; B2, 50 mg; vitamin B6, 24 mg vitamin B12; 51 mg niacin; 1.5 mg folic acid; 15 mg; biotin,500 µg and 13.5 mg pantothenic acid.

***Calculated based on feed consumption Tables of NRC(1994).

Results and Discussion

Hematological tests:

The results in Table (2) indicate effect of adding Zeolite, *Saccharomyces cerevisiae* and *Milk Thistle* on some cellular blood characteristics. The results did not show significant differences between experimental treatments in measuring packed cell volume and blood hemoglobin. It is also noted that red blood cells recorded significant differences between different experimental treatments ($P \leq 0.05$). It is observed that birds of treatment T5 (addition of

Milk Thistle powder 10 g / kg) recorded highest value, reaching 4.053 million cells / ml of blood, and mentioned treatment did not record any significant difference (P≤0.05) with birds of treatments T3, T4, T6, T7, T8, T9, which recorded blood cell counts of (3.396, 3.916, 3.526, 3.886, 3.863, 3.280) million cells / ml of blood, respectively. Significant differences were recorded between birds of treatment T5 and control treatment T1 and treatment T2 (addition of aflatoxin B1 toxins), which recorded (2.776, 2.583) million cells / ml blood, respectively. Regarding number of white blood cells, it is noted that birds of treatment T2 recorded highest value 38.60 thousand cells / ml blood, with a significant difference (P≤0.05) from all experimental treatments, with exception of T1 (control) treatment, which recorded 37.30 thousand cells / ml blood. It is also noted that birds of treatments T3 and T4 did not differ significantly with birds in control treatment, while they recorded significant differences with birds in treatments T5, T6, T7, T8, and T9. Birds in treatments T5, T6, and T7 recorded white blood cell counts of (30.96, 30.97, 30.33) thousand cells / ml blood, respectively, compared to birds of treatments T8 and T9, which recorded lowest white blood cell counts of (28.03 and 28.27) thousand cells / ml blood, respectively. The results also included ratio of heterophil / lymphocyte (H/L) cells, where it is noted that birds of treatment T2 recorded highest value with a significant difference (P≤0.05) compared to birds in treatments T1, T3, T4, T5, T6, T7, T8, and T9, which recorded (0.543, 0.543, 0.533, 0.510, 0.506, 0.460, 0.380, 0.32) respectively. The results also showed that birds of treatments T1, T3, T4, T5, and T6 recorded a significant increase at expense of birds of treatments T7, T8, and T9. It is also noted that birds of treatment T7 recorded a significant increase of 0.460 compared to birds of treatments T8 and T9, which recorded (0.380, 0.320) respectively.

Table (2) Effect of Zeolite, *Saccharomyces cerevisiae*, *Milk Thistle* supplementation to broiler diets contaminated with aflatoxin B1 on Blood hematological parameters.

Treatments	RBC	PCV %	Hb	WBC	H/L
T1	**2.776** ±0.13 B	**24.00** ± 2.0	**7.70** ± 0.35	**37.30** ±1.15 AB	**0.543** ±0.024 B
T2	**2.583** ±0.18 B	**24.00** ± 0.58	**7..36** ± 0.12	**38.60** ±0.98 A	**0.810** ±0.046 A
T3	**3.396**±0.14 AB	**25.66** ± 1.45	**8.10** ± 0.20	**32.47** ±0.69 B	**0.543** ± 0.03 B
T4	**3.916** ±0.03 AB	**25.66** ± 0.33	**8.13** ± 0.12	**32.23** ±0.95 B	**0.533** ±0.022 B
T5	**4.053** ±0.01 A	**25.00** ± 0.58	**8.63** ± 0.20	**30.96** ±1.35 C	**0.510** ±0.011 B
T6	**3.526** ±0.02 AB	**26.66** ± 0.88	**8.86** ± 0.30	**30.97** ±0.50 C	**0.506** ±0.041 B
T7	**3.886** ±0.05 AB	**25.66** ± 1.21	**8.66** ± 0.33	**30.33** ±0.48 C	**0.460** ± 0.01 C
T8	**3.863** ±0.03 AB	**25.00** ±0.57	**8.46** ± 0.12	**28.03** ±0.47 D	**0.380** ± 0.02 D
T9	**3.280** ±0.07 AB	**25.67** ±0.88	**8.53** ± 0.29	**28.27** ±0.51 D	**0.320** ±0.051 D
P- Value	**0.05**	**N.S**	**N.S**	**0.05**	**0.05**

* T1: control diet without any additives, T2: diet contaminated with 2 mg/kg aflatoxin B_1, T3: diet contaminated with aflatoxin B_1 plus 4 g/kg natural Zeolite, T4: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae*, T5: diet contaminated with aflatoxin B_1 plus 10 g/kg *Milk Thistle* powder, T5: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 5 g/kg *Saccharomyces cerevisiae* , T7: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 10 g/kg *Milk Thistle* powder, T8: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae* and 10 g/kg *Milk Thistle* powder and T9: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite, 5 g/kg *Saccharomyces cerevisiae*, and 10 g/kg *Milk Thistle* powder.

The experimental data indicate a decrease in number of red blood cells and an increase in white blood cells for treatment T2, which may be due to negative role of aflatoxin B1. This could be due to reduced availability of amino acids as a result of damage to lining of digestive tract, leading to a lack of essential nutrients required by body to maintain vital functions, including production of red blood cells (Chen *et al*., 2014). It may also be due to its effect on red blood cell-producing cells in bone marrow, which is reflected negatively on all these parameters (Ali *et al*., 2017). Additionally, aflatoxin B1 is a toxin that affects function of kidneys, which produce Hemopoietin that stimulates bone marrow to form red blood cells. The effect of aflatoxin on kidneys may lead to a decrease in production of this hormone, and therefore a decrease in formation of red blood cells in birds consuming aflatoxin (de-Oliveira *et al*.,

2020; Yang *et al*., 2020). The decrease in number of red blood cells (RBCs) may also be due to effect of aflatoxin B1 toxins on production of red blood cell-stimulating factor (ESF), leading to a decrease in production of RBCs. In the other treatments, reason may be due to role of additives in restricting fungal toxins and preventing their negative effects (Zhang *et al*., 2020), or decrease in number of red blood cells in treatment T2 (fungal toxins without other additives) may be due to inhibition of Glutathione production by fungal toxins. The function of Glutathione is protecting red blood cells from effects of toxic substances, which makes red blood cells more sensitive to toxins and thus reduces their numbers (Wu, 2018). Recent studies have indicated that aflatoxin B1 toxins contribute to negative changes in red blood cells (Abd Allah *et al*., 2017). Other studies have indicated a significant decrease in blood parameters in animals fed diets contaminated with aflatoxin B1 compared to control treatment (Ramamurthy and Rajakumar, 2016). Iron is one of factors that helps stimulate oxidation and when cells are exposed to free radicals, this leads to damage to their membranes due to breakdown of unsaturated fatty acids, which results in a loss of selective permeability of cells that controls entry and exit of materials from and to cell, thus reducing lifespan of blood cells, which leads to a decrease in their numbers and an increase in number of white blood cells and an increase in stress levels in birds, which is confirmed by increase in H/L ratio, which is one of measures of stress in birds, where it is observed that T2 treatment birds recorded highest value, which may be due to birds' exposure to aflatoxin B1 toxins without presence of antioxidants or any other additions, compared to rest of experimental treatments that were exposed to same level of contamination but with addition of Zeolite, *Saccharomyces cerevisiae* and *Milk Thistle* powder to diets (Saleemi *et al*., 2020). The individual and synergistic role of additives used in diet had a significant effect in reducing negative effects of aflatoxin, as it is observed that blood parameters and hemoglobin were not affected, which may be due to role of additives (Zeolite, *Saccharomyces cerevisiae* and *Milk Thistle* powder) in limiting effect of aflatoxin toxins and protecting cells and preventing their absorption by intestinal villi and their excretion from body (Prasai *et al*., 2017).

Blood Biochemical tests :

The results of table (3) indicate effect of adding Zeolite, *Saccharomyces cerevisiae* and *Milk Thistle* powder on some biochemical blood characteristics. It was observed that experimental treatment birds did not show significant differences between them in measurement of blood glucose concentration, uric acid, and creatinine. On the other hand, results indicated recording of significant differences between treatment birds in measurement of total protein concentration, and appearance of significant differences (P≤0.01) in favor of birds of treatment T9 (addition of Zeolite , *Saccharomyces cerevisiae* , *Milk Thistle* powder), as it recorded a significant increase in concentration of total protein, reaching 5.30 g/100 ml of blood, with a significant difference from birds of treatments T1, T2, T3, T4, which recorded (2.98, 2.45, 3.02, 3.73, 3.71) g/100 ml of blood, respectively. It is noted that birds of treatments T6, T7, T8 did not show any significant differences between and with birds of treatment T9, as birds of these treatments recorded a total protein concentration of (4.21, 4.62, 4.12) g/100 ml of blood, respectively. From obtained results, it can be observed that birds of treatments T4, T5 recorded a significant increase (P≤0.01) at expense of treatments T1, T2, T3. It can also be observed that birds of treatment T2 recorded lowest total protein concentration of 2.45 g/100 ml of blood, with a significant decrease compared to birds of treatments T1, T3, which did not differ significantly between them and recorded an average total protein concentration of (2.98, 3.02) g/100 ml of blood, respectively. Regarding results of albumin, it was observed that birds of treatment T9 recorded highest value with a significant increase (P≤0.01) compared to experimental treatments, recording 2.82 g/100 ml of blood. This was followed by birds of treatments T7 and T8, which recorded (2.12, 2.18) g/100 ml of blood respectively, without any significant differences being recorded with birds of treatment T4, which recorded 2.00 g/100 ml of blood, and without achieving any significant differences with birds of treatments T1, T2, T3, T5, and T6, which recorded albumin values of (1.18, 1.06, 1.04, 1.02, 1.09) g/100 ml of blood, respectively. The results also showed effect of adding Zeolite and *Saccharomyces cerevisiae* on blood globulin, where it was observed that birds of treatment T6

(addition of Zeolite with *Saccharomyces cerevisiae*) recorded highest value, reaching 3.12 g/100 ml of blood, followed by birds of treatments T5, T7, and T9, which recorded (2.69, 2.50, 2.48) g/100 ml of blood, with a significant difference (P≤0.01) compared to birds of treatments T1, T2, T3, T4, and T8, which recorded (1.80, 1.38, 1.83, 1.94) g/100 ml of blood, respectively. We also note from results that birds of treatment T2 recorded lowest average without achieving any significant differences with control treatment birds.

Table (3) Effect of Zeolite, *Saccharomyces cerevisiae*, *Milk Thistle* supplementation to broiler diets contaminated with aflatoxin B1 on biochemical parameters.

Treatments	Glucose (100gm/ml)	Protein (100 gm/ml)	Albumin 100) gm/ml)	Globulin (100 gm/ml)	Uric acid	Creatinine
T1	268.7 ± 22.6	2.98±1.86 C	1.18±1.15 C	1.80 ±1.0 CD	1.830 ± 0.038	0.403 ± 0.33
T2	270.2 ± 13.1	2.45±2.60 D	1.06±0.88 C	1.38±0.33 D	1.670 ± 0.25	0.390 ± 1.76
T3	255.0±19.4	3.02±1.73 C	1.04±2.03 C	1.98±2.03 C	1.843 ± 0.11	0.473 ± 0.88
T4	276.0 ± 6.17	3.73±1.76 B	1.90±0.88 BC	1.83 ±1.0 C	1.743 ± 0.032	0.453 ± 1.53
T5	274.0 ± 18.04	3.71±1.86 B	1.02±0.58 C	2.69±0.58 B	1.686 ± 0.029	0.486 ± 0.33
T6	278.99 ± 13.07	4.21±2.91 AB	1.09±0.88 C	3.12±1.73 A	1.670 ± 0.081	0.436 ± 0.58
T7	272.32 ± 9.73	4.62±2.52 AB	2.12±1.20 B	2.50±1.45 B	1.750 ± 0.089	0.466 ± 0.88
T8	261.05 ± 11.87	4.12±0.89 AB	2.18±0.33 B	1.94±0.67 C	1.830 ± 0.029	0.490 ± 0.58
T9	253.99 ± 14.32	5.30±1.73 A	2.82±0.67 A	2.48±0.58 B	1.770 ± 0.087	0.483 ± 1.86
P- Value	N.S	0.01	0.01	0.05	N.S	N.S

* T1: control diet without any additives, T2: diet contaminated with 2 mg/kg aflatoxin B_1, T3: diet contaminated with aflatoxin B_1 plus 4 g/kg natural Zeolite, T4: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae*, T5: diet contaminated with aflatoxin B_1 plus 10 g/kg *Milk Thistle* powder, T5: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 5 g/kg *Saccharomyces cerevisiae* , T7: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 10 g/kg *Milk Thistle* powder, T8: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae* and 10 g/kg *Milk Thistle* powder and T9: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite, 5 g/kg *Saccharomyces cerevisiae*, and 10 g/kg *Milk Thistle* powder.

** N.S : Non significant .

The reason for superiority of addition treatments in concentrating total protein, albumin, and globulin may be attributed to vital role played by (Zeolite, *Saccharomyces cerevisiae*, and *Milk Thistle* powder) in reducing effects of mycotoxins. Zeolite forms a colloidal complex that surrounds aflatoxin, impeding or preventing its transfer through cells of digestive tract, thereby reducing or preventing negative effects of mycotoxins on liver cells (Prasai *et al*., 2017). Additionally, *Saccharomyces cerevisiae* plays a prominent role in biochemical blood properties and reducing negative effects of aflatoxin, as addition of *Saccharomyces cerevisiae*, either individually or synergistically, helps to limit effect of mycotoxins, which may be due to polysaccharides present in cell walls of yeast, which compensate for high effort expended by body to reduce effect of aflatoxin (Matur *et al*., 2011). Or it may be due to *Saccharomyces cerevisiae* in relation to mycotoxins and reduction of negative effects in body cells, especially digestive tract, and preventing their transfer to blood stream and reaching body organs (Fowler *et al*., 2015), this was reflected in productive traits, cellular properties and biochemical blood. Also, leaves of *Milk Thistle* plant contain some biologically active compounds such as saponins and glycosides, and these substances have antimicrobial and antioxidant activity, and these substances play an important role in reducing activity of intestinal bacteria by increasing secretion of digestive enzymes such as amylase, lipase and protease and improving immune response as well as protecting lining tissue of digestive tract (El-Shehhat, 1986; Saad *et al*., 1988). The leaves of *Milk Thistle* plant also contain compound quercetin, which has many biological activities in body, including ability to protect against oxidation by suppressing free radicals, and it also regulates process of programmed cell death, especially liver cells, and this explains reason for increase in percentage of total protein, albumin and globulin (Khalaf, 2021). Or , reason for decrease in level of total protein and albumin in plasma of broiler chickens for second treatment fed (feed supplemented with mycotoxins) may be due to accumulation of lipid peroxides in liver cells, which leads to damage to liver cell membranes and a decrease in manufacturing function of liver to produce proteins (Elliott, 2019). Aflatoxin

B1 can be metabolized within body to AFla-8,9 epoxide, which has ability to bind to guanine nitrogenous base of DNA, RNA, leading to a change in sequence of nitrogenous bases and inhibition of protein synthesis (Li *et al.,* 2020).

Lipid Profile tests:

Regarding data in table (4) there were no significant differences between control birds and birds of additive treatments (Zeolite, *Saccharomyces cerevisiae*, *Milk Thistle* powder) individually or combination, despite fact that birds of these treatments recorded a significant decrease compared to birds of treatment T2 (addition of aflatoxin toxins), which recorded highest value. The results also showed significant differences between different experimental treatments in measurement of triglyceride concentration, and birds of treatment T2 which recorded highest concentration of 297.33 mg/100 ml of blood, with a significant difference from birds in treatments T3, T4, T5, T6, T7, T8, T9, which recorded (213.33, 200, 202.67, 203, 212, 210.33, 214.67) mg/100 ml of blood respectively, and which did not show any significant differences with birds of treatment T1, which recorded 238.67 mg/100 ml of blood. Results also showed measurement of blood cholesterol concentration for different experimental treatments, where it was noted that birds of treatment T2 (addition of aflatoxin B1 toxins at a concentration of 2 mg/kg of feed) recorded highest value of 254.14 mg/100 ml of blood, with a significant decrease compared to birds of treatments T3, T4, T5, T6, T7, T8, T9, which recorded (194.99, 194.34, 192.13, 190.27, 187.27, 186.33, 181.87) mg/100 ml of blood respectively. The data also showed that control treatment recorded 200.73 mg/100 ml of blood and did not achieve any significant differences with birds of treatments T2, T3, T4, T5, T6, while birds of treatments T7, T8, T9 recorded best cholesterol concentration values with (187.27, 186.33, 181.87) mg/100 ml of blood, with a significant decrease ($P \leq 0.05$) compared to different experimental treatments.

Table (4) Effect of Zeolite, *Saccharomyces cerevisiae*, *Milk Thistle* supplementation to broiler diets contaminated with aflatoxin B1 on lipid profile parameters.

Treatments	Cholesterol (mg/100ml)	Triglycerides (mg/100ml)	HDL (mg/100ml)	LDL (mg/100ml)	VLDL (mg/100ml)
T1	**200.73** ± 4.48 **AB**	**238.67**±4.67 **AB**	**44.67** ±2.60	**108.33**±2.03 **B**	**47.73** ±1.21
T2	**254.14** ± 3.33 **A**	**297.33** ±7.80 **A**	**43.00** ±2.52	**161.67**±3.18 **A**	**49.47** ±1.45
T3	**194.99** ± 306 **B**	**213.33**±4.41 **B**	**43.00** ±1.53	**109.33**±1.76 **B**	**42.66** ±0.33
T4	**194.34** ± 3.51 **B**	**200.00** ±8.14 **B**	**44.67** ±2.19	**109.67**±3.48 **B**	**40.00** ±1.73
T5	**192.13** ± 3.46 **B**	**202.67**±4.26 **B**	**44.33** ±1.45	**107.27**±3.76 **B**	**40.53** ±1.51
T6	**190.27**± 4.84 **B**	**203.00** ±4.51 **B**	**43.00** ±2.00	**106.67**±0.67 **B**	**40.6** ±0.67
T7	**187.27**± 3.06 **C**	**212.00**±7.51 **B**	**42.00** ±0.58	**102.87**±1.20 **B**	**42.4** ±1.86
T8	**186.33** ± 3.48 **C**	**210.33**±2.89 **B**	**44.00** ±2.52	**100.26** ±1.76 **B**	**42.06** ±0.88
T9	**181.87** ± 6.08 **C**	**214.67** ±6.36 **B**	**42.67** ±1.76	**96.27** ±6.48 **B**	**42.93** ±1.45
P- Value	**0.05**	**0.05**	**N.S**	**0.05**	**N.S**

* T1: control diet without any additives, T2: diet contaminated with 2 mg/kg aflatoxin B_1, T3: diet contaminated with aflatoxin B_1 plus 4 g/kg natural Zeolite, T4: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae*, T5: diet contaminated with aflatoxin B_1 plus 10 g/kg *Milk Thistle* powder, T5: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 5 g/kg *Saccharomyces cerevisiae* , T7: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite and 10 g/kg *Milk Thistle* powder, T8: diet contaminated with aflatoxin B_1 plus 5 g/kg *Saccharomyces cerevisiae* and 10 g/kg *Milk Thistle* powder and T9: diet contaminated with aflatoxin B_1 plus 4 g/kg Zeolite, 5 g/kg *Saccharomyces cerevisiae*, and 10 g/kg *Milk Thistle* powder.

The effect of additions (Zeolite, *Saccharomyces cerevisiae*, *Milk Thistle* powder) was evident in lowering values of triglyceride concentration, cholesterol, low-density lipoprotein values, as results obtained from experiment showed a significant deterioration in fat properties, especially for second treatment which indicates negative efficacy and harmful effect of aflatoxin which causing oxidative stress and an increase in production of free radicals that lead to lipids breakdown, which causes a decrease in concentration of lipids in body, or it may be due to function in digestive processes in intestines

of lipids as a result of damage to intestinal cell membranes due to their attack by free radicals, or due to changes in metabolism of lipoproteins (Shabani *et al*., 2010). Mycotoxins are among most stress factors, and that leads to secretion of corticosteroid hormone by adrenal cortex, which has negative feedback on activity of thyroid gland, causing an increase in value of cholesterol and triglycerides (Sakamoto *et al*., 2018). The exposure of liver cells to damage due to aflatoxin B1 as a result of oxidative stress caused by fungal toxins leads to a decrease in effectiveness of liver in performing its vital function (Sakamoto *et al*., 2018). The harmful effects of aflatoxin on liver can be inferred from observation that protein concentration has decreased significantly in aflatoxin treatment for this experiment.

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