مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الثالث والعشرون - المجلد الثامن - علوم الحياة - تموز 2024 م
doi.org/10.52866/esj.2023.08.23.15

Lycopene as a Minimize Harmful Effects of Fungal Toxins and Enhancing Productive Performance of Broiler Chickens

Ali Esam Hameed * , Baraa Hameed Mousa **
* E-mail: ali22g4010@uoanbar.edu.iq
** E-mail: ag.baraa.hameed@uoanbar.edu.iq
Department of Animal Production, Faculty of Agriculture, University of Anbar, Iraq

Abstract :

Lycopene is a natural, fat-soluble red pigment that acts against free radicals from oxidative stress also combats fungal toxins and helps minimize their harmful effects.The aim of study was to determined effect of adding levels of lycopene 0 , 50 , 100 , 150 and 200 mg/kg feed to broiler's diets contaminated aflatoxin B_1 with 2 mg/kg feed on Productive performance. A total of 180 chicks, one-day-old (unsexed), were distributed in a completely randomized design that were divided to six treatments with three replicates (10 chick / replicate). All diets were formulated to meet the same requirements. Broiler were fed with water and food *ad libitum*. Indicated that addition of lycopene to contaminated feeds with Aflatoxin B1 led to significant increase ($P\leq0.05$) in live body weight and body weight gain. Moreover, a significant decrease ($P\leq0.05$) was observed in feed consumption for treatment T2 which birds fed diets contaminated with aflatoxin B_1. A significant improvement ($P\leq0.05$) in feed conversion ratio (FCR) for birds fed diets supplemented with Lycopene as compared with T1(control) and T2 (birds fed diets contaminated with Aflatoxin B_1). The addition of lycopene extract has shown a clear impact in reducing the effects of aflatoxin B_1, which is produced by fungal toxins. Aflatoxin B1 is considered the greatest threat to poultry breeders and meat producers, as it can contaminate feed.

Keywords: Lycopene, Aflatoxin B1, Productive performance, Broiler.

دور اللايكوبين في تقليل الآثار الضارة للسموم الفطرية وتعزيز الأداء الإنتاجي لفروج اللحم

علي عصام حميد ، براء حميد موسى
قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة الأنبار ، العراق

مستخلص

اللايكوبين هو صبغة حمراء طبيعية ذائبة في الدهون تعمل ضد الجذور الحرة الناتجة عن الاجهاد التأكسدي وتحارب السموم الفطرية وتساعد في تقليل آثارها الضارة. الهدف من هذه الدراسة هو دراسة تأثير إضافة مستويات مختلفة من اللايكوبين (0، 50، 100، 150 و 200 ملغم/ كغم علف) إلى أعلاف دجاج اللحم الملوثة بألافلاتوكسين B1 وبمعدل 2 ملغم/ كغم علف على الأداء الإنتاجي. تم توزيع مجموعة 180 كتكوت في اليوم الأول من العمر (غير محدد الجنس) بتصميم عشوائي كامل تم تقسيمها إلى ستة معاملات مع ثلاث مكررات (10 كتاكيت / مكرر). تم تركيب جميع العلف لتلبية متطلبات واحتياجات الطيور وحسب دليل التربية. أظهرت النتائج أن إضافة اللايكوبين إلى الأعلاف الملوثة بأفلاتوكسين B1 أدت إلى زيادة معنوية ($P\leq0.05$) في وزن الجسم الحي وزيادة وزن الجسم. علاوة على ذلك، لوحظ انخفاض معنوي ($P\leq0.05$) في استهلاك العلف للمعاملة T2 التي تم تغذية طيورها بأعلاف ملوثة بأفلاتوكسين B1. كما لوحظ تحسن معنوي ($P\leq0.05$) في معامل تحويل العلف (FCR) للطيور التي تم إطعامها بأعلاف مكملة باللايكوبين مقارنة بالمعاملة السيطرة T1 والمعاملة T2 الطيور التي تم إطعامها بأعلاف ملوثة بأفلاتوكسين B1). أظهرت إضافة مستخلص الليكوبين تأثيرًا واضحًا في تقليل آثار أفلاتوكسين B1 الناتجة عن السموم الفطرية.

Introduction

Fungal toxins are widely spread in animal feed and Aflatoxin B_1 toxins are among the most widespread and dangerous mycotoxins. They are secondary metabolites primarily produced by the fungi *Aspergillus flavus* and *parasiticus*. Aflatoxins have been associated with various diseases, such as aflatoxicosis in poultry (AFSSA,2009). Fungal toxins are highly prevalent in animal feed and have a remarkable ability to proliferate in grain crops. They can grow in processed and raw materials, leading to the deterioration of food products by altering their quality, nutritional value, and causing the secretion of toxic substances harmful to public health (Abrehame *et al.,* 2023). Numerous important strategies have been implemented to find appropriate solutions to mitigate the effects of fungal toxins on poultry health. One of these strategies involves the use of natural antitoxin substances, such as dietary supplements like lycopene. Lycopene is one of the most important types of carotenoids, produced by certain plants and microorganisms during photosynthesis to protect them from light activity and increase light sensitivity (Nabi *et al.,* 2020). Lycopene is classified as a natural antioxidant that provides protection against cellular damage caused by free radicals and oxidative stress (Hidayat *et al.,* 2023). The addition of lycopene to poultry feed enhances growth, productivity, meat quality, and acts as a powerful antioxidant capable of scavenging free radicals and reducing oxidative stress (Sarker *et al.,* 2021). Karadas *et al*.(2016) stated that lycopene is considered one of the most effective antioxidants within the carotenoid family. It has a higher ability than α-tocopherol to scavenge reactive oxygen species (ROS) and more than ten times the ability of β-carotene to alleviate oxidative damage to tissues and cells. Lycopene also possesses anti-inflammatory properties. Additionally, Karaca *et al.*,(2021) indicated that lycopene promotes the levels of enzymatic antioxidants such as glutathione and catalase, reduces hydrogen peroxide levels, and maintains cell membrane permeability. Hence, lycopene can be considered a preventive factor against aflatoxin poisoning. The protective role of lycopene in broiler feed is important and beneficial for public health, particularly due to the formation of free radicals during increased temperature, stress, rapid growth, high production, and metabolism (Muhammed *et al.,*

2023). Therefore, the aim of this study is to investigate the impact of adding pure lycopene and its role in reducing aflatoxin B_1 contamination in feed and its influence on the production performance of broiler chickens.

Material 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. One Hundred-eighty unsexed with an initial weight 42 gm. were randomly distributed to six treatments with 3 replicates (10 chicks/ replicate). Temperature of field was controlled by thermostat and gradually decreased from 35°C on beginning of experiment 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). Lycopene has been purchased from Guangzhou Ur, Trading. Co, Ltd, China. Treatments were as follow: T1: basal diet without any addition , T2: addition aflatoxin B_1 with 2mg/kg feed , T3: addition lycopene 50 mg. / kg feed + addition aflatoxin B_1 with 2mg/kg feed ,T4: addition lycopene 100 mg. / kg feed + addition aflatoxin B_1 with 2mg/kg feed, T5 : addition lycopene 150 mg. / kg feed + addition aflatoxin B_1 with 2mg/kg feed , T6: addition lycopene 200 mg. / kg feed + addition aflatoxin B_1 with 2mg/kg feed. All chicks were individually weighed and Feed Intake (FI) was recorded at weekly intervals. Based on the recorded data, Feed Intake (FI), Feed Conversion Ratio (FCR), and Body Weight Gain (BWG) were subsequently calculated based on the performance values.

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 the cultivation medium Potato Dextrose Agar (PDA), and then the strain was grown on the grains of corn as a primary development medium, while the main development medium was rice.

Aflatoxin toxins were measured in the extract of each sample in two ways: The first method was by using (Enzyme-Linked Immune Sorbent (ELISA) according to the method of West *et al.*,(1973) where 5 gm. of ground rice powder were weighed and then

25 ml of methanol 70: 30% distilled water was added to it with stirring the sample to ensure mixing with the extraction solution for a period of three minutes. Then the sample was filtered with (Whitman No.$_1$) filter paper to obtain the extract of each sample and the amount of aflatoxin B_1 toxin was measured according to the method recommended by the company that supplied the aflatoxin measuring kit. The second method is by high Performance Liquid Chromatography (HPLC) Adopting the method by Seitz and Mohr (1977).

The data were statistically analyzed using the statistical program of SAS to study the effect of different treatments in the studied traits under study according to the complete randomized design (CRD) and compare the significant differences between means with Duncan polynomial test (1955).

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

Ingredients	Starter 1-14 day	Grower 15-28 day	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% Crude Protein, 5% Calcium, 3.7% Meth., 4.12% Meth. + Cys., 3.85% Lys. , 4.68% Available Phosphorus, ME 2107 Kcal kg^(-1), 2.50 mg Na, 1.70 mg threonine, 0.42mg Tryp., 4.20 mg choline , each 1 kg of protein concentrate contain: 100000 IU vit. A, 33000; IU vit. D3, 100 mg; vit. E, 2.55 mg; vit. K3, 25 mg; vit. B1, 10 mg; B2, 50 mg; vit. B6, 24 mg vit. B12; 51 mg niacin; 1.5 mg folic acid; 15 mg; biotin,500 µg and 13.5 mg pantothenic acid.

***Calculated was basis on Tables of NRC (1994).

Results

Results in table 2. indicated significant differences (P≤0.05) between treatments at second week of bird's age in live body weight. All lycopene supplementation treatments recorded live weights 467.1, 459.9, 487.7, and 472.1 gm., respectively, while the second treatment (T2) had lowest live body weight, 396.5 gm. At third week of bird's age, T3 and T5 showed a significant increase (P≤0.05) in live body weight compared to control treatment and T2, respectively. Moving to fourth week, it is observed that high concentrations of lycopene T5 and T6 resulted in live body weights of 1605.2 and 1591 gm. respectively . At the fifth week of bird's age, results showed significant differences between different treatments. High dosage of lycopene in T6 recorded highest live body weight with 2327.2 gm, with a significant difference (P≤0.05) compared to control treatment and T2, which recorded live body weights of 1977.8 and 2004.5 gm. respectively. The results also demonstrated that T3, T4, and T5 achieved 2178, 2166.2, and 2197.9 gm. respectively. Additionally, the statistical analysis of table (2) revealed a significant increase (P≤0.05) in live body weight at the sixth week of bird age for treatment T6, reaching 3198 gm. compared to treatments T1 and T2, which had live body weights of 2639 and 2453.3 gm., respectively. Similarly, T3, T4, and T5 achieved 3040.2, 3024.4, and 3014.8 gm. respectively.

Table 3. demonstrated body weight gain rates of different experimental treatments for birds. It can be observed from the table that lycopene supplementation treatments showed significant weight gain with values of (425.1, 417.9, 436.7, 430.1) gm. compared to the second treatment, which included aflatoxin B_1 supplementation at a concentration of 2 mg/kg feed and recorded a weight gain rate of 354.5 gm. At the third week, all lycopene supplementation treatments exhibited significant weight gain compared to the control treatment and the second treatment (aflatoxin B_1 supplementation). Treatments T3, T4, T5, and T6 achieved body weight gain rates (450, 438.8, 427.7, 819.8) gm. respectively, while treatments T1 and T2 recorded body weight gains (389.6, 386) gm. respectively. At fourth week, birds fed lycopene at concentrations of 100, 150, and 200 mg. showed increase signifi-

cant (P≤0.05) in body weight gain rates with (677.2, 698.8, 699.1) gm. respectively, compared to control treatment and second treatment (aflatoxin B_1 supplementation), which recorded body weight gain (542.3, 463.5) gm. respectively. Regarding the results at fifth week, it can be observed that T2 and T6 achieved significant body weight gain rates 758.5 and 736.2 gm. respectively. At sixth week of bird's age, significant differences were observed between different experimental treatments, all lycopene treatments recorded increase significant at (P≤0.05) in body weight gain rates (862.2, 858.2, 816.9, 870.8) gm. respectively, compared to control treatment, which recorded 661.2 gm.

Results in table (4) indicated that during the second and third weeks of bird age, there were no significant differences (P≤0.05) in feed consumption between different treatments. However, at fourth week, significant differences were observed between the treatments. Birds in T5 and T6 showed a significant decrease (P≤0.05) in feed consumption rates, with values of (790 and 771) gm. respectively, compared to the control treatment and the second treatment (T3), which recorded feed consumption rates of (867, 861, 880) gm. respectively. In the fifth week, birds in T5 and T6 continued to exhibit a significant decrease in feed consumption rates, with 952 and 958 gm. respectively compared to birds in T1 and T2, which recorded 1164 and 1187 gm. respectively. At the sixth week of bird's age, it can be observed that birds fed with feed contaminated with aflatoxin B_1 (T2) had the lowest feed consumption rate, with a value of 1176 gm.

Results of statistical analysis in table (5) indicated a significant improvement for lycopene addition treatments. Feed conversion ratios were recorded for addition all lycopene treatments as (0.74, 0.79, 0.72, 0.74) gm. feed consumed /gm. bodyweight gain, compared to birds in (T2) aflatoxin B_1 addition treatment, which recorded a feed conversion ratio of 0.97 gm. feed consumed / gm. body weight gain. In third week of study, significant improvements were observed for lycopene addition treatments feed conversion ratios were achieved as (1.09, 1.12, 1.15, 1.15) gm. feed consumed / gm. body weight gain, compared to control treatment and second treatment, which recorded feed conversion ratios of (1.51 and 1.54) gm. feed consumed / gm.

body weight gain, respectively. The significant differences for these treatments continued during fourth week. In fifth week, it was observed that birds in treatment T6 recorded best feed conversion ratio with 1.30 gm. feed consumed/ gm. body weight gain, compared to all experimental treatments. At 42 days of age, it was noticed that birds in T6 recorded best feed conversion ratio of 1.39 gm. feed consumed / gm. body weight gain, followed by birds in treatments T3, T4, and T5, which recorded feed conversion ratios of 1.52, 1.41, 1.50 gm. feed consumed / gm. body weight gain, respectively compared to birds in T2, which recorded a feed conversion ratio of 2.62 gm. feed consumed / gm. body weight gain.

Discussion

The reason for the improvement in feed conversion ratio of adding lycopene can be attributed to high efficiency of lycopene in feeds. Lycopene acts as an antioxidant and plays a role in protecting fats and polyunsaturated fatty acids (PUFA) from oxidation and rancidity in feeds. It inhibits lipid peroxidation and suppresses free radicals that attack and break down fats and PUFA, which results in a significant decrease in the nutritional value of fats and the availability of energy represented by them. Lycopene prevents hydrogen atoms from dissociating from double bonds sites of PUFA, there by inhibiting oxidation and formation of lipid peroxides, this leads to increased utilization of feed fats and increased utilization of energy derived from nutrient metabolism, including fats and that positive effect reflects on feed conversion ratio of transactions adding lycopene (Arain *et al.,* 2018). Alternatively, the improvement in productivity may be attributed to lycopene content in rations, which plays an important role inside and outside cells. It enhances utilization of nutrients, resulting in improved productivity of broiler chickens. Lycopene is a natural antioxidant and contains more than two aromatic rings with several hydroxyl groups, which helps in hydrogen atom donation and the saturation of free radicals, there by inhibiting their activity (Wu *et al.,* 2024). Lycopene extract significantly and effectively contributes to increasing live body weight and weight gain, as well as improving feed conversion ratio by enhancing the availability of nutrients in the feed and increasing the digestibility coefficient of

nutrients by trapping them in digestive tract. This is achieved through binding of nutrients to villi of small intestines (Long *et al.,* 2024) . Lycopene may also be attributed to its ability to meet the actual needs of broiler chickens and enhance the utilization of nutrients while mitigating the detrimental effects of aflatoxin in feeds, this positively affects the bird's ability to achieve optimal production and increase metabolic efficiency. Additionally, lycopene may have an inhibitory effect on bacterial infections, there by promoting the bird's health, which positively impacts its productive performance (Kulawik *et al.,* 2023). This is evident in absence of the negative effects of aflatoxin in the feeds containing lycopene, unlike the clear negative effects on productive performance of birds in (T2) group that only received aflatoxin B1, especially considering that birds consumed 2 ppm of aflatoxin B1/kg feed, which had a negative impact on their productive performance, as shown by data tables at end of study. Lycopene helps in inhibiting the detrimental effects of free radicals, increasing activity of cellular antioxidants, suppressing lipid peroxidation, and limiting cellular oxidative stress. Antioxidants sustain the necessary raw materials and increase their biological representation in broiler chicken diets. They protect lipoproteins and other fat compounds within the blood from oxidation, resulting in an abundance of these materials. This, reflects positively on live body weight, weight gain, and feed conversion ratio in a shorter time compared to control and second treatment groups. The decline in productivity in treatment T2 can be attributed to the negative impact of aflatoxin B1 on digestive system. Aflatoxin B1 decreases activity of digestive enzymes such as lipase, protease, and amylase, which are necessary for the digestion of nutrients required by the body to provide essential nutrients (Ali and Mousa, 2023). Additionally, a significant decline in productive traits of broiler chickens in treatment T2 was observed throughout the study weeks due to harmful effects of aflatoxin B_1 toxins, this led to a decrease in live body weight and weight gain. The negative impact of fungal toxins may be attributed to the daily intake of aflatoxin B_1 throughout the study period, resulting in the accumulation of aflatoxin toxins in liver, causing damage in liver cells and bird's inability to eliminate them (Tilley *et al.,* 2017). This

negatively affected live body weight, feed conversion ratio, and collapse of body's natural defence lines, which are inversely related to increased aflatoxin contamination levels. The effectiveness of lycopene added to diets helped reduce impact of fungal toxins and improve productive traits during study period. Lycopene acts as a substance that absorbs fungal toxins and prevents their absorption by villi in small intestines (Ogundeji *et al.,* 2023).

Table 2: Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B_1 on Live body weight ± Standard Error.

Treatments	Week 2	Week 3	Week 4	Week 5	Week 6
T1	a 438.9 ±6.7	b 828.5 ± 15.1	b 1280.8 ± 20.1	b 1977.8 ± 29.1	b 2639 ± 15.0
T2	b 396.5 ±5.9	c 782.5 ± 17.2	b 1246.0 ± 17.8	b 2004.5 ± 23.6	c 2453.3 ± 12.5
T3	a 467.1 ±6.1	a 917.5 ± 13.5	ab 1550.2 ±22.3	ab 2178.0 ±18.7	ab 3040.2 ±18.0
T4	a 459.9 ±5.8	ab 898.7 ±15.8	ab 1575.9 ±20.9	ab 2166.2 ± 13.6	ab 3024.4 ±16.9
T5	a 478.7 ±8.0	a 906.4 ± 13.4	a 1605.2 ± 20.1	ab 2197.9 ±18.9	ab 3014.8 ±16.6
T6	a 472.1 ±5.5	ab 891.9 ١١,٤±	a 1591.0 ± 18.6	a 2327.2 ± 15.4	a 3198.0 ± 17.5
P- Value	0.00	0.00	0.00	0.00	0.00

* T1 : control, T2(addition Aflatoxin B_1 with 2 ppm),
T3: (addition Aflatoxin B_1 with 2 ppm + lycopene 50 mg / kg feed),
T4: (addition Aflatoxin B_1 with 2 ppm + lycopene 100 mg / kg feed),
T5 (addition Aflatoxin B_1 with 2 ppm + lycopene 150 mg / kg feed),
T6: (addition Aflatoxin B_1 with 2 ppm + lycopene 200 mg / kg feed).
** The various letters indicate significant differences between treatments within one columns' at ($P \leq 0.005$).

Table 3 : Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B_1 on body weight gain ± Standard Error.

Treatments	Week 2	Week 3	Week 4	Week 5	Week 6
T1	ab 396.9 ± 21.9	b 389.6± 15.1	b 452.3 ± 21.3	ab 697 ±11.4	b 661.2 ±9.51
T2	b 354.5± 9.4	b 386 ± 16.9	b 463.5 ± 25.5	a 758.5 ±19.5	c 448.8 ±10.9
T3	a 425.1± 12.9	a 50.4 ± 21.0	ab 632.7±26.5	b 627.8 ± 27.2	a 862.2 ±13.3
T4	a 417.9± 19.9	a 438.8 ± 39.9	a 677.2 ±29.9	c 590.3 ±10.1	a 858.2±14.01
T5	a 436.7± 13.3	a 427.7 ± 31.4	a 698.8 ±14.2	c 592.7 ±15.1	a 816.9 ±15.0
T6	a 430.1± 59.1	a 419.8 ± 13.3	a 699.1 ±17.8	a 736.2 ±26.7	a 870.8 ±22.2
P- Value	0.000	0.000	0.000	0.000	0.000

* T1 : control, T2(addition Aflatoxin B_1 with 2 ppm),
T3: (addition Aflatoxin B_1 with 2 ppm + lycopene 50 mg / kg feed),
T4: (addition Aflatoxin B_1 with 2 ppm + lycopene 100 mg / kg feed),
T5 (addition Aflatoxin B_1 with 2 ppm + lycopene 150 mg / kg feed),
T6: (addition Aflatoxin B_1 with 2 ppm + lycopene 200 mg / kg feed).
** The various letters indicate significant differences between treatments within one columns' at ($P \leq 0.005$).

Table 4 : Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B_1 on Feed Consumed ± Standard Error.

Treatments	Week 2	Week 3	Week 4	Week 5	Week 6
T1	330±0.44	592±0.026	a 867±0.09	a 1164±0.34	a 1290±0.434
T2	344±0.053	595±0.007	a 861±0.13	a 1187±0.27	b 1176±0.339
T3	315 ±0.52	493±0.033	a 880±0.05	ab 1109±0.49	a 1315±0.439
T4	331±0.028	492±0.011	ab 830±0.27	ab 1055±0.15	ab 213±0.541
T5	317±0.043	495±0.008	b 790±0.38	b 52±0.057	ab 1230±0.312
T6	322±0032	495±0.009	b 771±0.021	b 958±0.17	ab 213±0.429
P- Value	N.S	N.S	0.000	0.000	0.000

* T1 : control, T2(addition Aflatoxin B_1 with 2 ppm),
T3: (addition Aflatoxin B_1 with 2 ppm + lycopene 50 mg / kg feed),
T4: (addition Aflatoxin B_1 with 2 ppm + lycopene 100 mg / kg feed),
T5 (addition Aflatoxin B_1 with 2 ppm + lycopene 150 mg / kg feed),
T6: (addition Aflatoxin B_1 with 2 ppm + lycopene 200 mg / kg feed).
** The various letters indicate significant differences between treatments within one columns' at ($P \leq 0.005$).

Conclusion

Lycopene extract had a significant effect in mitigating the harmful impact of aflatoxin B_1 toxins in broiler feed. Furthermore, lycopene supplementation improved productive characteristics of birds. Lycopene supplements can be used as natural additives to minimize impact of common fungal toxins and control diseases resulting from aflatoxin poisoning and that may be enhance bird's health.

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