EVALUATION OF THE EFFICACY OF LIQUORICE EXTRACT AND *SACCHAROMYCES CEREVISIAE* TO SUPPRESS THE TOXIC EFFECTS OF AFLATOXIN IN BROILERS

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ABSTRACT

The amelioration of aflatoxicosis in broilers was examined by the dietary addition of mold killer, *Saccharomyces cerevisiae* yeast, or liquorice extract. A total of 750 Fawbro broiler chicks, three weeks old were randomly divided into 5 treatment groups (control; T1, Aflatoxin (AF); T2, AF+mold killer; T3, AF+yeast; T4 and AF+liquorice extract; T5) each consisting of 150 chicks. The various parameters studied were: Live body weight (BW), weight gain (WG), feed consumption (FC), feed conversion ratio (FCR), mortality (M), Productive Index (PI), Economic Figure (EF), dressing percentage with (DPV) or without viscera (DPWV), and relative weights of liver (L), heart (H), gizzard (G), spleen (S) and abdominal fat (AFT).

Compared to control group (T1), AF treatment (T2) significantly ($p<0.05$) decreased BW, WG, FC, FCR, PI, EF, DPV and DPWV; and increased ($p<0.05$) M, L, S and AFT. The addition of mold killer (T3), yeast (T4) or liquorice extract (T5) to an AF–containing diet significantly ($p<0.05$) improved the adverse effects of AF on these characteristics. However, liquorice extract (T5) surpasses all other treatments and restore the means of these productive characters to the control values.

These results, as a conclusion, clearly demonstrated that liquorice extract (450 mg/kg of diet) is effective in preventing the deleterious effects of AF on broiler chickens.

تقييم فعالية مستخلص عرق السوس والخميرة *Saccharomyces cerevisiae* في الحد من التأثيرات السمية للافلاتوكسين في فروج اللحم

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المستخلص

أجريت هذه الدراسه لبحث امكانية تقليل التأثير السلبي للتسمم بالافلاتوكسين B1 عن طريق اضافة مادة قاتلة للفطريات Mold killer أو الخميرة *Saccharomyces cerevisiae* أو مستخلص عرق السوس الى عليقة فروج اللحم. واستخدم فيها 750 فروج لحم فلوبرو بعمر ثلاث اسابيع. تم توزيع الافراخ عشوائياً على خمس معاملات يتكون كل منها من 150 طير : مجموعة المقارنة (T1) ومجموعة المعاملة بالافلاتوكسين (T2) ومجموعة المعاملة بالافلاتوكسين + مادة قاتلة للفطريات (T3) ومجموعة المعاملة بالافلاتوكسين + الخميرة (T4) ومجموعة المعاملة بالافلاتوكسين + مستخلص عرق السوس .(T5) وتضمنت الدراسة تقويم الصفات الانتاجية التالية : معدل وزن الجسم والزيادة الوزنية واستهلاك العلف وكفاءة التحويل الغذائي ونسبة الهلاكات والدليل الانتاجي والمؤشر الاقتصادي ونسبة التصافي مع أو من دون الاحشاء الداخلية والوزن النسبي لكل من الكبد والقلب والقانصة والطحال ودهن البطن.

اشارت نتائج الدراسة الى ان المعاملة بالافلاتوكسين (T2) ادت الى انخفاض معنوي (أ < 0.05) في وزن الجسم والزيادة الوزنية واستهلاك العلف وكفاءة التحويل الغذائي والدليل الانتاجي والمؤشر الاقتصادي ونسبة التصافي مع او من دون الاحشاء الداخلية والى ارتفاع معنوي (أ < 0.05) في نسبة الهلاكات والوزن النسبي للكبد والطحال ودهن البطن مقارنةً بمجموعة المقارنة (T1) . من ناحية ثانية ، فأن اضافة المادة القاتلة للفطريات (T3) او الخميرة (T4) او مستخلص عرق السوس (T5) ادت الى تحسن معنوي في التأثيرات السلبية للتسمم بالافلاتوكسين في هذه الصفات. فضلاً على ذلك ، فأن معاملة عرق السوس (T5) قد تفوقت على باقي المعاملات واستعادت قيم مجموعة المقارنة (T1) للصفات الانتاجية التي شملتها الدراسة الحالية.

يستنتج من الدراسة الحالية ، ان اضافة مستخلص عرق السوس بتركيز 450 ملغم/كغم من العلف الملوث بالافلاتوكسين كانت فعالة في الحد من التأثيرات السلبية للتسمم بالافلاتوكسين في فروج اللحم.

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Introduction

Aflatoxins (AF) are a group of extremely toxic chemicals produced by some species of fungi in the genus *Aspergillus* and occur as natural contaminants of poultry feeds (6). AF cause severe economic losses in the poultry industries and cause a variety of effects, including anorexia, with lowered growth rate, poor feed utilization, decreased weight gain and egg production, increased susceptibility to environmental and microbial stress, and increased mortality (14). Also associated with aflatoxicosis is anaemia, inhibition of immune function and haemorrhage (13).

Practical and cost - effective methods for detoxifying of AF – containing feed and feed stuffs are in a great demand. Since the beginning of 1990s the adsorbent – based studies have been performed for removing AF from contaminated fed and minimizing the toxicosis of AF in poultry. However, some authors are concerned about the possible disadvantages of adsorbents such as required high inclusion rates and negative interactions with feed nutrients (25). Many specialists are of the opinion that the best approach for decontamination should be degradation by biological matters giving a possibility for removal of AF under mild condition, without using harmful chemicals without losses in nutritive value and palatability of detoxified feed and feedstuffs (7). The yeast (*Saccharomyces cerevisiae*) was found to have beneficial effect on physiological status in broilers exposed to AF (3). A recent study made with liquorice extract showed significant improvements to counteract aflatoxicosis in broiler chickens (4).

In an effort to develop a practical method for AF detoxification, therefore, the objective of this study using broiler chickens was to examine the toxic effects of AF on productive performance of broilers, to evaluate the repressive efficacy of dietary liquorice extract or yeast (Saccharomyces *cerevisiae*), and to compare the efficiency of these two materials in counteracting the aflatoxicosis in broilers.

Materials and Methods

This study was conducted at the Animal Production farm/State Board of Agricultural Research during the period from 21/3/2002 to 27/4/2002. A total of 750 Fawbro broilers, three weeks old were divided at random into 5 treatments with 3 - replicates of equal groups (150 chicks per each treatment group). All birds fed a starter diet during 3rd week of age (beginning of experiment ; 22.7 % crude protein and 2867.4 k cal ME/kg of diet) and finisher diet (20.6 % crude protein and 2922 k cal ME/kg of diet) until the end of experiment (7 weeks of age).

Birds in treatment 1 (T1) fed a basal diet and used as a control group. Those in treatment 2 (T2) were fed a diet contaminated with AF, while birds in T3 were fed a diet contaminated with AF and supplemented with 150 mg mold killer/kg of diet (Choong ang Biotech company, Korea). However, birds in T4 were fed a diet contaminated with AF and treated with *Saccharomyces cerevisiae* yeast. The commercial bread yeast (Pakmaya) was grown on Sabround Dextrose agar. The microbial count was taken per each gram of commercial yeast by using the procedure of diffusion on Petri dishes (11). The level of yeast in the diets was determined so that 1 gm of commercial yeast powder contains 256 X 10^8 germ cells of *S. cerevisiae* bread yeast. Besides, T5 birds were fed a diet contaminated with AF and supplemented with liquorice extract at a level of 450 mg/kg of diet. This level of liquorice extract was chosen on the basis that it recorded the best results as regards productive performance of broilers in comparison with other levels (150 and 300 mg/kg) that used in our previous studies (2).

AF used in this study was AFB1 which was brought from the Department of Plant Protection, College of Agriculture, University of Baghdad. AF was produced from *Aspergillus parasitcus* culture through fermentation of rice by the method of Shotwell et al. (32). Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. The total AF content in the rice powder was analyzed spectrophotometrically (18).The rice powder was incorporated into the basal diet to provide the required amount of 2 mg AF/kg feed.

Productive traits involved in the present study were : Live body weight (BW), weight gain (WG), feed consumption (FC), feed conversion ratio (FCR), and mortality rate (M). However, Productive Index (PI) and Economic Figure (EF) were calculated according to Naji and Hana (19). At the end of experiment, 18 birds per each treatment (6 birds of each replicate) were sacrificed to determine dressing percentage with (DPV) or without viscera (DPWV), and weights

of visceral organs, *viz.* liver (L), heart (H), gizzard (G), spleen (S), in addition to abdominal fat (AFT). The results obtained were statistically analyzed by using SAS (30). The statistical significance is based on $p < 0.05$.

Results and Discussion

The results presented in Tables 1 – 5 show the effects of dietary treatments on BW, WG, FC, FCR, M, PI and EF. Feeding the diet with AF alone (T2) significantly ($p < 0.05$) suppressed all these productive traits from the fourth week onwards compared to control group (T1). Supplementation of mold killer (T3), yeast (T4) or liquorice extract (T5) significantly ($p < 0.05$) improved BW, WG, FC, FCR, M, PI and EF in comparison with T2 group. However, T5 surpasses all other treatments and restore the means of characters mentioned hereinabove to the control values (Tables 1 – 5).

The most prevalent symptom of aflatoxicosis in poultry is poor performance and reduced growth rate. The failure in BW, WG and FCR will lead to economic losses and also sever AF-dependent diseases in poultry flocks. The adverse effects seen in the present study in relation to productive characteristics involved may be due to anorexia, listlessness, inhibition of protein synthesis and lipogenesis (22, 26). However, impaired liver functions and protein/lipid/carbohydrate utilization mechanisms may have also affected the growth performance and general health of birds (16, 23).

Stanley et al. (33) pointed out that live yeast can be used as a performance promoter in broilers exposed to AF. The positive effects of yeast have been later attributed to Mannan Oligosaccharide (MOS) derived from cell walls of yeast. The researchers discovered and extracted MOS and used for removing pathogenic bacteria from the intestine (10) and immuno – modulation (12) in poultry. The studies performed by MOS extracted from *S. cerevisiae* yeast with different concentrations of AF in broilers (27, 29) showed that yeast extract partially and/or completely reversed the detrimental effects of AF on performance, immune response and biochemistry – haematology of birds.

Al–Daraji et al. (5) found that supplementation of the liquorice extract, especially at the level of 450 mg/kg of diet effectively diminished the adverse effects of aflatoxicosis on haematological and biochemical traits of broilers. However, these authors concluded that the role of liquorice extract in AF detoxification may be attributed to two mechanisms : First, it may have selective binding capacity for AF molecules in gastrointestinal tract, and the second that liquorice shows some anti – infective properties, hence this herb destroys or prevent the growth of fungi on the diet (35). In laboratory and animal studies, liquorice has stopped or slowed the growth of certain bacteria, fungi, and parasites (1). Several animal studies have also revealed a possibly strong antiviral and fungicide effects for true liquorice (9). In these studies, true liquorice component that belong to isoflavonoid class of chemicals, appear to have several anti–infective effects that include interference with oxygen utilization by infective–organisms. Additionally, true liquorice may have some ability to improve functioning of the immune system (31).

Feeding AF alone (T2) also caused significant ($p < 0.05$) decreases in DPV and DPWV, while significant ($p < 0.05$) increases were found in relative weights of L, S and AFT compared with T1 group (Table 6). However, there were no significant differences observed between T1 and T2 group regarding relative weights of H and G. Furthermore, inclusion the mold killer (T3), yeast (T4) or liquorice extract (T5) in the AF – containing diet significantly ($p < 0.05$) ameliorated the adverse effects of AF on DPV, DPWV, L, S and AFT, with the exception for S when the differences between T2, T3 and T4 treatments lack the significance ($p>0.05$). Besides, there were no significant differences between experimental treatments in regard to H and G. However, liquorice treatment (T5) was superior to other treatments (T3 and T4) with relation to DPV, DPWV, L, S, and AFT and it also restore the means of these characteristics to the control values (Table 6).

Chronic and sub – clinical aflatoxicosis cases may be diagnosed by determining organ weight alterations when major symptoms became apparent. The changes in organ weights as a result of toxic effects of AF are well – investigated and well – known subject and these were clearly seen in the present study. The increases in relative weights of L, S and AFT could be attributed to an increase in lipid deposition due to impaired fat metabolism (34). Safameher et al. (28) found that livers of the intoxicated chicks

were larger, yellowish, fatty and more friable than those of control chickens. Ortatatli et al. (24) pointed out that AF can cause important pathological changes in L such as enlarging, paleness, hydropic/fatty degenerations, kidney and spleen lesions, reproductive changes, impairment in the humoral and cellular immune responses. Mashaly et al. (15) reported that AF feeding at 50 g/kg diet resulted in a significant decline in body, carcass and L weights, rate of liver protein and RNA synthesis, and muscle RNA synthesis. Waldroup (36) indicated that some of the most common effects of aflatoxicosis are: pale and enlarged L, swollen of kidney and spleen, oral lesion, increased susceptibility to bruising, decrease bone strength and increased intestinal fragility.

Liquorice extract supplementation significantly improved carcass traits included in this study in comparison with T2 group. These beneficial effects may due to its ability to trap the AF irreversibly. Ngo et al. (21) studied three liquorice compounds (extract 348, carbenoxolone, and glycyrrhetinic acid) for their effects on AFB1 – induced mutagenesis using *Salmonella typhimurium* as the bacterial tester strain, and rat liver supernatant (S–9) as the metabolic activation system. They found that all three compounds exhibited a concentration– dependent inhibition of S–9 mutagenesis induced by AFB1. However, these three compounds also significantly decreased the activation of AFB1 to mutagenic/carcinogenic metabolites. Muravev et al. (17) reported that liquorice has superb anti–inflammatory and emollient actions on the skin, and may be used on any inflammatory skin problem such as eczema or psoriasis. However, certain constituents in liquorice appear to be anti – fungal and anti viral. By conducting experiments on rats with toxic hepatic damage induced by tetrachloromethane, Nasyrov et al. (20) have shown that the derivatives of glycyrrhizinic acid promote a decrease in the rate lipid peroxide oxygenation in the hepatic tissue homogenate and the blood serum, in the inhibition of organo - specific enzyme activity (AST, ALT, BHMT) and increase in choleresis. However, results of this study also testify to the hepatoprotective activity of the derivatives of glycyrrhizinic acid. Wang et al. (37) pointed that glycyrrhizin could protect the liver from hepatotoxin – induced liver injury and cirrhosis, improve ALT levels and act as an anti fibrotic agent. Bown (8) reported that liquorice is a very sweet, moist, soothing herb that detoxifies and protects the liver, kidney and spleen, and is also powerfully anti – inflammatory, being used in conditions as varied as arthritis and mouth ulcers.

The results clearly demonstrated that growth performance (BW, WG, FC and FCR), carcass traits (DPV and DPWV 0 and relative organ weights (L, S and AFT) of broilers were negatively affected by feeding of AF contain diet for 5 weeks ; and the addition of liquorice extract (450 mg/kg) to AF– containing diet significantly recovered and ameliorated the adverse effects of AF on productive performance of broilers. These improvements obtained in this study contribute a safe and practical decontamination procedure, and may contribute to a solution of AF problem in poultry when used in conjunction with other AF prevention practices.

Table 1. Influence of dietary supplementation with *Saccharomyces cerevisiae* or licorice extract on the mean body weight (g) of broilers during aflatoxicosis

Treatments / Age (weeks)	T1	T2	T3	T4	T5
3	A 345.3 ± 16.2	A 338.4 ± 18.6	A 342.1 ± 17.3	A 343.8 ± 18.1	A 345.1 ± 17.6
4	A 627.1 ± 25.3	C 591.2 ± 28.1	B 613.4 ± 25.7	AB 619.7 ± 24.4	A 625.9 ± 24.9
5	A 935.7 ± 30.2	C 869.6 ± 34	B 911.2 ± 30.2	A 930.3 ± 29.7	A 934.4 ± 30.0
6	A 1275.5 ± 35.6	D 1157.8 ± 37.4	C 1213.5 ± 35.6	B 1259.0 ± 33.8	A 1274.3 ± 34.7
7	A 1628.8 ± 48.6	D 1464.7 ± 53.4	C 1560.8 ± 51.5	B 1607.7 ± 47.0	A 1625.9 ± 49.3

*** Means in the same row with different superscripts refer to significant differences ($p < 0.05$).**

Table 2. Influence of dietary supplementation with *Saccharomyces cerevisiae* or licorice extract on the weight gain (g) of broilers during aflatoxicosis

Treatments / Age (weeks)	T1	T2	T3	T4	T5
3	A 175.3 ± 10.6	A 168.4 ± 14.0	A 172.1 ± 13.2	A 173.8 ± 11.2	A 171.4 ± 12.4
4	A 281.8 ± 13.7	C 252.7 ± 16.3	B 271.3 ± 13.8	AB 275.9 ± 14.6	A 280.7 ± 15.4
5	A 308.5 ± 18.3	C 278.4 ± 22.1	B 297.7 ± 14.9	A 310.5 ± 16.6	A 308.4 ± 17.5
6	A 339.8 ± 20.4	D 288.1 ± 25.0	C 302.3 ± 20.5	B 328.7 ± 21.4	A 339.9 ± 18.7
7	A 353.3 ± 23.0	C 306.9 ±23.2	B 347.2 ± 22.5	B 348.6 ± 24.8	A 351.5 ± 22.3

*** Means in the same row with different superscripts refer to significant differences ($p < 0.05$).**
**** T1= Birds fed a basal diet, T2= Birds fed diet contaminated with aflatoxin, T3=Birds fed diet contaminated with aflatoxin and treated with mold killer, T4= Birds fed diet contaminated with aflatoxin and treated with *Saccharomyces cerevisiae*, T5= Birds fed diet contaminated with aflatoxin and supplemented with 450 mg/kg licorice extract.**

Table 3. Influence of dietary supplementation with *Saccharomyces cerevisiae* or licorice extract on the feed consumption (g/bird) of broilers during aflatoxicosis

Treatments / Age (weeks)	T1	T2	T3	T4	T5
3	A 405.3 ± 25.9	C 387.1 ± 35.9	B 395.5 ± 32.2	A 401.8 ± 29.4	A 402.5 ± 27.3
4	A 590.1 ± 40.6	D 548.8 ± 46.9	C 575.4 ± 44.1	B 581.7 ± 37.8	A 589.4 ± 42.7
5	A 735.7 ± 53.9	B 701.4 ± 65.8	B 707.7 ± 57.4	A 742.0 ± 58.1	A 734.3 ± 56.0
6	A 835.8 ± 65.8	C 762.3 ± 70.7	B 796.6± 68.6	A 826.7 ± 61.6	A 834.4 ± 63.0
7	A 880.6 ± 87.5	C 821.1 ± 93.1	B 856.1 ± 72.1	AB 867.3 ± 81.9	A 878.5 ± 13.3

*** Means in the same row with different superscripts refer to significant differences ($p < 0.05$).**

Table 4. Influence of dietary supplementation with *Saccharomyces cerevisiae* or licorice extract on the feed conversion efficiency (g feed/g of wt.gain) of broilers during aflatoxicosis

Treatments / Age (weeks)	T1	T2	T3	T4	T5
3	A 2.31 ± 0.18	A 2.31 ± 0.24	A 2.30 ± 0.20	A 2.31 ± 0.15	A 2.35 ± 0.17
4	B 2.11 ± 0.13	A 2.17 ± 0.18	B 2.12 ± 0.14	B 2.11 ± 0.13	B 2.10 ± 0.12
5	E 2.37 ± 0.19	A 2.78 ± 0.27	B 2.61 ± 0.19	C 2.58 ± 0.18	D 2.38 ± 0.20
6	C 2.46 ± 0.20	A 2.65 ± 0.31	A 2.63 ± 0.21	B 2.52 ± 0.22	C 2.46 ± 0.23
7	B 2.49 ± 0.21	A 2.68 ± 0.27	B 2.47 ± 0.19	B 2.49 ± 0.22	B 2.49 ± 0.23

*** Means in the same row with different superscripts refer to significant differences ($p < 0.05$).**
**** T1= Birds fed a basal diet, T2= Birds fed diet contaminated with aflatoxin, T3=Birds fed diet contaminated with aflatoxin and treated with mold killer, T4= Birds fed diet contaminated with aflatoxin and treated with *Saccharomyces cerevisiae*, T5= Birds fed diet contaminated with aflatoxin and supplemented with 450 mg/kg licorice extract.**

Table 5. Influence of dietary supplementation with *Saccharomyces cerevisiae* or licorice extract on certain productive traits of broilers during aflatoxicosis

Treatments / Traits	T1	T2	T3	T4	T5
Final body weight (g)	A 1628.8 ± 48.6	D 1464.7 ± 53.4	C 1560.8 ± 51.5	B 1607.7 ± 47.0	A 1625.9 ± 49.3
Mean weight gain (3 – 7 weeks ; g)	A 291.7 ± 16.3	C 258.9 ± 19.4	B 278.1 ± 16.4	A 287.5 ± 16.0	A 290.4 ± 15.7
Mean feed consumption (3 – 7 ; g)	A 3447.5 ± 160	D 3220.7 ± 181	C 3331.3 ± 168	B 3419.5 ± 166	A 3439.1 ± 167
Mean feed conversion efficiency (3 – 7 weeks)	C 2.35 ± 0.13	A 2.52 ± 0.17	B 2.43 ± 0.20	BC 2.40 ± 0.15	C 2.36 ± 0.14
Total mortality (%)	C 1.39 ± 0.09	A 2.69 ± 0.15	B 1.62 ± 0.07	C 1.40 ± 0.05	C 1.41 ± 0.05
Productive Index	A 195.2 ± 13.4	C 161.6 ± 16.3	B 180.5 ± 14.8	B 188.7 ± 13.3	AB 190.1 ± 14.4
Economic Figure	A 196.0 ± 15.0	C 162.1 ± 16.1	B 181.0 ± 15.2	B 189.2 ± 14.9	AB 191.0 ± 15.2

*** Means in the same row with different superscripts refer to significant differences ($p < 0.05$).**
**** T1= Birds fed a basal diet, T2= Birds fed diet contaminated with aflatoxin, T3=Birds fed diet contaminated with aflatoxin and treated with mold killer, T4= Birds fed diet contaminated with aflatoxin and treated with *Saccharomyces cerevisiae*, T5=Birds fed diet contaminated with aflatoxin and supplemented with 450 mg/kg licorice extract.**

Table 6. Influence of dietary supplementation with *Saccharomyces cerevisiae* or licorice extract on carcass characteristics and organ weights of broilers during aflatoxicosis

Treatments / Age (weeks)	T1	T2	T3	T4	T5
Dressing percentage (with viscera)	A 72.8 ± 5.6	D 71.9 ± 6.7	C 72.5 ± 5.7	B 72.6 ± 5.1	A 72.8 ± 5.5
Dressing percentage (without viscera)	A 65.1 ± 5.3	D 63.9 ± 6.6	C 64.7 ± 5.1	B 64.8 ± 4.8	A 65.1 ± 4.4
Abdominal fat (%)	C 1.04 ± 0.009	A 1.11 ± 0.013	AB 1.09 ± 0.007	C 1.06 ± 0.008	C 1.05 ± 0.009
Liver weight (%)	C 3.11 ± 0.05	A 3.29 ± 0.07	B 3.17 ± 0.04	B 3.16 ± 0.04	C 3.12 ± 0.05
Gizzard weight (%)	A 2.80 ± 0.02	A 2.83 ± 0.02	A 2.80 ± 0.02	A 2.81 ±0.17	A 2.79 ± 0.18
Spleen weight (%)	B 0.15 ± 0.001	A 0.18 ± 0.003	A 0.17 0.001	A 0.17 ± 0.002	B 0.13 ± 0.001
Heart weight (%)	A 0.57 ± 0.004	A 0.60 ± 0.007	A 0.60 ± 0.005	A 0.58 ± 0.004	A 0.57 ± 0.006

*** Means in the same row with different superscripts refer to significant differences ($p < 0.05$).**
**** T1= Birds fed a basal diet, T2= Birds fed diet contaminated with aflatoxin, T3=Birds fed diet contaminated with aflatoxin and treated with mold killer, T4= Birds fed diet contaminated with aflatoxin and treated with *Saccharomyces cerevisiae*, T5= Birds fed diet contaminated with aflatoxin and supplemented with 450 mg/kg licorice extract.**

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