

EFFECT OF SACCHAROMYCES CEREVISIAE AND VITAMIN C SUPPLEMENTATION ON PERFORMANCE OF BROILERS SUBJECTED TO OCHRATOXIN A CONTAMINATION

By

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Abstract : *Dried yeast Saccharomyces cerevisiae (SC) and vitamin C (Vit. C) were used in this study as a feed supplements to ameliorate the deleterious effect of Ochratoxin A (OTA) on broiler performance and relative organs weight. A total of 368 Ross male broiler chicks were randomly distributed according to diet supplementation into eight groups (46 chicks each) as follow. T1: fed a broiler diet without any supplementation (control) ; T2: fed the control diet supplemented with 3 g SC / kg diet; T3: fed the control diet supplemented with 300 mg Vit.C / kg diet; T4: fed the control diet supplemented with 3 g SC + 300 mg Vit.C / kg diet; T5: was fed the control diet contaminated with 200 ppb of OTA. While, T6: fed the control diet contaminated with 200 ppb OTA and supplemented with 3 g SC / kg diet; T7: fed the control diet contaminated with 200 ppb OTA and supplemented with 300 mg Vit.C / kg diet and T8: fed the control diet contaminated with 200 ppb OTA and supplemented with 3 g SC+300 mg Vit.C / kg diet. Results showed that feeding OTA at 200 ppb (T5) decreased significantly body weight and feed consumption. Moreover, a significant increase in relative weights of kidney, liver, gizzard, proventriculus, in addition to high mortality percent and worse feed conversion ratio were observed in OTA-fed chicks. On the other hand, addition of either SC alone or in combination with-Vit C may ameliorate the toxic effects of OTA. However, Vit. C alone did not prevent the negative effects of OTA observed in chicks.*

INTRODUCTION

Mycotoxins are secondary metabolites synthesized by different filamentous fungi that grow on organic material frequently occurring in agricultural products and foodstuffs (Serra *et al.*, 2005). The most common mycotoxins are aflatoxins, ochratoxins, tricothecenes, zearalenone and fumonisins (Engelhardt *et al.*, 1999).

Over 300 mycotoxins have been reported in the literature. Target sites and the mechanisms of toxicity vary for each one, due to their chemical structure. The food contamination are found not only in cereals (wheat, maize and barley), but also in beans, dried fruits, coffee and cocoa. It is also found in animal blood and tissues (Miraglia *et al.*, 1993).

The family of ochratoxins consists of three members known as ochratoxin A, ochratoxin B and ochratoxin C but ochratoxin A (OTA) is the most toxic one (Chang *et al.*, 1979). They are the second major group of mycotoxins characterized after the discovery of aflatoxins. OTA is an isocoumarin derivative linked through the carboxy group to a L-phenylalanine (Engelhardt *et al.*, 1999). The fungi responsible for the production of OTA (*Aspergillus ochraceus*, *Aspergillus carbonarius*, *Penicillium verrucosum*, *Penicillium viridicatum*, *Penicillium nordicum*, and others) are found all over the world because they are able to develop under quite different conditions of moisture, pH and temperature (Zimmerli and Dick, 1996).

OTA causes significant losses to the poultry industry due to its effects on performance and health. It causes a reduction in growth rate, feed consumption, poor feed conversion ratio and higher mortality (Verma *et al.*, 2004; and Elaroussi *et al.*, 2006 and 2008). suppression of immune function (Stoev *et al.*, 2000, 2002; Santin *et al.*, 2002; and Politis *et al.*, 2005) and impairment of blood coagulation (Raju and Devegowda, 2000). OTA is a teratogenic (Mayura *et al.*, 1984), genotoxic (Pfohl-Leszkowicz *et al.*, 1991) and carcinogenic (Kanisawa and Suzuki, 1978). It is a known as a renal and hepatic carcinogen. Also, OTA induces degenerative changes and an increase in the weight of the kidney and liver, as well as a decrease in the weights of the lymphoid organs (Stoev *et al.*, 2000, 2002. and Elaroussi *et al.*, 2006 and 2008).

According to FAO, when all prevention methods are correctly carried out, 25% of the world grain production is still contaminated with mycotoxins. Prevention and control of mycotoxins is not easy task. A number of approaches have been tried but because of the large number of

toxins and their interactions, a single solution to the problem does not exist (Mannon and Johnson, 1985).

Lately, the more promising and practical approaches to counteract mycotoxins are the use of organic and inorganic adsorptives and vitamin supplements of livestock feeds. In this way, however studies showed that aluminosilicates can reduce the effects of aflatoxin due to its capacity to bind aflatoxin (Kubena *et al.*, 1990). However, these adsorbents did not ameliorate the deleterious effects of ochratoxin (Santin *et al.*, 2002), probably due to variation in the structure of mycotoxins. On the other hand, a live yeast, *Saccharomyces cerevisiae* was found to alleviate the adverse effects of aflatoxicosis in poultry (Stanley *et al.*, 1993), and these beneficial effects have been attributed to cell wall of *Saccharomyces cerevisiae*.

The addition of naturally occurring inert adsorbents to mycotoxin-contaminated feed has been a popular approach to decrease the toxicity as well as the carry-over of mycotoxins from contaminated feed to animal by-products. These adsorbents act by decreasing bioavailability (by adsorption through animal's gastrointestinal tract) and distribution through the target organs (Galvano *et al.*, 2001; and Council for Agricultural Science and Technology (CAST, 2003).

At the same time, supplementation of vitamin C has produced the beneficial effects in layers exposed to dietary OTA under normal (25°C) and high (33°C) ambient temperatures. OTA decreased egg weight, egg mass, feed intake and increased shell elasticity at both temperatures. Addition of vitamin C tended to counteract these negative effects and also all the effects of OTA on electrolyte concentration and AST activity were moderated by supplementing Vit.C into the diets. (Haazele *et al.*, 1993)

In this respect, the present study was aimed to determine if the addition of *Saccharomyces cerevisiae*(SC) and vitamin C either alone or in combination would ameliorate the deleterious effects of ochratoxin A in male broiler chicks.

MATERIALS AND METHODS

This study was carried out at Poultry Research Unit, Biological Application Department, Nuclear Research Center at Inshas, to evaluate the effect of *Saccharomyces cerevisiae* (SC) and vitamin C (Vit.C) supplementation on performance of broilers subjected to ochratoxin A (OTA) contamination. A total number of three hundred and sixty eight day-old male Ross broiler chicks were used. At 3- days of age, chicks were weighed and randomly allotted to eight groups (46 chicks each) and treated

as shown in (Table 1) until 5 weeks of age. Chicks were housed in batteries and kept under standard hygienic and similar environmental conditions and were supplied with feed and water *ad libitum* throughout the whole experimental period. Commercial broiler starter and grower diets were used in this study and were free from any medication and tested to be free from other mycotoxins by immunoaffinity, which showed them to be below the detection limit. The ochratoxin A and other mycotoxins were assayed prior to start of the trial and again at all feed addition to chicks to provide the levels employed of ochratoxin A per kilogram of finished feed. Chicks were fed a commercial starter diet (22.2 % crude protein and 2925 kcal ME / kg feed) from day 1 to 19 days, then a grower diet (20.8 % crude protein and 3076 kcal ME/kg feed) from day 20 to 5 weeks (Table 2). These commercial broiler starter and grower diets (to which OTA was added), were formulated to meet or exceed the nutritional requirements of broilers recommended by the National Research Council (NRC,1994).

Ochratoxin A (OTA) was supplied by *Aspergillus ochraceus* NRRL 3174 culture. Production and extraction of OTA were performed by the method of Davis *et al.* (1969) and purification and clean-up of the toxin were performed according to Nesheim (1969). Quantitative determination of OTA was conducted using a basic methanolic extraction, monoclonal antibody immunoaffinity column clean-up and a fluorometric detection method according to Scott and Kanhere (1995). Pure chloroform-extracted dry OTA was dissolved in 95% ethanol and mixed with a small portion of feed, the ethanol was allowed to evaporate and then it was mixed with the remainder of the feed in a horizontal mixer to provide 200 ug OTA/kg finished feed. During the preparation of the control diet, an equal amount of ethanol was added and allowed to evaporate in a fashion similar to that of the OTA-treated feed.

MEASUREMENTS

Birds were individually weekly weighed from the beginning till the end of the experiment to the nearest gram to determine the average weekly body weight. Feed consumption was recorded daily and the mean was calculated weekly up to the end of the experiment. Feed conversion ratio was calculated for each group at the end of the experiment as gram feed per gram body gain. Mortality rate was calculated as a percentage of total number of birds weekly for each group through the experimental periods.

At the end of each week, five birds from each group were selected at random, weighed, starved for 12 hours then sacrificed, dissected and the liver, kidney, gizzard and proventriculus were extracted and blotted dry then

organ weights were determined as relative weights with respect to the live body weight.

STATISTICAL ANALYSIS

Data that were collected in the study were statistically analyzed using the one – way analysis of variance using the general linear models (GLM) of (SAS, 2000). The statistical model used was as follow:

$$Y_{ij} = U + T_i + e_{ij}$$

Where

Y_{ij} : Performance traits measured on the J^{th} broilers in the i^{th} treatment

U: Overall mean

T_i : Effect of the treatments ($i = 1, 2, 3, \dots$)

e_{ij} : Random error effect

Significant differences among means were separated using the Duncan Multiple Range Test, (Duncan, 1955).

RESULTS AND DISCUSSION

1. Productive performance:-

1.a. Body weights:-

Ochratoxin might cause significant losses to poultry industry due to reduced performance and health problems in the exposed birds. Results in (Table 2) showed that body weight was decreased ($p < 0.05$) significantly in OTA- treated group (T5) throughout the duration of the experiment as compared to all experimental groups.

Supplementing broiler diets with *Saccharomyces cerevisiae* (SC) either alone (T2) or in combination with Vit C (T4) in absence of OTA recorded significantly a higher body weight ($p \leq 0.05$) than all experimental groups. There was no significant difference between Vit C group (T3) and control group (T1). At the same time, *Saccharomyces cerevisiae* (SC) either alone (T6) or in combination with Vit C (T8) showed to minimize the deleterious effects of OTA on body weight, also addition of Vit C alone (T7) may counteract the toxic effects of OTA on body weight but to a lesser extent when compared with T6 (SC) and T8 (SC + Vit.C).

The reduction in body weight due to ochratoxicosis in broilers in the present study is in agreement with the previous reports of Kubena *et al.*, (1997); Raju and Devegowda (2000), Stoev *et al.*, (2002), and Verma *et al.*,

(2004). Also, Garcia *et al.*, (2003) pointed out that broiler chicks fed OTA-contaminated diet at level of 567 ppb had a lower body weight than the control birds. Furthermore, Elaroussi *et al.*, (2006) reported a significant decrease in body weight of broiler chicks fed a contaminated diet with OTA at level of 400 and 800 ppb.

The depression in body weight occurred during ochratoxicosis in this study may be attributed to many factors. OTA affect protein synthesis through competitive inhibition of phenylalanine-t-RNA-synthesis by phenylalanine moiety of the toxin. (Konrad and Roschenthaler, 1977 and Bung *et al.*, 1978). Moreover, ochratoxin A interferes with DNA, RNA and protein synthesis and affect carbohydrate metabolism, particularly glucogenolysis. The loss in body weight during ochratoxicosis may be due to the reduction in feed intake which caused decrease in total serum proteins (Prior *et al.*, 1980, 1981).

On the other hand, Santin *et al.* (2001) showed that cell wall of *Saccharomyces cerevisiae* improve the intestinal mucosa aspects and suggested that it might be the explanation for the improve in performance of broilers supplemented with *Saccharomyces cerevisiae*. Furthermore, Cooney (1980) explained the ability of active yeast to alleviate the aflatoxicosis effects, via chelating aflatoxins, which is transported to and eliminated via intestinal tract. Live yeast (*saccharomyces cerevisiae*) at level of 0.1% was found to alleviate the adverse effects of aflatoxicosis on body weight (Stanley *et al.*, 1993).

Glucomannans extracted from the external parts of *Saccharomyces cerevisiae* are able to bind certain mycotoxins. This great binding capacity results from the large area available for exchange, thus 500 g of glucomannan have the same capacity adsorption as 8 kg of clay (Ahokas *et al.*, 1998).

In respect to vitamin C, McKee and Harrison, (1995) found that dietary supplementation with vitamin C increased the growth rate by 4.5% and improved the tolerability to stress and reduced the mortality by 5%. Moreover, Kultu, (2001) stated that ascorbic acid supplementation increased body weight gain, carcass weight, while reduced carcass crude fat content, and concluded that ascorbic acid supplementation improved the performance of broiler chicks with experimentally induced hypothyroidism. Also, Mona *et al.*, (2004) reported that ascorbic acid improved average body weight and this effect may be related to collagen synthesis, calcium and vitamin D3 metabolism as well as carnitine synthesis required for oxidation of fatty acid to obtain energy.

1.b. Feed consumption (FC) and feed conversion ratio (FCR)

Results in table (3) showed that the amount of feed consumed (FC) per chick per week was negatively affected with OTA contamination. There was a trend for reduced feed consumption throughout the experimental period in birds fed the OTA-containing diet (T5). Addition of *Saccharomyces cerevisiae* either alone or with Vit C (T2 and T4, respectively) in broiler diets without OTA increased feed consumption compared to the control group (T1), while, adding Vit C alone (T3) did not differ than the control. At the same time, addition of *Saccharomyces cerevisiae* either alone or in combination with Vit C (T6 and T8, respectively) to broiler diets contaminated with OTA showed to reduce the toxic effects of OTA on broilers while addition of Vit C (T7) alone did not exerts that effects in presence of OTA contamination.

Results of FCR (Table 3) showed that the quantity of feed required per unit of weight increased in OTA-fed group (T5) (1.865) than the control group (1.679). Broiler group received Vit C alone in their diet in the absence of OTA (T3) had the same feed conversion ratio of the control group. While, the combination of Vit C and *Saccharomyces cerevisiae* (T4) improved feed conversion. On the other hand, supplementing broiler diets with *Saccharomyces cerevisiae* alone (T6), and combined with Vit.C (T8) in the presence of OTA improved feed conversion ratio compared to the unsupplemented OTA group (T5)

The reduction in feed intake in broiler chicks consuming OTA in their diet observed in the current study are supported and came in a close agreement with those reported by Prior *et al.* (1980 and 1981) who found that OA caused lower feed consumption in both broiler chicks and laying hens. They also reported that the cause of this poor acceptability of OA-contaminated diet was not ascertained. In this respect, Raina *et al.* (1991) showed that broiler chicks receiving OA in their diets showed poor feed conversion and reduced feed consumption rate.

El-Karim *et al.* (1991) and Elkady (1993) found that feed conversion ratio was increased in the OA-fed birds and this increment may be due to impaired protein metabolism., feed intake was decreased significantly by 21 % and feed: gain ratio increased by 17 % in male Leghorn chicks fed dietary OA-supplementation for 14 days from hatch compared to that of chicks received OA-free diets (Hoehler and Marquardt, 1996).

On the other hand, Prior *et al.*, (1980) observed that the loss in body weight during ochratoxicosis was not due to a direct effect of OTA, but rather to the reduced feed intake that led to a decreased total serum proteins

or hypoproteinaemia.. Furthermore, the FCR was altered in a manner consistent with dietary OTA level and agreed with the findings of several others (Raju and Devegowda, 2000; Garcia *et al.* , 2003; Verma *et al.*, 2004 ,and Elaroussi *et al.*, 2006).

At the same time, Santin *et al.*, (2001,2003 and 2006) reported that cell wall of *Saccharomyces cerevisiae* improved the intestinal mucosa aspects and it might be the explanation for the improve in performance of broilers. The cell wall of yeast is normally constituted of mannan oligosaccharides and the use of theses compost have been shown to improve feed conversion of birds (Savage and Zakrzewska, 1997 and Fritts and Waldroup, 2003).

1.c. mortality percentage

It can be clearly observed that birds received diet supplemented with OTA recorded the highest mortality percent than the other groups followed by T7 (OTA +Vit.C) when compared with other groups. The mortality percentage which occurred in the T3 during the experimental period lied under the normal range. While, mortality percent of the group received OTA (T5) was above the normal range by about 6-8 % (Table 4). Addition of *Saccharomyces cerevisiae* either alone or in combination with vit.C through OTA contamination may counteract the adverse effect of OTA on broiler mortality. On the other hand, Vit.C supplementation may contribute to prevent the toxic effects of OTA on mortality percent by about 50% (14.1 and 7.23 % for T5 and T7, respectively)

The increase in mortality percentage of broilers fed OTA-supplemented diet was in accordance with the findings of many investigators. In this respect, Gibson *et al.* , (1989) found that feeding OTA contaminated diets at 2 to 4 parts/ 10^6 , reported mortality that reached 21.9% in OTA-treated broiler chicks versus 2.5% for the controls. Niemiec *et al.*, (1991) reported that OTA caused high mortality percent being 22.2 % when broiler chicks was fed contaminated diet with 1.5 ug OA / g diet for 5 weeks of age. McKee and Harrison (1995) found that dietary supplementation with vitamin C increased the growth rate by 4.5% and improved the tolerability to stress and reduced the mortality by 5%.. Furthermore, Elaroussi *et al.*, (2006) reported that mortality percent reached about 5.23% and 12.98 % in broiler chicks fed a diet supplemented with OTA at levels of 400 and 800 ppb, respectively compared with 1.25 % for control.

2. Relative organ weights:

2.a. Kidney and liver weights:-

Kidney is a target organ for the toxic action of OTA and the primary area of attack of OTA is the kidney. Ochratoxin A caused significant increase in the relative kidney weight in ochratoxicated birds group (T5) birds when expressed as a percent of live body weight. In control group, the relative kidney weight was decreased as the age of the birds increased, these status were quite converse in OA-treated group (Table 5)

The effects of OTA on the relative kidney weight was started from the 2nd week and continue through the experimental period. On the other hand, supplemented broiler diets with *Saccharomyces cerevisiae* and / or vitamin C in the absence of OTA was differ but not significantly than control birds. At the same time, addition of *Saccharomyces cerevisiae* alone or in combination with Vit.C in presence of OTA may prevent the toxic action of the toxin on relative kidney weight, while addition of Vit.C alone with OTA did not prevent any changes of kidney weight resulted from the toxin.

Liver is the principle detoxification organ in the body. Ochratoxin A is known to be primarily nephrotoxin in poultry species and the kidney is a target organ for the toxic action of OA. In addition to that, it was found also in this investigation that OA caused detrimental effects in broilers liver that lead to the conclusion that OA had also hepatotoxic properties. This hepatotoxic action was manifested by the significant increase in the relative liver weight in broilers that were subjected to OA toxicity in their diets (T5) compared to the controls (T1). This increase in the relative liver weight was started at the third week of age and continued throughout the experimental period. Also, supplementing broiler diets with *Saccharomyces cerevisiae* and / or Vit.C in absence of OTA (T2, 3 and 4) did not differ significantly than control group (T1). Addition of *Saccharomyces cerevisiae* either alone or in combination with Vit.C in the broiler diet contaminated with OTA (T6 and T8) may alter the toxic effects of OTA on the relative liver weight especially for T8, while T6 did not express the same action as T8. On the other hand, supplementing broiler diet with Vit.C alone in the presence of OTA (T7) did not shows any significant alteration in the relative liver weight compared to ochratoxicated group (T5).

The increase in the kidney and liver relative weights in OTA treated birds (T5) came in agreement with several previous reports using dietary 1,2.and 4 mg OTA / kg feed (Huff *et al.*, 1988); 2 mg / kg (Santin *et al.*, 2002); 130, 300 and 800 ug / kg OTA and 1000-5000 ug / kg *penicillic acid* (PA) (Stoev *et al.*, 2000 and 2004) ; 400 and 800 ug OTA / kg diet

(Elaroussi *et al.*, 2008). While, other investigators using OTA at 2 mg / kg (Raju and Devegowda, 2000) or 4 mg / kg (Verma *et al.* , 2004) reported an increase only in kidney weight.

The reported enlargement in the liver and kidney in OTA groups is probably due to the fact that these organs are involved in detoxification and elimination of OTA. Ochratoxin A is known to have direct toxic action (Stoey *et al.*, 2000) and high rate of accumulation in these two organs (Biro *et al.* , 2002). The current results are in agreement with those of Huff *et al.* (1975) who reported that the most sensitive indicators of ochratoxicosis in broiler chicks were reduction in growth rate and enlargement in kidney which occurred at dose as low as 1 ppm OA. They suggested that the kidney enlargement could be the result of oedema and general increase in the protoplasm. The present results also agrees with prior observation of acute ochratoxicosis in broiler chicks (Huff *et al.*, 1992; Elkady, 1993 and Elissalde *et al.*,1994)

In this respect, live yeast (*Saccharomyces cerevisiae*) at level of 0.1 % was found to alleviate the adverse effects of aflatoxicosis on relative liver weight of broilers (Stanley *et al.*, 1993).Furthermore, Baptista *et al.*,(2002) reported that dehydrated active yeast was able to reduce the hepatotoxicity caused by aflatoxin in hepatocytes.

2.b. Gizzard and proventriculus weights:-

Results in Table (6) present the changes in the relative weights of gizzard and proventriculus in their response to ochratoxin A contamination and the efficacy of *Saccharomyces cerevisiae* and vitamin C either alone or in combination to ameliorate the toxic effects of OTA. The present results showed that the relative gizzard weight increased significantly in broiler chicks that were subjected to OTA intoxication in their diets (T5) when compared with the control birds (T1) or with other groups. This increase was seen from the 1st week of age and was more pronounced with the experimental duration, which confirms that the effect of OTA was time dependent.

On the other hand, supplementing broiler diets with *Saccharomyces cerevisiae* and / or vitamin C in the absence of OTA (T2, T3 and T4) was differ but not significantly than control birds(T1). Significant differences was observed in the relative gizzard weight group of T6 and T8 when compared with the group T5 as a results of addition of *Saccharomyces cerevisiae* either alone or in combination with Vit.C in presence of OTA, which indicate that the addition of SC may ameliorate the toxic action of the OTA on relative gizzard weight. No significant differences were observed

between T5 and T7, indicating that addition of Vit.C alone to OTA-supplemented birds in their diets did not prevent any changes of gizzard weight resulted from the toxin.

In respect to the relative proventriculus weight, Table (6) demonstrated that there was a significant increase in the relative proventriculus weight of the OTA-treated birds (T5) when compared with that of control birds (T1) or with other group birds. This increase was also observed from the 1st week of age and was more severe with time.

Supplementing broiler diets with *Saccharomyces cerevisiae* and / or Vit.C without presence of OTA (T2, 3 and 4) did not differ significantly than control birds (T1). Furthermore, addition of *Saccharomyces cerevisiae* either alone or in combination with Vit.C in the broiler diet contaminated with OTA (T6 and T8) may alter the toxic effects of OTA on the relative proventriculus weight. On the other hand, supplementing broiler diet with Vit.C alone in the presence of OTA (T7) did not shows any significant alteration in the relative proventriculus weight compared to the ochratoxicated group (T5).

The significant increase in the gizzard and proventriculus weight due to OTA in the present study was in accordance with that found by other workers exposing birds to dietary OTA at 1 to 8 ppm (Elkady, 1993; Elissalde *et al.*, 1994; Stoev *et al.*, 2002; Elaroussi *et al.*, 2006). Raju and Devegowda (2000) reported that feeding broiler chicks the OTA-contaminated diet at 2 ppm from 1 to 35 days of age resulted in a 14.6 % increase in the gizzard weight. They suggested that the toxin affinity for the gastrointestinal tract affected several physiological systems that led to a dose-related weight alterations during ochratoxicosis.

Moreover, Huff *et al.*, (1988) attributed the gizzard sensitivity to OTA to the toxin irritative properties when in direct contact. They ranked the relative sensitivity of the organs to OTA from the most sensitive to the least sensitive as follows: gizzard > kidney > spleen > liver, based upon the time at which the significant changes occurred on the relative organ weight. They added that the effects of OTA on the upper gastrointestinal tract are due to irritation from direct contact with OA, as some gizzard erosion is present.

Preliminary data reported by Huff *et al.*, (1992) stated that commercial male broiler chicks exposed to intoxication with graded levels of ochratoxin A up to 8 ug / g feed in their diets from hatching until 3 weeks of age showed a significant increase in relative proventriculus weight. They indicated that the enlargement of the proventriculus in broilers suggest some affinity of the toxin

for the gastrointestinal tract. They added that the alteration which occurred in the proventriculus during ochratoxicosis was dose related.

The presence of OTA in the broiler diets lead to significant increase in the relative proventriculus weight in a predictable manner. This response of this organ has been postulated to reflect the cytotoxic effects of mycotoxin elicited by direct exposure of the upper digestive tract to this mycotoxin during the digestive process which confirms the sensitivity of the upper alimentary tract to OTA.(Huff *et al.*,1992)

CONCLUSION

Finally, it can be speculated that the mycotoxin ochratoxin A is one of the most potent important mycotoxins in broilers due to its toxicity even at lowered levels. Ochratoxin A produce large deleterious and negative effects on performance, internal organ weight of broiler chickens with the effects becoming more severe with prolonged exposure to it. The addition of *Saccharomyces cerevisiae* either alone or combined with Vit.C may be efficient in reducing the negative effects caused by OTA and enhance broiler's tolerance to toxicity. While, supplementing broiler diet with Vit.C alone did not exhibit any counteract to OTA.

Table (1) Grouping Experimental design

Treatments	Code	Dietary treatments
T1	(control)	NRC recommended diet without any supplementation
T2	(SC)	Control diet + 3 g <i>Saccharomyces cerevisiae</i> / kg diet
T3	(Vit C)	Control diet + 300 mg Vit.C / kg diet
T4	(SC+ Vit C)	Control diet + 3 g <i>Saccharomyces cerevisiae</i> + 300 mg Vit.C / kg
T5	(OTA)	Control diet + 200 ppb ochratoxin A / kg diet
T6	(OTA+SC)	Control diet + 200 ppb ochratoxin A + 3g <i>Saccharomyces</i>
T7	(OTA+Vit C)	Control diet + 200 ppb ochratoxin A + 300 mg Vit.C / kg diet
T8	(OTA+SC+Vit C)	Control diet + 200 ppb ochratoxin A + 3g <i>Saccharomyces cerevisiae</i> + 300 mg Vit.C / kg diet

Table (2) Formula and chemical composition of the experimental starter and grower diets.

Item	Starter	Grower
Yellow corn	56.4	58
Soybean meal 44 %	32	30
Corn Gluten meal 60 %	5	4
Oil	1.5	3.5
mono calcium phosphate	1.8	1.6
Calcium carbonate	1.7	1.5
Lysin	0.23	0.23
D.L. Methionin	0.3	0.25
Sodium chloride	0.3	0.25
Choline Chloride	0.2	0.18
Premix (Vitamin)	0.15	0.15
Premix (Mineral)	0.15	0.15
Anticoccidial (Declomix)	0.27	0.27
Calculated analysis	100	100
Crude protein (%)	22.2	20.8
Metabolizable energy (Kcal / kg)	2925	3076

Table (3) Effect of ochratoxin A , *Saccharomyces cerevisiae* and vitamin C supplementation on body weight (g) of broilers.

Age (week) / Treatments	3-days	Week 1	Week 2	Week 3	Week 4	Week 5
T1	*60.5435 ^a ± 0.5	179.804 ^{bc} ± 2.7	453.39 ^{abc} ± 7.4	836.35 ^{bc} ± 10.1	1352.94 ^{bc} ± 17.8	1880.27 ^b ± 25.2
T2	60.4348 ^d ± 0.5	188.696 ^a ± 2.4	468.12 ^{ab} ± 6.1	872.18 ^a ± 9.4	1406.16 ^a ± 17.6	1952.38 ^a ± 20.4
T3	60.5000 ^a ± 0.4	180.239 ^{bc} ± 2.7	449.79 ^{bc} ± 6.8	841.36 ^b ± 10.2	1360.19 ^b ± 18.4	1874.24 ^b ± 23.1
T4	60.6522 ^a ± 0.5	184.870 ^{ab} ± 2.7	471.85 ^a ± 7.4	885.8 ^a ± 9.9	1415.03 ^a ± 16.9	1966.77 ^a ± 20.1
T5	60.6957 ^a ± 0.5	170.348 ^d ± 2.9	391.19 ^c ± 7.9	698.43 ^c ± 12.7	1069.21 ^c ± 13.8	1401.91 ^d ± 17.1
T6	60.5870 ^a ± 0.5	176.891 ^{bcd} ± 2.8	439.80 ^c ± 6.9	809.07 ^c ± 10.5	1308.42 ^c ± 15.6	1758.60 ^c ± 23.8
T7	60.6304 ^a ± 0.6	172.848 ^{cd} ± 2.6	418.40 ^d ± 7.6	750.24 ^d ± 11.2	1122.20 ^d ± 14.3	1457.71 ^d ± 19.8
T8	60.6087 ^a ± 0.5	178.370 ^{bc} ± 2.4	443.91 ^c ± 7.9	820.82 ^{bc} ± 10.9	1315.87 ^{bc} ± 16.0	1769.58 ^c ± 23.7

* Values are means ± SE

a,b,c ... Means in the same column with different superscript are significantly different (P< 0.05)

Table (4) Effect of ochratoxin A , *Saccharomyces cerevisiae* and vitamin C supplementation on Feed consumption (g) and cumulative feed conversion ratio (FCR) of broilers.

Feed consumption (g / chick)						
Age (week) Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Feed conversion ratio(FCR)
T1	161.8	376.9	614.6	890.7	1114.3	1.68
T2	169.3	378.8	625.8	909.2	1136.3	1.65
T3	163.6	372.1	617.9	897.0	1085.3	1.67
T4	165.5	386.2	637.9	902.7	1129.5	1.64
T5	159.2	314.2	525.4	716.3	900.4	1.87
T6	161.3	366.1	598.4	878.3	1023.2	1.72
T7	160.6	342.4	565.1	724.5	910.9	1.86
T8	162.3	369.1	608.3	871.2	1025.7	1.72

Table (5) Effect of ochratoxin A, *Saccharomyces cerevisiae* and vitamin C supplementation on mortality percentage of broilers

Treatments	Mortality percent %				
	Age (weeks)				
	1	2	3	4	5
T1	0.00 (0/46)	0.00 (0/41)	0.00 (0/36)	0.00 (0/31)	0.00 (0/26)
T2	0.00 (0/46)	0.00 (0/41)	0.00 (0/36)	0.00 (0/31)	0.00 (0/26)
T3	0.00 (0/46)	0.00 (0/41)	0.00 (0/36)	0.00 (0/31)	3.85 (1/26)
T4	0.00 (0/46)	0.00 (0/41)	0.00 (0/36)	0.00 (0/31)	0.00 (0/26)
T5	0.00 (0/46)	2.44 (1/41)	0.00 (0/35)	3.33 (1/30)	8.33 (2/24)
T6	0.00 (0/46)	0.00 (0/41)	0.00 (0/36)	0.00 (0/31)	3.85 (1/26)
T7	0.00 (0/46)	0.00 (0/41)	0.00 (0/36)	3.23 (1/31)	4 (1/25)
T8	0.00 (0/46)	0.00 (0/41)	0.00 (0/36)	0.00 (0/31)	0.00 (0/26)

Table (6) Effects of ochratoxin A, *Saccharomyces cerevisiae* and vitamin C supplementation on relative kidney and liver weights of broilers

Treatments	Relative kidney weight (g /100g)					Relative liver weight (g /100g)				
	Age (week)					Age (week)				
	1	2	3	4	5	1	2	3	4	5
T1	0.725 ^a ± 0.032	0.695 ^b ± 0.038	0.667 ^{cd} ± 0.034	0.617 ^{bcd} ± 0.032	0.621 ^{bc} ± 0.035	3.855 ^a ± 0.197	3.2533 ^{ab} ± 0.159	2.555 ^c ± 0.121	2.311 ^d ± 0.115	2.466 ^{cd} ± 0.160
T2	0.750 ^a ± 0.039	0.711 ^{ab} ± 0.035	0.633 ^d ± 0.032	0.585 ^d ± 0.029	0.588 ^c ± 0.033	3.9736 ^a ± 0.227	3.0419 ^b ± 0.110	2.631 ^c ± 0.140	2.396 ^d ± 0.101	2.3216 ^d ± 0.133
T3	0.717 ^a ± 0.051	0.704 ^{ab} ± 0.039	0.678 ^{cd} ± 0.031	0.632 ^{bcd} ± 0.035	0.597 ^c ± 0.038	4.022 ^a ± 0.195	3.222 ^{ab} ± 0.10	2.726 ^{bc} ± 0.175	2.517 ^{bcd} ± 0.129	2.4041 ^{cd} ± 0.144
T4	0.734 ^a ± 0.031	0.682 ^b ± 0.042	0.696 ^{cd} ± 0.034	0.608 ^{cd} ± 0.028	0.581 ^c ± 0.029	3.9072 ^a ± 0.187	3.101 ^{ab} ± 0.117	2.448 ^c ± 0.190	2.4191 ^c ± 0.122	2.2443 ^d ± 0.122
T5	0.801 ^a ± 0.042	0.827 ^a ± 0.04	0.853 ^a ± 0.032	0.842 ^a ± 0.027	0.829 ^a ± 0.027	3.948 ^a ± 0.299	3.5619 ^a ± 0.157	3.3639 ^a ± 0.222	3.4238 ^a ± 0.111	3.6518 ^a ± 0.137
T6	0.769 ^a ± 0.037	0.738 ^{ab} ± 0.032	0.746 ^{bc} ± 0.038	0.709 ^b ± 0.034	0.699 ^b ± 0.036	4.122 ^a ± 0.221	3.411 ^{ab} ± 0.161	2.912 ^{abc} ± 0.146	2.811 ^{bc} ± 0.159	3.094 ^b ± 0.196
T7	0.786 ^a ± 0.044	0.772 ^{ab} ± 0.045	0.819 ^{ab} ± 0.041	0.826 ^a ± 0.029	0.805 ^a ± 0.031	4.00 ^a ± 0.269	3.504 ^a ± 0.128	3.199 ^{ab} ± 0.194	3.365 ^a ± 0.118	3.602 ^a ± 0.160
T8	0.763 ^a ± 0.049	0.744 ^{ab} ± 0.04	0.728 ^{bcd} ± 0.031	0.688 ^{bc} ± 0.032	0.702 ^b ± 0.036	3.892 ^a ± 0.249	3.395 ^{ab} ± 0.174	2.851 ^{abc} ± 0.133	2.913 ^b ± 0.146	2.860 ^{bc} ± 0.150

* Values are means ± SE

a,b,c ...Means in the same column with different superscript are significantly differ (P ≤ 0.05)

Table (7) Effects of ochratoxin A, *Saccharomyces cerevisiae* and vitamin C supplementation on relative gizzard and proventriculus weights of broilers

Treatments	Relative gizzard weight (g/100g)					Relative proventriculus weight (g/100g)				
	Age (week)					Age (week)				
	1	2	3	4	5	1	2	3	4	5
T1	6.104 ^{bc} ± 0.126	4.539 ^{bcd} ± 0.117	3.672 ^{bcd} ± 0.116	3.047 ^{cd} ± 0.139	2.606 ^d ± 0.140	0.900 ^{abc} ± 0.026	0.611 ^{bc} ± 0.032	0.495 ^{bc} ± 0.020	0.431 ^{bc} ± 0.023	0.393 ^d ± 0.025
T2	6.278 ^{bc} ± 0.205	4.325 ^{cd} ± 0.162	3.236 ^d ± 0.119	2.895 ^d ± 0.128	2.387 ^d ± 0.098	0.878 ^{bc} ± 0.036	0.581 ^c ± 0.031	0.504 ^{bc} ± 0.033	0.389 ^c ± 0.027	0.380 ^d ± 0.02
T3	4.515 ^c ± 0.106	4.515 ^{bcd} ± 0.171	3.492 ^{bcd} ± 0.164	3.014 ^{cd} ± 0.178	2.725 ^{cd} ± 0.161	0.915 ^{abc} ± 0.028	0.629 ^{bc} ± 0.029	0.488 ^c ± 0.028	0.419 ^{bc} ± 0.03	0.400 ^{cd} ± 0.024
T4	6.333 ^{bc} ± 0.162	4.124 ^d ± 0.168	3.400 ^{cd} ± 0.136	2.761 ^d ± 0.162	2.513 ^d ± 0.140	0.848 ^c ± 0.036	0.593 ^{bc} ± 0.028	0.466 ^c ± 0.025	0.406 ^{bc} ± 0.02	0.362 ^d ± 0.028
T5	6.843 ^a ± 0.153	5.237 ^a ± 0.135	4.536 ^a ± 0.132	3.925 ^a ± 0.123	3.626 ^a ± 0.11	0.996 ^a ± 0.019	0.738 ^a ± 0.033	0.617 ^a ± 0.021	0.601 ^a ± 0.027	0.611 ^a ± 0.019
T6	6.441 ^{abc} ± 0.177	4.733 ^{bc} ± 0.145	3.820 ^{bc} ± 0.146	3.422 ^{bc} ± 0.173	3.162 ^b ± 0.132	0.931 ^{abc} ± 0.031	0.645 ^{abc} ± 0.032	0.540 ^{abc} ± 0.027	0.485 ^b ± 0.025	0.479 ^b ± 0.021
T7	6.602 ^{ab} ± 0.116	4.966 ^{ab} ± 0.176	4.423 ^a ± 0.158	3.652 ^a ± 0.093	3.588 ^a ± 0.131	0.963 ^{ab} ± 0.030	0.687 ^{ab} ± 0.030	0.580 ^{ab} ± 0.026	0.562 ^a ± 0.029	0.547 ^a ± 0.023
T8	6.472 ^{ab} ± 0.169	4.665 ^{bc} ± 0.162	3.883 ^b ± 0.184	3.353 ^{bc} ± 0.149	3.113 ^{bc} ± 0.178	0.925 ^{abc} ± 0.029	0.652 ^{abc} ± 0.04	0.526 ^{bc} ± 0.03	0.475 ^b ± 0.03	0.466 ^{bc} ± 0.026

* Values are means ± SE

a,b,c ... Means in the same column with different superscript are significantly differ ($P \leq 0.05$)

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الملخص العربي

تأثير إضافة الخميرة (*Saccharomyces cerevisiae*) وفيتامين ج على أداء كتاكيت التسمين المعرضة للتلوث بالاوكراتوكسين

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أجريت هذه الدراسة لمعرفة تأثير إضافة الخميرة الجافة النشطة و / أو فيتامين ج كإضافات غذائية لعلائق كتاكيت التسمين المعرضة للتلوث بسموم الاوكراتوكسين على الأداء الانتاجى والوزن النسبى لبعض الأعضاء الداخلىة. استخدم فى هذه الدراسة عدد ٢٦٨ كتكوت تسمين ذكور (ROSS) تم توزيعهم عشوائيا عند عمر ٣ ايام تبعا للمعاملات الغذائية الى ٨ مجموعات (بواقع ٤٦ كتكوت لكل مجموعة) كالاتى:-

- ١- المجموعة الأولى (كنترول):- تم تغذيتها على العليقة الأساسية خالية من اى إضافات
- ٢- المجموعة الثانية:- تم تغذيتها على عليقة الكنترول بالإضافة الى ٢ جم خميرة جافة نشطة / كجم عليقة
- ٣- المجموعة الثالثة:- تم تغذيتها على عليقة الكنترول بالإضافة الى ٣٠٠ مجم فيتامين ج / كجم عليقة
- ٤- المجموعة الرابعة:- تم تغذيتها على عليقة الكنترول بالإضافة الى ٢ جم خميرة جافة نشطة + ٣٠٠ مجم فيتامين ج / كجم عليقة.
- ٥- المجموعة الخامسة:- تم تغذيتها على عليقة الكنترول ملوثة ب ٢٠٠ جزء فى البليون اوكراتوكسين / كجم عليقة.

- ٦- المجموعة السادسة: تم تغذيتها على عليقة الكنترول ملوثة بـ ٢٠٠ جزء في البليون اوكراتوكسين + ٣ جم خميرة جافة نشطة / كجم عليقة
 - ٧- المجموعة السابعة: تم تغذيتها على عليقة الكنترول ملوثة بـ ٢٠٠ جزء في البليون اوكراتوكسين + ٣٠٠ مجم فيتامين ج / كجم عليقة
 - ٨- المجموعة الثامنة : تم تغذيتها على عليقة الكنترول ملوثة بـ ٢٠٠ جزء في البليون اوكراتوكسين + ٣ جم خميرة جافة نشطة + ٣٠٠ مجم فيتامين ج / كجم عليقة.
- وتم تربية الطيور في البطاريات تحت نفس الظروف من الرعاية حتى نهاية التجربة عند ٥ اسابيع وقد أوضحت النتائج ان:-
- تغذية كتاكيت التسمين على علائق ملوثة بسموم الاوكراتوكسين بمستوى ٢٠٠ جزء في البليون أدى الى انخفاض معنوى فى كل من وزن الجسم واستهلاك العليقة وزيادة معنوية فى نسبة النفوق ومعدل التحويل الغذائى وكذلك فى الوزن النسبى لكل من الكلى والكبد والقانصة والمعدة الغدية.
 - إضافة الخميرة الجافة النشطة بمفردها مع فيتامين ج الى العلائق الملوثة بالاوكراتوكسين أدى الى تقليل التأثيرات السامة للاوكراتوكسين على المقاييس السابقة.
 - إضافة فيتامين ج بمفرده لم يقلل من التأثيرات الضارة الناتجة من تلوث العلائق بسموم الاوكراتوكسين.