BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES AFTER ADDITION OF *SACCHAROMYCES CEREVISIAE* TO BROILER RATION CONTAMINATED WITH AFLATOXIN

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Abstract

In this study, 40 broiler chicks (native breed x saso) of 21 days age were used to evaluate the effect of dry yeast (*Saccharomyces cerevisiae*) on biochemical constituents in relation to aflatoxicosis. Chicks were divided into four groups each of 10 chicks; 1st group received a standard ration and kept as normal control, 2nd group was fed a diet contaminated with aflatoxin at a level of 5mg (AF) B1/kg, the 3rd group received a diet containing dry yeast at a level of 0.05% /kg of feed and the 4th group received a diet containing aflatoxin at a level of 5 mg (AF) B_1/kg and dry yeast at a rate of 0.05% /kg of feed. This feeding program continued for 4 weeks, the obtained data revealed that the chicks kept on diet contaminated with aflatoxin (group 2) possess. the lowest values of serum total protein, albumin, globulin, cholesterol, calcium, phosphorus, iron and copper. On the other hand group2 showed significant increase in liver enzymes, serum alanin amino transferase (ALT), aspartate amino transferase (AST) and gamma glutamyl transferase (GGT) and significant increase in kidney function tests as (urea, uric acid and creatinine) while chicks fed ration containing dry yeast with or without aflatoxin (group 3&4) showed a significant improvement of the values of biochemical constituents and returned to normal control values due to the detoxifying effect of dry yeast. At the same, time there were the histopathological changes in liver and kidney in aflatoxicated group (G2). The addition of Saccharomyces cerevisiae could eliminate the pathological action of aflatoxicosis in chicks.

Key words: Broiler, aflatoxin *Saccharomyces cerevisiae* biochemical parameters, hisopathology.

INTRODUCTION

The aflatoxins are a group of secondary fungal metabolites which are formed after logarithmic phase of growth of either *Aspergillus parasiticus* or *Aspergillus flavus*, they

are found as natural contaminants in many types of poultry feeds (Edds and Bortell, 1983). One day old duckling and turkey poults are extremely susceptible to aflatoxicosis (Brawn and Abrams, 1965). The different types of aflatoxin cause economic losses in poultry industry due to negative effects on chicken growth, body weight and body gain (Lanza *et al.*1980, Huff *et al.*, 1986 Quezada *et al.*, 2000 and Rosa *et al.*,2001). In laying hens aflatoxicosis causes poor egg production and decreased egg weight and percent of hatchability (Smith and Hmilton1970). Aflatoxin causes decrease in serum protein (Quezada *et al.*, 2000 and Rosa *et al.*, 2001).

Based on growing concern regarding the use of antibiotics in animal production there is much interest in exploring alternatives to antimicrobial feed additives (Martin *et al.*1999). Dry yeast contains several other minerals including selenium, zinc, phosphorus, magnesium, it is often used for loss of appetite, supplement for chronic acne and treatment of diarrhea, as well as it reduces mortality percentage in layer and broiler. This could be attributed to its ability to promote the multiplication of the normal lactic fermenting microflora in the gut (Silverstro, 1983); this microflora will colonize the gut of the bird and provide significant reduction against colonization and subsequent infection by various Salmonellae, *E coli* and other enteric pathogens (Snoeyenbos, 1987). Dry yeast contains the most important organic acid considered as detoxifying agent which is significantly diminishing some deleterious effects caused by aflatoxin in chickens (Kubena *et al.*,1990)

The aim of the present study was conducted to evaluate the effect of *Saccharomyces cerevisiae* in protection of chickens against aflatoxicosis.

MATERIALS AND METHODS

1-*Saccharomyces cerevisia*: (THEPAX) was supplied by Doxal, Italia, S.P.A. as a pure powder.

2-Chicks: Forty broiler chicks (native breed x saso) of 21 days old were divided into four groups, each of 10 chicks and kept under strict hygienic condition.

3-Aflatoxin production: Production of aflatoxin was performed according to the method of Davis *et al.* (1966) and the amount was estimated with thin layer chromatography according to Schuller and Van Egmond (1981).

4-Experimental design: Chicks were divided into four groups, 1st group received a standard ration and kept as normal control, 2nd group was fed a diet contaminated with aflatoxin (AF) at a level of 5mg AF(B1), 3rd group received a diet containing dry yeast (*Saccharomyces cerevisiae*) at a level of 0.05% per kg of feed,4th group received a diet contaminated with aflatoxin at a level of 5 mg AF B1/kg and dry yeast at a level of 0.05% per kg of feed; this feeding program continued for 4 weeks .

5-Sampling: Forty serum samples were collected for biochemical studies, samples from liver, kidney and spleen were collected and kept in formalin saline 10% for histopathological studies.

6-Procedures

Biochemical studies: GGT, ALT, AST, glucose, total protein, albumin, calcium, phosphorus, uric acid, and creatinine were estimated using standard kits supplied by bio-merieux (Poains, France), iron and copper were estimated using atomic absorption.

7-Histopathological studies: Tissue specimens were fixed in formalin 10% salin and embedded in parafin wax, tissue sections were cut at 4-5 µm thickness and routine dehydrated cleared in ethyl alcohol and xylol. Slides were stianed with hematoxylin and eosin and were examined using light microscope for histopathological results and photographed (Harris,1988).

8-Statistical analysis: Data were analysed statistically using the liner model program of SAS (1990). The differences among means were tested using Duncans Multiple Range Test (Duncans, 1955).

RESULTS

Tables 1 and 2 show the data presented the biochemical parameters of serum in different groups. AST, in group 2 showed significant increase compared with all test groups, while, groups 3 and 4 showed no significant change compared with control group1. ALT in group 2 showed significant increase compared with all experimental groups, group 4 showed no significant change compared with control group1, group 3 showed significant decrease compared with group 2.GGT in group 2 showed significant increase compared with all test groups, while, groups 3 and 4 showed no significant change compared with control group 1.Total protein showed significant decrease in group 2, while, group 4 showed no significant changes compared with control group 1, group 3 showed significant increase compared with all test groups .Albumin in group 2 showed significant decrease compared with control group 1 and returned towards normal control levels in groups 3 and 4. Globulin showed significant decrease in group 2 compared with all test groups, no significant changes were observed between groups 3 , 4 and control group 1,group 3 showed significant increase compared with all experimental groups. Urea, uric acid and creatinine showed significant increase in group 2, while, groups 3 and 4 showed non significant change compared with normal control group 1.Glucose in group 2 showed significant increase compared with control group1 , no significant change between group 4 and control group1,group 3 showed significant decrease compared with all experimental groups .Cholesterol showed significant decrease in group 2, group 4 showed no significant change compared with control group 1, while, group 3 showed significant increase compared with group 2. Calcium showed significant decrease in group 2 compared with control group 1, while, no significant changes were observed between groups 3. and 4 compared with control group 1 .Phosphorus showed significant decrease in group 2, while, returned towards normal levels control in groups 3 and 4 . Copper showed significant decrease in group 2, while, groups 3 and 4 showed no significant change compared with control group 1.Iron in group 2 showed significant decrease, no significant change was observed between group 4 and control group 1, while, serum iron level returned to normal control in groups 3 and 4.Histopathological changes are illustrated in figures 1, 2, 3 and 4. Histopathological examination of kidney showed subcapsular coagulative necrosis of the renal tubules, coagulative

necrosis of the epithelium lining of the renal tubules with highly infiltration of lymphocytes and congesion of renal blood vessels, thickening of pariatal layer of the Bowman's capsule and hyper cellularity of the glomeruli; some glomeruli showed pale eosinophilic structurless substance (Fig1). Examination of liver showed centrolobular coagulative necrosis, congestion of central veins and portal blood vessels, the portal area highly infiltrated with lymphocytes and hetrophils which extended to the adjacent hepatic parenchyma, proliferation of bile ductules was observed and the epithelium linning of such bile ductules showed large vesicular nucleus with prominent nuclei,as well as there was fatty change and necrosis of hepatocytes (Figs 2&3). Histopathological examination of spleen showed depletion of the lymphocytes from the white pulp with apoptosis of lymphocytes (Fig. 4).

Table 1. Biochemical parameters in serum of broilers fed ration containing dry yeast with or without aflatoxin.

Parameters	G_1	G_4	G_3	G_4
AST iu/l	155.64 ± 1.65^{b}	178.25 ± 2.64^{a}	153.17 ± 1.76^{b}	157.16 ± 1.71^{b}
AlT iu/l	44.86 ± 0.76^{b}	63.37 ± 0.82^{a}	41.38 ± 0.89^{c}	45.64 ± 0.60^{b}
GGT iu/l	23.89 ± 0.92^{b}	32.61 ± 0.72^{a}	21.92 ± 0.85^{b}	24.15 ± 0.96^{b}
T.protein gm/dl	4.08 ± 0.12^{b}	2.65 ± 0.16^{c}	5.11 ± 0.11^{a}	3.87 ± 0.12^{b}
Albumin gm/dl	1.74 ± 0.06^{b}	0.89 ± 0.05^{c}	2.01 ± 0.05^{a}	1.69 ± 0.05^{b}
Globulin gm/dl	2.35 ± 0.13^{b}	1.76 ± 0.16^{c}	3.09 ± 0.13^{ab}	2.18 ± 0.15^{b}
A/G ratio	0.76 ± 0.05^{ab}	0.57 ± 0.08^{b}	0.66 ± 0.04^{b}	0.82 ± 0.09^{a}
Urea mg/dl	2.87 ± 0.27^{b}	8.14 ± 0.32^{a}	2.42 ± 0.19^{b}	3.02 ± 0.25^{b}
Creatinine mg/dl	1.006 ± 0.04^{b}	1.84 ± 0.01^{a}	0.93 ± 0.03^{b}	1.02 ± 0.03^{b}
Glucose mg/dl	182.02 ± 1.84^{b}	234.48 ± 2.46^{a}	174.24 ± 1.96^{c}	183.27 ± 2.19^{b}
Total cholesterol mg/dl	197.92 ± 2.47^{a}	130.36 ± 4.56^{c}	180.90 ± 3.23^{b}	199.19 ± 3.39^{a}

Results are presented as mean $\pm$ S.E. S.E. = standard error

Different letters in the same row means significant difference at ($p \leq 0.05$) while the same letters mean non significant difference at ($P > 0.05$) .

G_1= was given standard ration only (control) .

G_2 = was given standard ration + AF .

G_3 = was given standard ration + dry yeast .

G_4 = was given standard ration + dry yeast + AF .

Table 2. Serum analysis of minerals in broilers fed ration containing dry yeast with or without aflatoxin .

Parameters	G_1	G_4	G_3	G_4
Total calcium mg/dl	10.81 ± 0.85^{a}	6.69 ± 0.33^{b}	11.54 ± 0.82^{a}	10.48 ± 0.78^{a}
Inorganic Phosphorus mg/dl	6.96 ± 0.86^{ab}	4.71 ± 0.66^{b}	8.06 ± 0.95^{a}	6.69 ± 0.89^{ab}
Copper mg/l	1.72 ± 0.008^{a}	1.07 ± 0.02^{b}	1.73 ± 0.02^{a}	1.69 ± 0.009^{a}
Iron mg/l	1.85 ± 0.01^{b}	1.04 ± 0.007^{c}	1.89 ± 0.02^{a}	1.82 ± 0.01^{b}

Results are presented as mean $\pm$ S.E. S.E. = standard error

Different letters in the same row means significant differnce at ($p \leq 0.05$) while the same letters mean non significant difference at ($P > 0.05$)

G_1= was given standard ration only (control) .

G_2 = was given standard ration + AF .

G_3 = was given standard ration + dry yeast .

G_4 = was given standard ration + dry yeast + AF .

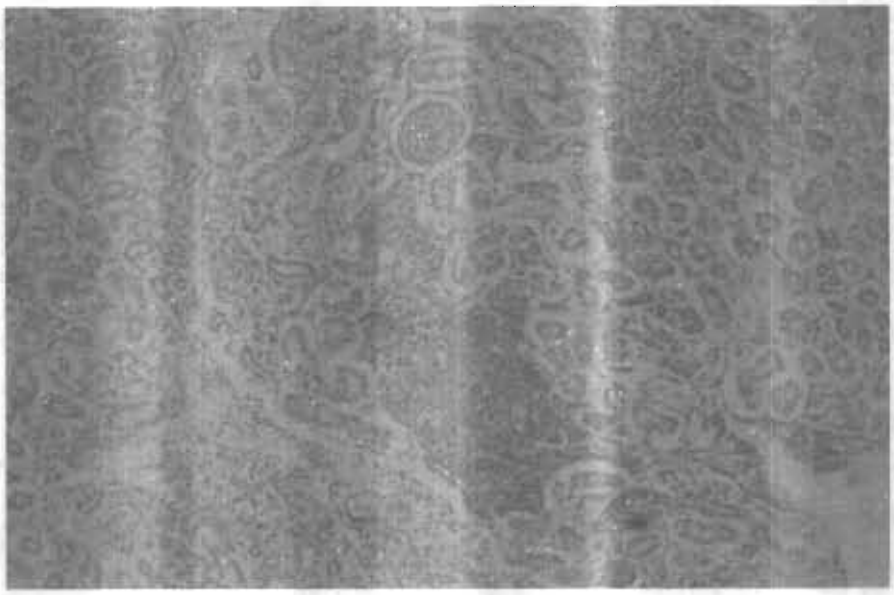

Fig. 1. kidney of chick fed ration contaminated with AFB1 (group2), showing focal coagulative necrosis of some renal tubules besides mononuclear cell infiltration (H& E. x 300)

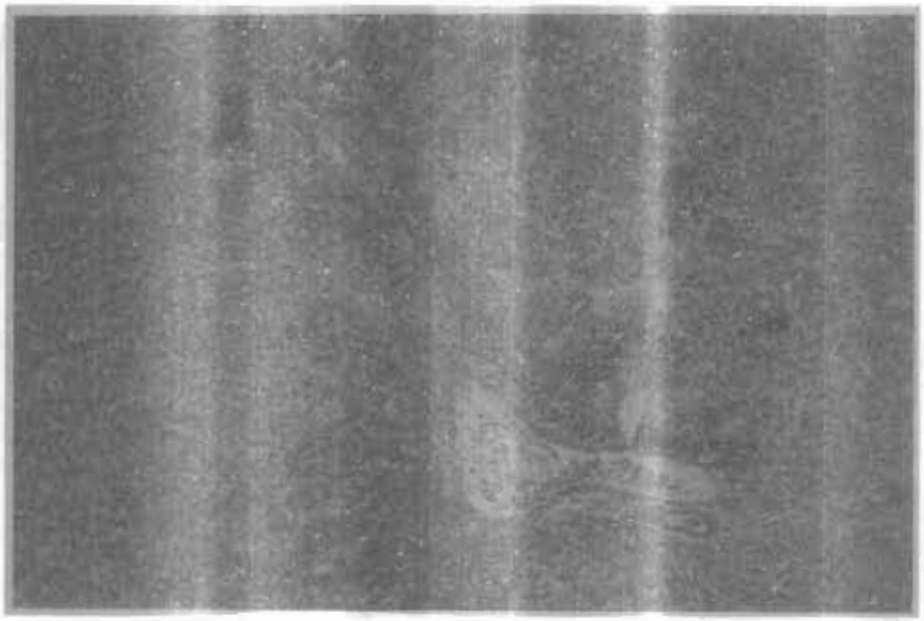

Fig. 2. liver of chick fed ration contaminated with AFB1 (group2) showing congestion and centrolobular coagulative necrosis of hepatocytes . The portal area shows numerous heterophils and mononuclear cell infiltration (H&E. x 300)

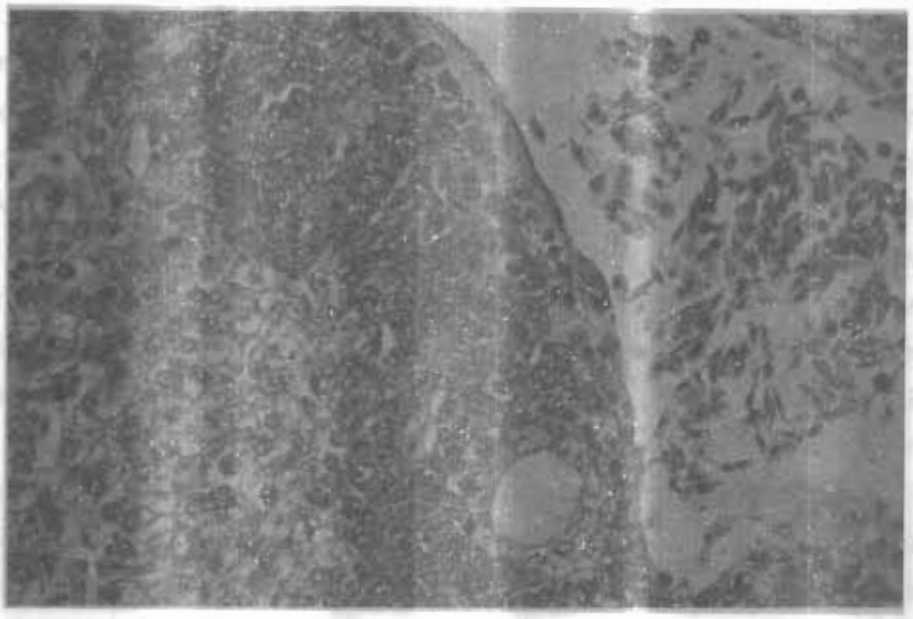

Fig. 3. liver of chick fed ration contaminated with AFB1 (group2) showing congestion of the portal blood vessels besides heterophils and mononuclear cell infiltration. The adjacent hepatocytes show hydropic degeneration (H&E. x 1200)

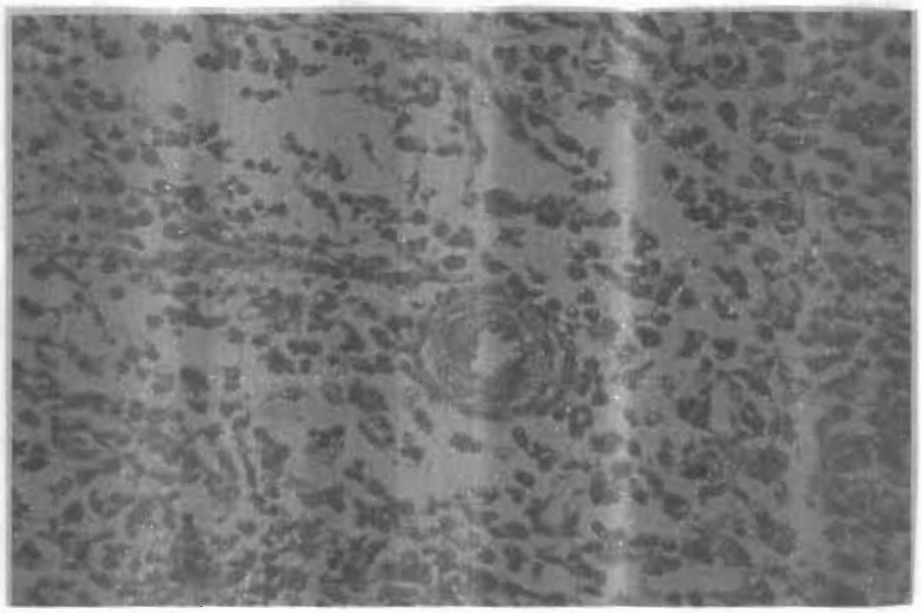

Fig. 4. Spleen of chick fed ration contaminated with AFB1(group2) showing severe depletion and necrosis of the lymphocytes from the white pulb (H&E. x 1200)

DISCUSSION

The present investigation demonstrates significant increase in serum liver enzymes (ALT andAST) in aflatoxicated chicks (G2), may be attributed to the hepato toxic effect of aflatoxin which lead to disturbance of liver function. These results agree with (Aletor *et al.*,1981 and Kramer, 1989). Addition of dry yeast with or without aflatoxin in groups 3 and 4 improved the liver enzymes (ALT, AST). Our results are in agreement with Kukuruzinska and Lennon (1995), as well as liver enzyme (GGT) showed significant increase in group 2, while, addition of dry yeast decreasd serum (GGT) level. Decreased serum protein in aflatoxin treated group (G2) may be attributed to impairment of hepatic protein synthesis as suggested by Tung *et al.* (1975), and Beer *et al.* (1990). Addition of dry yeast in groups 3 and 4 corrected serum protein level. Serum calcium, phosphorus, copper and iron showed significant decrease in group 2 which is attributed to inhibit absorption of these minerals from intestine. The same results were suggested by Raoa and Joshi (1991), and Fernandez *et al.* (1993). Addition of dry yeast with or without aflatoxin in groups 3and 4 returned serum mineral levels towards normal control. Our results are similar to those obtained by Moonsie-Shageev and Mowat (1993). Globulin in group 2 showed significant

decrease indicating immunosuppressive effect of aflatoxin. Our findings were in agreement with Rosa *et al.* (2001). Addition of dry yeast with or without aflatoxin in groups 3 and 4 increased serum globulin level which can be attributed to presence of some immuostimulating factors in *Saccharomyces cerevisiae* as V. E. like substance and selenium. These results are similar to those obtained by Jurgens *et al.*(1997). Significant increase in serum urea, uric acid and creatinine in group 2 showed that kidney function was impaired. This result agreed with Cortina and Sangabriel (1972). Use of dry yeast corrected the kidney function. Glucose in group 2 showed significant increase. This may be attributed to impaired reabsorption of glucose in convoluted tubules. Simillar results were obtained by Abd el Hamid *et al.* (1994). Dry yeast in groups 3 and 4 lead to decrease serum glucose level which is attributed to enhancing the response to insulin by hepatocytes. The data obtained support the hypothesis that chromium or some other factors present in *Saccharomyces cerevisiae* potentiates the peripheral effects of insulin and improved glucose tolerance (Yoshida *et al.*, 2002). Total cholesterol showed significant decrease in aflatoxicated group (G 2) as mentioned by Abd el Hamid *et al.* (1994). Amaya Frfan (1999), mentioned that the sign of acute imbalance of lipid metabolism can be the result of chemical modification (blocking) of key lysyl residues on the LDL protien B-100 by the activated (AFB1), lipid starvation of peripheral tissues takes place, while, fat accumulates in the liver. The addition of dry yeast in groups 3 and 4 improved the serum cholesterol suggested the presence of cholesterolemic factor as chromium in *Saccharomyces cerevisiae* cells. These results were confirmed by histopathological finding as kidney in (group2) showed coagulative necrosis of the epithelium lining of renal tubules and degeneration of the glomeruli. These results were in accordance with those of Abd El Hamid *et al.* (1992) and Fernandes *et al.*(1993). Liver tissues in (group2) showed centrolobular coagulative necrosis with congesion of central veins and portal blood vessels which were in agreement with Kramer (1989), Fernandez *et al.* (1993) and Rosa *et al.* (2001). Spleen showed apoptosis of lymphocytes and depletion of lymphocytes from white pulp which indicated the immunosuppressive effect of aflatoxin. The histopathological changes disappeared in chicks received dry yeast in their ration. It is concluded that, the addition of yeast *Saccharomyces cerevisiae* at the level of 0.05% feed could eliminate histopathological effects of aflatoxins B1 in diet.

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دراسات بيوكيميائية وهستوباثولوجية بعد إضافة خميرة الخبز على علائق التسمين الملوثة بسم الافلاتوكسين

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أجريت هذه الدراسة على ٤٠ من دجاج التسمين الهجين (بلدى×ساسو) عمر ٢١ يوماً قسمت إلى ٤ مجموعات كل مجموعه تحتوى على ١٠دجاجات المجموعة الأولى (الضابطة) تم تغذيتها على علف بادى خالى من أى سموم فطرية، المجموعة الثانية تم تغذيتها على علف بادى +سم الافلاتوكسبن (٥مجم /كجم علف) والثالثة تم تغذيتها على علف بادى +خميرة الخبز بمعدل (٠,٠٥% /كجم علف) والمجموعة الرابعة تم تغذيتها على علف بادى + سم الافلاتوكسين(٥مجم/كجم علف) +خميرة الخبز (٠,٠٥% /كجم علف) وأستمر هذا البرنامج لمدة ٤أسابيع ثم تم جمع عينات سيرم لعمل القياسات البيوكيميائية وكذلك عينات من الكلى والكبد والطحال لعمل الدراسات الهستوباثولوجية وقد أظهرت المجموعة الثانية نقصاً معنوياً فى معدل البروتين والألبومين والجلوبيولين والكوليسترول والمعادن مثل الكالسيوم والفوسفور والحديد والنحاس بينما كان هناك زيادة معنوية فى السكر وأنزيمات الكبد (ألانين أمينو ترانسفيريزوالجاماجلوتاميل ترانسفيريزواسبرتات امينو ترانسفيريز) ووظائف الكلى مثل (البولينا والكرياتينين وحمض اليوريك) ولكن إضافة الخميرة إلى العلف في وجود الافلاتوكسين أو بدونه أدت إلى تحسين كل القياسات البيوكيميائية السابقة وقد أظهر الفحص الهستوباثولوجى وجود تكسير فى خلايا الكبد والكلى ولكن إضافة خميرة الخبز إلى العليقة أدى إلى اختفاء كل التغيرات الهستوباثولوجية.