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Effects of Additives Mannan and β-Glucan as a Prebiotics on Broilers Diets Contaminated with Aflatoxin

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ABSTRACT

This study was carried out to determine the effects of different levels of mannan and β-glucan as a prebiotics on some liver and kidney function, blood parameters, immune globulins concentration and body weight on broilers fed diets contaminated with 1mg aflatoxin / kg diet. Eighty broilers were randomly distributed into eight similar groups each group fed commercial broiler diet as the follows {negative control group fed an regular broilers ration free from prebiotics and aflatoxin, posative control group fed diet contaminated with aflatoxin (1mg/kg) and free from prebiotics, groups from 3 to 8 fed diet contaminated with aflatoxin (1mg/kg) and contain different concentration of mannan and β-glucan in premix (premix added as a 1.5 gm/kg diet)}. Results showed that positive control significantly increased ($P < 0.05$) serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT), compared with negative control and the best effect of adding mannan and β-glucan recorded with group 5, 6 where the results were close of negative control. The same trend recorded with results obtained from serum urea and creatinine. Results of hematological analysis showed that positive control caused a significant decrease ($P < 0.05$) in values of packed cell volume (PCV), Red blood cells counts (RBC), Hemoglobin (Hb), and noticed a significant increase ($P < 0.05$) occurrence in values of total white blood cell (WBC). Generally the addition of mannan and β-glucan to diets contaminated with aflatoxin significantly improved the adverse effects of aflatoxin on hematological parameters. This study demonstrates an increase in serum IgGs concentrations with positive control and group8 without significant differences with negative control or other groups. The average body weight gain (g) increased ($P < 0.05$) with broilers fed negative control diet compared with positive control. The addition of both mannan and β-glucan to diets led to improvement in increase body weight gain and decreases in relative liver whight ratio.

INTRODUCTION

Aflatoxins (AF) are secondary toxic metabolites produced by fungi belonging to the genus *Aspergillus*, and can occur as natural contaminants of poultry feeds. Aflatoxins may cause serious economic losses in the poultry industry Oguz and Kurtoglu, (2000), reduction of the immune function and cause hepatotoxicosis and hemorrhage Oguz *et al.,* (2003),. The detoxification of AF-containing feeds and feedstuffs are in great demand. The worldwide increase in the use of prebiotics and its evaluation in diets for breeders are of integral part of the poultry and livestock production particular interest because it not only shifts industry to treat and prevent infectious diseases and toward beneficial as growth promoters at sub therapeutic levels in feeds organisms Ortatatli and Oguz, (2001).

prebiotics are today the most frequent components used for the elaboration of functional foods Spring *et al* (2000). β-glucans classification as prebiotics, β-glucans are long chains of D-glucose monomers called polysaccharides and β-glycosidic bonds link these glucose monomers. β-glucans differ greatly in terms of molecular mass, solubility and viscosity. They can occur in the bran of cereal grains such as barley and oats or the cell wall of baker's yeast and in certain fungi, mushrooms and bacteria. β-glucans are often found in cell walls and function to maintain the rigidity and shape of the cell Sandula *et al*., (1995). It is recognized that mushrooms and yeast contain mainly (1-3) (1-6)-β-glucans, while cereals contain (1-3)(1-4)-β-glucans (Mantovani et al., 2008). The solubility of β-glucans is directly impacted by the degree of polymerization with highly branched (1-6)-β-glucans having the greatest solubility Zekovic *et al*., (2005).

β-glucans are considered as biological response modifiers, and are attracting attention from the pharmaceutical and functional food industries because of their beneficial effects on human and animal health. Their biological effects are influenced by their degree of branching, molecular mass and tertiary structure Oliveira *et al* (2009). Production of β-glucans confers

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to *Pediococcus* and *Lactobacillus* strains (Visser 1984). Dekker and Medlen,(2003). Found that β-glucans synthesis from *Lactobacillus paracasei* NFBC338 had higher survival during gastrointestinal passage or technological process. Yeast cell wall derived mannan oligosaccharides (MOS) powerful antigenic and stimulating products which help to maintain the balance between feed intake and products Shashidhara, and Devegowda (2003). Supplementation of poultry diets with MOS results in improved production in terms of body weight gain and feed conversion, partly due to its hypothesized nutrient. In addition, feeding MOS to animals increased the immunoglobulin levels in their plasma, bile, and colostrums Savage *et al*., (1996).

The aim of the present study was to investigate the effect of mannan and β -glucan on kidney and liver function, some hematology parameter, serum IgGs concentrations and the body weight gain parameter on broilers fed diets contaminated with aflatoxin 1mg/kg feed.

MATERIALS AND METHODS

The Experimental Animal:-

A total number of eighty broilers at one day old are used in this work they collect from farm near Alexandria. Broilers were housed in floor pens in an environmentally controlled broiler house. They received a commercial broiler starter diet, formulated, as recommended by (NRC) 1994. Lighting was provided for 23 h/d. At 7-d of age and adaptation to laboratory conditions inside prepared hatches, it was given vaccines to ensure the avoidance of diseases hazard or death, and then the treatments starts to proceed from the 7^{th} day till the 45^{th} day and received commercial diet to cover the nutrient requirements of broilers as recommended by (NRC) 1994. Ingredients diet g / kg (Yellow maize 550 / Soybean meal 257 / cotton seed meal 52 / Fish meal 50 / Vegetable (sunflower) oil 50 / Limestone 9 / Dicalcium phosphate 7 / Salt 3.7/ Vitamin and Mineral 17.5/ premix 1.5 / D-L Methionine 1.2 / L-lysine 1.1).

Table 1. Chemical composition of commercial diet

Item	OM	CP	CF	EE	NFE	Ash
diet	94.7	3.53	23.12	6.50	56.25	5.30

Proximate analyses were carried out according to A.O.A.C. (1995).

From 7^{th} day to 45^{th} total number of (80) chicks of similar weights were distributed randomly into 10 broilers for each group. The birds were assigned to the following treatment groups: group (1) Negative control, group (2) Positive control fed diet which contain aflatoxin add (1mg/kg diet) between 7 to 45 days of age, and 6 groups (from group 3 to group 8) fed diets containing treatments with different concentration of Mannan and β Glucan in premix (premix added as a 1.5 gm/kg feed) as showed in the table (2):-

Hematological Analysis:-

Blood sample were collected every week from the wing veins of individual chickens in all groups and received in anti-coagulants tubes. Packed cell volume PCV was measured using wintrobe hematocrit tubes that was filled with homogeneous ant coagulated blood samples and then centrifuged for five minutes at 4000 rpm and the packed cell volume was obtained by measuring the percentage of the packed cell volume relative to the whole sample volume Wintrob, (1965). Hemoglobin concentration was measured by the cyanomethoglobin method Tietz (1982). The red blood cell count was determined by diluting blood samples with formal citrate solution (3g sodium citrate, formaldehyde 40% W/V and 100 ml distilled water). Red blood cell (RBC's) and white blood cells (WBC's) were counted and determined using a hemocytometer using a 40x objectives according to method describe by Wintrobe (1965). The serum obtained was stored at 20°C until usage for the determination of liver and kidney functions. Liver function was assessed by measuring the activity of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GTP) according to Reitman and Frankle (1957). Kidney function was evaluated by measuring blood urea according to the method of Patton and Crouch. (1977). Createnine was measured according to Bartels and Bohmer (1972).

Immune Globulin Analysis:-

Bovine polyclonal immune globulin G (IgGs) was obtained from Sigma (St. Louis, MO). Blood samples of chicks randomly selected from each pen, were collected for determination of serum IgGs concentrations. The method used LC-MS/MS according to Julien *et al*.,(2002).

Physiological Parameters:-

a) The body weight Gain:-A random five chicks from every group were chosen to measure Body Weight by measuring the weight of every one using normal balance and weight.
b) Relative Liver Weight Ratio:-After sacrificing the chicks, the livers were extracted, weighed and calculated as relative to the living body weight.

Data were statistically analyzed using the method of least squares analysis of variance using General Linear Models (GLM) procedure (SAS, 2000).

Table 2. Concentration of Mannan and β-Glucan in premix added to diets expremintal groups

Groups	Mannan % In premix	β-Glucan % In premix	Aflatoxin con. (mg/kg feed)
1(N.C.)	0	0	0
2(P.C.)	0	0	1
3	3	1	1
4	10	0	1
5	17	25	1
6	25	25	1
7	25	30	1
8	32	38	1

Duncan's Multiple Range Test (Duncan, 1955) was used to compare among means of each trait.

RESULTS AND DISCUSSION

Table (3) presents the effect of mannan and β-glucans on liver function of aflatoxin intoxicated broilers. The results of Blood Serum Glutamic Oxaloacetic Transaminase (SGOT) showed that group (1) negative control recorded value 297u/l comparison with group (2) positive control recorded 342u/l these results explain the effect of mycotoxin on liver function. These results in-agreement with Ghosh (2007) who found an increase in SGOT and SGPT was observed at 0.75 ppm aflatoxin in chicken diets. However, groups from 3 to 8 showed intermediate results between the negative and positive control While, groups (4, 5 and 6) showed an improvement in SGOT and the result was close to the negative control. The same trend was recorded with the results obtained from the analysis of Blood Serum Glutamic Pyruvic Transaminase (SGPT) of broilers. These result was in-agreement with previous studies suggested that the best approach for decontamination would be biological degradation which would allow the inactivation of aflatoxin by prebiotics Fritts and Waldroup, (2003); Shashidhara and Devegowda, (2003). The results of kidney function showed in table (2). Results of blood urea and creatinine of broilers indicated that negative control recorded lowest results compared to positive control this result in-agreement with Sajid *et al* (2012) who reported increased level of urea and creatinine with layers feed diets contaminated with Aflatoxin in. Also Hassan *et al* (2012) found that increased serum creatinine and urea levels indicated to inflammatory or degenerative changes in the kidney. Our results indicated that added prebiotics to contaminated food with aflatoxin could ameliorate the aflatoxin-induced increase in uric acid and creatinine concentrations and return these to levels similar to negative control this results in-agreement with Kasman *et al* (2012) who found a significant decrease in serum level of urea and creatinine when supplementation probiotics to chickens fed diets contaminated with aflatoxin. However groups 7, 8 were recorded the highest ($P < 0.05$) results than negative control of blood urea mg/dl. On the other hand no significant differences ($P < 0.05$) obtained of blood creatinine values among groups from 3 to 8 and negative control.

Table (4) presents values of hematological indices of controls and treated groups. Table revealed that treatments groups induced a significant variation in blood characteristic among controls and treatments groups. Significant decreases were noted in the following hematologic parameters: Red blood cell (RBC), hemoglobin (Hb) and packed cell volume (PCV) with positive control group than negative control group. The result observed that red blood cell counts, (PCV) and hemoglobin concentrations were different to those reported by (Betina 1998), who observed no significant differences ($P<0.01$) between hens and broiler chickens fed 1,500g of aflatoxin1/kg diet and the control negative group. While our results was in-agreement with Jeff (2014) who found significant decreases in heamatological parameters with positive group feed diet containing 1g of aflatoxin1/kg of diet. On the other hand the results of white blood cells indicated that the presences of aflatoxin alone in the diet with positive group caused a significant ($P<0.05$) increase compared with those fed diets free from aflatoxine or those received mannam and β-glucans treatment this results was in- agreement with Shlig (2009). Generally improvement observed with groups that fed diets contain prebiotics (mannam and β-glucans) and the better improvement recorded with groups 4 and 6. These results in-agreement with Gezen *et al* (2004) who reported that addition prebiotics to diets contaminated with aflatoxin was effective of reducing the impact of toxins.

Table 3. Overall mean and their ±SE of liver and kidney functions in serum blood of Broilers fed the diets.

Groups	SGOT (U/L)	SGPT (U/L)	Urea mg/dl	Creatinine g/dl
G1(N.C)	297.32±8.29^{d}	12.16±0.45^{c}	16.58±0.68^{b}	0.29±0.03^{b}
G2(P.C)	342.32±12.54^{a}	18.89±0.58^{a}	18.35±0.55^{a}	0.42±0.04^{a}
G3	329.52±9.52^{b}	15.46±0.53^{b}	14.50±0.39^{c}	0.32±0.02^{ab}
G4	317.92±8.98^{bc}	13.12±0.66^{c}	15.20±0.25^{bc}	0.26±0.03^{b}
G5	303.42±14.74^{d}	14.55±0.59^{bc}	14.74±0.11^{c}	0.31±0.02^{ab}
G6	310.48±9.77^{c}	14.02±0.44^{bc}	15.68±0.91^{bc}	0.29±0.01^{b}
G7	321.16±12.32^{bc}	17.56±0.56^{a}	17.05±0.14^{a}	0.36±0.03^{ab}
G8	333.04±11.52^{b}	17.68±0.71^{a}	17.28±0.54^{a}	0.35±0.01^{ab}

^{abcd} Means within the same rows with different superscript are significantly differ (P<0.05).

Table 4.Effect of prebiotics on some heamatological parameters blood of Broilers fed the diets containing aflatoxin. (Mean± SE)

Groups	RBC x (10^6/mm^3)	Hb (g/100ml)	PCV %	WBC X(10^3/mm^3)
G1(N.C)	4.82±0.11^{a}	11.6±0.44^{a}	44.48±0.85^{a}	28.21±0.51^{b}
G2(P.C)	3.54±0.16^{b}	9.40±0.32^{b}	36.58±0.72^{c}	34.80±0.55^{a}
G3	3.00±0.18^{c}	9.30±0.35^{b}	46.5±0.76^{a}	27.52±0.62^{b}
G4	2.96±0.12^{c}	9.20±0.47^{b}	37.62±0.73^{c}	32.52±0.64^{a}
G5	3.62±0.14^{b}	10.0±0.33^{ab}	40.54±0.71^{b}	31.60±0.69^{ab}
G6	3.98±0.21^{ab}	9.80 ±0.34^{ab}	38.03±0.64^{bc}	29.46±0.54^{b}
G7	3.66±0.13^{b}	9.8±0.36^{b}	39.88±0.55^{b}	28.33±0.53^{b}
G8	3.40±0.15^{b}	10.2±0.41^{ab}	41.28±0.68^{ab}	32.11±0.44^{a}

^{abc} Means within the same rows with different superscript are significantly differ (P<0.05).

The effects of the prebiotic on serum IgGs concentrations of broilers are shown in Figure (1). There are differences in IgGs concentrations between the control groups (negative and positive) and experimental groups but the differences were not significant (P < 0.05). Higher IgGs concentrations levels were recorded with positive control and group 8 without significant differences with other groups. However, groups (4, 5, and 6) gave close result of the negative control. these results were in agreemnet with Choi *et al* (2010). Who reported that the aflatoxin - exposed diets had no significant effect on the IgG serum concentrations of mice. Also, Cetin *et al*. (2005) reported that IgG levels were not significantly increase by mannanoligosaccharide (MOS) supplementation in turkeys fed diets contaminated with aflatoxin. Similarly, some studies emphasize that aflatoxin did not change the humoral immunity Ballou,(1970) and Chen *et al* (2003). While, Marin *et al* (2003). Found that chikcens fed diets contminted with aflatoxin (2mg/kg diet) increase significant (P<0.05) serum IgG concentrations. Moreover, some other studies showed an increase in plasma globulin titers (IgM and IgG) in pigs exposed to dietary aflatoxin Thiel *et al* (1981) and Oguz *et al* (2003). The effects of humoral immune to AFB1 addition may be depend on the type, the dose, the duration of exposure, the susceptibility of each species (pigs, rats, chicken) Emea, 1999, These results confirmed that low dose aflatoxin dose not cause a significant modulation of the humoral immune response in broiler chicken.

The effect of additive perbiotics on body weight gain (BWG) of broilers fed diets containing aflatoxin showed in figure (2). The results indicated that the mean body weight gain (g) of broilers in different groups showed that significant increased with broilers fed control negative diet or those fed diets containing perbiotics additive compared to positive group. This result was in-agreement with Oguz and Parlat (2004) who found that the supplementation of mannanoligosaccharide(MOS) to the diets led to increased BWG. Also, Huff *et al* (2006) found that β-glucan supplementation protected broiler chickens against a decrease in growth after fed diets contaminated with aflatoxin. β-glucan also modulates the effects of the relative weights of the liver. However, significant decreased showed with broilers fed positive control. This adverse effects of aflatoxin on BWG were may be due to inhibition of protein synthesis and lipogenesis (Oguz and Kurtoglu, 2000; Oguz *et al.,* 2000). Impaired liver functions and protein/lipid utilization mechanisms may also have affected the

growth performance and general health (Ortatatli and Oguz, 2001). The effects of aflatoxin on growth performance in this study agreed with the previous studies, for example Kubena *et al.* (1993) and Miazzo *et al.* (2000) reported that BWG decreased by10% to 20% in broiler chickens given aflatoxin for 3 weeks ($P < 0.05$).

The results of relative liver weight ratio are presented in figure (3) there was a significant increase ($p< 0.05$) in the relative liver weight with broilers fed positive control, also showed abnormal hepatic lobules of liver, fatty liver, lipid vacuoles within hepatocytes, cirrhosis, globular red hyaline material, Kupffer Cells, pronounced, liver cell destruction have been observed as an abnormal liver view in inspection, which were characteristic of aflatoxin intoxication observed at the histopathological examination this results in-agremennt with Cardoso *et al.*(2011) who found that a significant increase in the relative liver weight and injury levels and abnormal liver histopathological examination. The liver is considered the principle target organ for flatoxin the diet containing aflatoxin alone resulted in a significant increase in the relative weight of liver Shlig *et al* (2009). However, significant decreases in liver weight showed with groups fed diets containing prebiotics additive. Groups 5, 6 negative controls had lowest relative liver weight ratio ($p< 0.05$) compared with other groups.

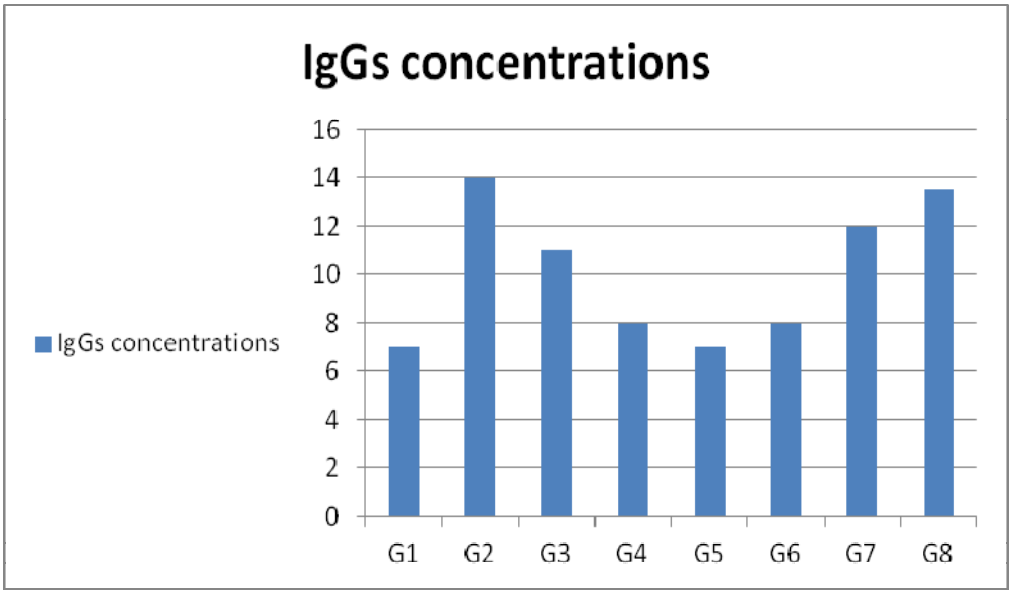

Figure 1. The analysis of Immune Globulins

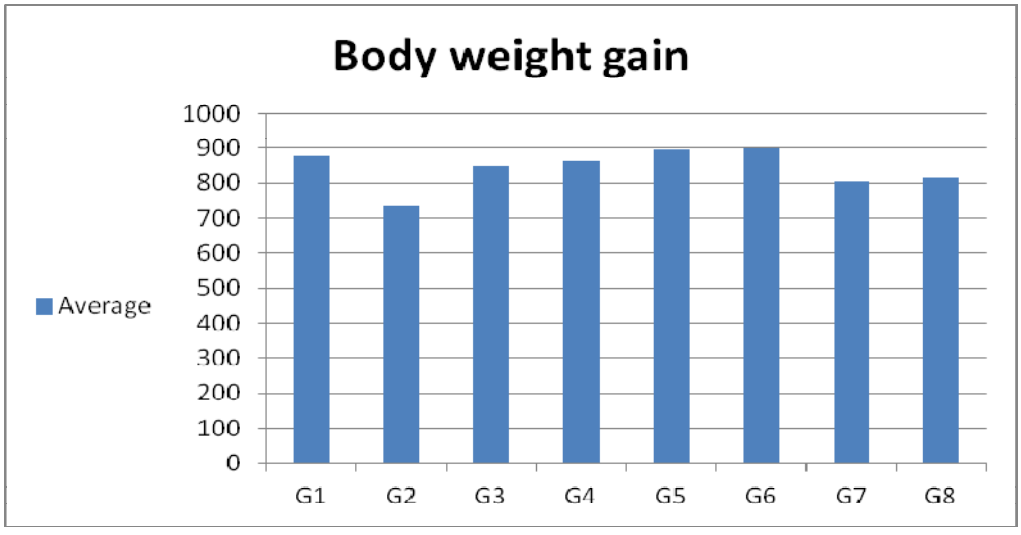

Figure 2. The Body Weight Gain (g) of different groups

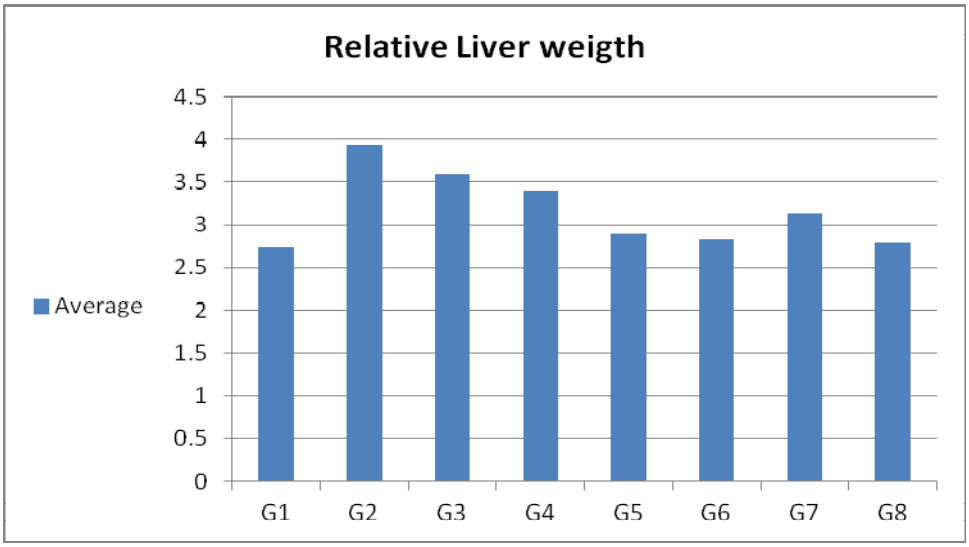

Figure 3. The Body Weight / Liver weight Ratio of different groups

CONCLUSION

The addition of different levels of mannan and β-Glucan as a prebiotics to broilers diets contaminated with aflatoxin might be reduced the effects of aflatoxin toxicity on liver and kidney function and body weight. Our data indicated that group (5) and group (6) were the most results which are meet the negative control results. Further works is needed to determine the optimum levels of mannan and β-Glucan to be used on other kinds of animals and on various types of production.

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الملخص العربي

تأثير إضافة المنان وβ-جلوكان باعتبارهما بريبايوتكس علي علائق دجاج اللحم الملوثة بالأفلاتوكسين

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أجريت هذه الدراسة لتحديد تأثير أستخدام مستويات مختلفة من المنان وβ-جلوكان باعتبارهما بريبايوتكس على وظائف الكبد والكلى وبعض مكونات الدم، وتركيز الجلوبيولين المناعي ووزن الجسم على دجاج اللحم الذي تم تغذيتة علي عليقة ملوثة ١mg الأفلاتوكسين/ كجم عليقة. واستخدم عدد ثمانين من دجاج اللحم وتم توزيعها عشوائيا إلى ثماني مجموعات. كل مجموعة تم تغذيتها علي عليقة تجارية الخاصة بالنظام الغذائي للدجاج اللحم على النحو التالي {المجموعة الأولي(مجموعة المقارنة السلبية) وهي التي تم تغذيتها علي العليقة التجارية فقط بدون إضافة البريبايوتكس او الأفلاتوكسين، العليقة الثانية (مجموعة المقارنة الايجابية) تم تغذيتها علي العيقة التجارية مضاف إليها ١mg الأفلاتوكسين/ كجم عليقة الحرة، والمجموعات من ٣ إلى ٨ تم تغذيتها علي عليقة التجارية مضاف المنان وβ-جلوكان باعتبارهما بريبايوتكس الأفلاتوكسين (1mg/kg). وتحتوي هذه العلائق على تراكيزات مختلفة من المنان وβ-جلوكان في صورة بريمكس والذي تم اضافته بنسبة (١.٥ جم / كجم علف)}. أظهرت النتائج الرئيسية لوظائف الكبد والكلى أن مجموعة المقارنة الإيجابية أظهرت زيادة معنوية ($P < 0.05$) في قيم (SGPT، SGOT) مقارنة مع مجموعة المقارنة السلبية وكان أفضل تأثير لإضافة المنان وβ جلوكان سجلت مع مجموعة ٥ و٦ حيث كانت النتائج قريبة من المجموعة المقارنة السلبية. وأوضحت النتائج نفس الأتجاه مع القيم التي تم الحصول عليها من اليوريا والكرياتيين. وبالنسبة لنتائج قياسات الدم أظهرت المجموعة المقارنة الأيجابية إنخفاضا معنويا ($P < 0.05$) في قيم حجم معبأة الخلية (PCV)، وخلايا الدم الحمراء (RBC)، الهيموغلوبين. وسجلت أيضا زيادة معنوية ($P < 0.05$) في قيم خلايا الدم البيضاء (WBC) وذلك عند مقارنتها بالمجاميع الاخري. عموما أظهرت النتائج أن إضافة المنان وβ جلوكان إلى العلائق الغذائية الملوثة بالأفلاتوكسين حسنت بشكل ملحوظ من الآثار الضارة للأفلاتوكسين على قياسات الدم. بالنسبة لدراسة تركيز المناعة أظهرت هذه الدراسة زيادة غير معنوية في تركيزات IgGs مع مجموعة المقارنة الإيجابية والمجموعة 8 عند مقرنتها بالمجاميع الأخري. وأظهرت نتائج معدل الزيادة في وزن الجسم (جم) زيادة معنوية ($P < 0.05$) في دجاج اللحم التي تغذت علي مجموعة المقارنة السلبية مقارنة مع مجموعة المقارنة الإيجابية. وأن إضافة كل من المنان وβ جلوكان إلى الوجبات الغذائية أدت إلى تحسن في زيادة وزن الجسم وإنخفاض فى وزن الكبد.