EFFECT OF PREBIOTIC (NUTRICELL) IN MINIMIZING THE ADVERSE EFFECTS OF OCHRATOXIN IN LAYING QUAILS

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Abstract

The aim of this study is to determine the most adverse effects of ochratoxin on egg production, egg weight, hematological and biochemical parameters, pathological changes and its residue in liver, kidneys, Muscles, and egg beside modulation of these adverse effects by using prebiotic (Nutricell). Quail hens received ochratoxin revealed significant reduction in RBCs, Hb, PCV, total proteins, egg production, and weight, beside elevation in AST, ALT, ALP, uric acid, and creatinine compared with control hens. Quail hens supplemented with prebiotic induced significant increase in RBCs, Hb, and PCV, total proteins, egg production, and weight beside insignificant increase in AST, ALT, ALP, uric acid, and creatinine. Laying quail hens received ochratoxin beside prebiotic induced insignificant elevation in RBCs, Hb and PCV, total proteins, AST, ALT, ALP, creatinine, uric acid, egg production and egg weight. Obtained results revealed that ochratoxin residues in examined samples of liver, kidney, and muscles were very high at 1stday post exposure and completely disappeared from all examined samples after 6th days post administration beside no residues were detected in the examined eggs.

The most frequent histopathological lesions in the liver of laying quail hens, showed congestion of the portal blood vessels, coagulative necrosis of hepatocytes, focal area of aggregation of leukocytes in the hepatic parenchyma. Congestion and leukocytic infiltration replaced the renal parenchyma in kidneys.

From this study we concluded that, prebiotic induces improvement in egg production, weight, hemato-biochemical parameters, and pathological changes induced by ochratoxin in laying quail hens.

INTRODUCTION

In recent years, a great attention was paid towards quail farming as a trial to fulfill the increasing demands for the animal proteins Seker, (2003). Quails become mature within 6 weeks and in full egg production by 50 days of age and their meat and eggs are considered of high protein content Azab, *et. al.,* (2001)

Mycotoxins are often found as natural contaminants in grains Walker, (2002) Ochratoxins are worldwide spread secondary metabolites produced by several toxigenic fungi such as *Aspergillus ochraceus* or *Penicillium verrucosum* Magan and Aldred, (2005). The family of ochratoxins consists of three members known as Ochratoxin A, B and C but ochratoxin A is the most toxic one Chang et al., (1981). It consists of dihydroisocoumarin

part coupled, via its 7 -carboxy group, with an L-beta-phenylalanine part Engelhardt et. al., (1999). It induces depressed growth Burns and Dwiveldi,(1986), nephrotoxicity Gill and Cross,(2002) and immunosuppression Santin et al.,(2002). Immunosuppressive effect of ochratoxins in chicken will lead to exacerbation of diseases Wang *et. al.,* (2009). Some authors suggested that ochratoxin interferes with synthesis of enzymes and other proteins by competitive inhibition of phenylalanine-t-RNA Ueno, (1991).

The harmful effects of mycotoxins were prevented by using some feed additives which have property to selectively bind mycotoxins and carry them out the organism without binding other beneficial elements as prebioti Cristina and Pantana,(2005). It is indigestible food ingredients which stimulate growth and activity of a selected number of bacteria in GIT tract Gibson and Robrfroid,(1995). Prebiotics improve bowl function enhanced resistance to invading pathogens Cashman,(2003). Prebiotics in poultry have beneficial effects on birds' performance Vegad, (2004).

This study was performed to evaluate the prophylactic efficacy of prebiotic in controlling hemato-biochemical parameters, egg production, egg weight and pathological adverse effect induced by ochratoxicosis in laying quails.

MATERIALS AND METHODS

Prebiotic(Nutricell-MOS®):was produced by Industrial Comercio Exprtacao Importace Ltda(ICC, Sao Paulo, Brazil).It Composed of Glucan 18.5%, Mannan oligopolysaccharide14.5%, Chitin 26.4%, Crude proteins 33.87%, Moisture 5.08% and NaCl, 1.56%.

Quails and Experimental design:-

A total of 160 laying quail hens were used in this investigation. Quails were housed under hygienic condition, fed on a balanced commercial ration and water was provided ad-libitum during experimental period. Ration used in this study was analysed to prove that it was free from Ochratoxin using immunoaffinity method described by Trucksess etal.,(1991) using Fluorometer.

Quails were divided into four equal groups (each of40).1st group was healthy and non-treated (control group), 2nd group received 2.5 mg ochratoxins/ kgm ration for 30 successive days, 3rd group received 1gm/kgm ration of prebiotic(Nutricell) for 30 successive days and 4th group received ochratoxin and prebiotic (Nutricell) by the same previous dose and period. All quail laying hens were left under observations during the experimental period to record egg production & egg production was calculated Summers, *et. al.,* (1976).

Hematological and biochemical examination

Two blood samples were taken from all quail hens after the1st, 6th and 15th days post ochratoxin and periodic supplementation. First sample was taken in a tube containing EDTA as an anticoagulant for erythrogram estimation according to Jain (2000). Second sample was taken to obtain clear serum for estimation of total proteins according to Doumas, etal.,(1981), AST and ALT after Reitman and Frankel, (1957), alkaline phosphatase, John (1982), uric acid (Coalombe and Faurean, 1963) and creatinine Husdan and Roporpot,(1968) .

Pathological examination: Specimens from liver and kidneys were collected from slaughtered laying quails. They were fixed in 10% buffered neutral formalin and embedded in paraffin Sections of 5 micron thickness, were prepared,stained by H &E and examined microscopically Bancroft etal.,(1990).

Ochratoxin residues:

A-Tissue: Five laying quail hens in all groups were slaughtered after the1st, 3rd, 6th& 15th days of ochratoxin and prebiotic supplementation, another five laying hens were slaughtered after the1st, 3rd, 6th& 15th days post ochratoxin and prebiotic supplementation i.e. after the experimental period (30 days) . Ochratoxin residues in muscles, liver, kidneys of slaughtered hens were estimated Jùrgensen (2004).

B-Egg: Ochratoxin residues in eggs of quail hens were estimated after recommended time according to AOAC,(1995). Egg samples were taken at1st, 3rd, 6th & 15th days of ochratoxin and prebiotic supplementation and another egg samples were taken after the1st, 3rd, 6th& 15th days post ochratoxin and prebiotic supplementation i.e. after the experimental period (30 days). Egg samples were weighed and homogenized. The samples were stored at -20°C till analysis.

Statistical analysis: obtained results were analyzed after Petrie and Watson (1999).

RESULTS

Ochratoxin displayed significant reduction in RBCs, Hb and PCV, total proteins, egg production%, egg weight beside significant elevation in ALT, AST, ALP, uric acid and creatinine at 1st and 6th day post ochratoxin feeding(Table 1, 2 & 4). Prebiotic elicited significant elevation in RBCs, Hb and PCV, total proteins, egg production %, egg weight, beside insignificant elevation in AST, ALT, ALP, uric acid and creatinine (Table 1, 2 & 4). Laying quail hens received ochratoxin and prebiotic together induced insignificant rise in RBCs, Hb and PCV, total proteins, AST, ALT, ALP, uric acid, creatinine, egg production and

egg weight (Table 1, 2 & 4). Ochratoxin residues in examined liver, kidney and muscles sample were high at 1st day post administration and disappeared from examined samples at 15th days post Ochratoxin supplementation beside no residues were detected in egg (Table 3&4).

Gross pathological lesions resulted from experimental ochratoxicosis, liver was enlarged, congested, and friable and in some cases pinpoint hemorrhages on surface with distended gall bladders and bronze discoloration. Kidneys were swollen and had hemorrhages on its surface, ureters were filled with urates. Microscopically, the liver of laying quail hens supplemented with ochratoxin (2.5 mg/kgm ration) shows congestion of the portal blood vessels besides focal area of aggregation of leukocytes in the hepatic parenchyma (Fig.1),moreover, focal areas of coagulative necrosis of hepatocytes were seen (Fig.2). Focal aggregations of mononuclear cells infiltrating the hepatic parenchyma were observed (Fig. 3). The kidneys of laying quail hens suffering from ochratoxicosis revealed congestion besides leukocytic infiltration replaced the renal parenchyma (Fig. 4), congestion of the renal blood vessels and leukocytic aggregation replaced some renal tubules (Fig. 5), leukocytic infiltration among the degenerated renal tubules (Fig. 6)

Table 1 Effect of ochratoxin and prebiotic on blood picture, in laying quail hens (n =5)

Parameters		RBcS (10^6/cm.m)	Hb (gm/dls)	P.C.V. %
Gp1(control)		4.09±0.17	11.21±0.40	36.13±0.68
Gp2	1day	3.29±0.25*	9.17±0.56*	33.69±0.46*
	6 day	3.38±0.18*	9.79±0.33	34.42±0.35*
	15 day	3.69±0.17	10.90±0.20	35.29±0.42
Gp3	1day	4.69±0.12*	13.38±0.63 *	38.60±0.53*
	6 day	4.55±0.10*	12.58±0.31 *	38.69±0.50*
	15 day	4.18±0.13	12.19±0.29	37.10±0.40
Gp4	1day	4.14±0.15	11.25±0.19	36.15±0.28
	6 day	4.08±0.18	11.19±0.27	36.09±0.36
	15 day	3.03±0.14	11.18±0.30	36.17±0.21

* Significant at P<0.05

Table 2. Effect of ochratoxin and prebiotic on liver and kidney function in laying quail hens (n=5).

Parameters		Liver Functions				Kidney Functions (mg/dl)	
		T.protein(g/dl)	AST(U/L)	ATL(U/L)	ALP (U/L)	uric acid	creatinine
Gp1(control)		4.89±0.28	42.26±0.98	33.11±0.89	55.34±0.61	2.50±0.11	1.07±0.16
Gp 2	1day	3.87±0.19*	46.19±0.95*	37.10±0.98*	58.51±0.91*	2.86±0.10*	1.59±0.13*
	6 day	3.95±0.27*	45.19±0.39	35.69±0.83*	57.69±0.80*	2.80±0.07*	1.65±0.15*
	15 day	4.44±0.16	43.07±0.60	34.05±0.89	56.10±0.98	2.57±0.18	1.27±0.18
Gp 3	1day	5.63±0.15*	42.83±0.30	3438±0.93	55.45±0.39	2.44±0.12	1.09±0.11
	6 day	5.57±0.12*	42.70±0.51	33.94±0.81	55.36±0.26	2.49±0.21	1.11±0.10
	15 day	5.12±0.29	42.42±0.40	33.27±0.59	55.26±0.17	2.51±0.11	1.10±0.12
Gp 4	1day	4.92±0.19	42.50±0.93	3312±0.54	55.49±0.81	2.56±0.15	1.08±0.15
	6 day	5.08±0.17	42.38±0.76	33.14±0.70	55.43±0.79	2.51±0.20	1.10±0.12
	15 day	5.10±0.16	42.29±0.59	33.04±0.48	55.33±0.59	21.48±0.16	1.08±0.15

- Significant at $P<0.05$

Table 3. Ochratoxin residues in liver, Kidney and muscles (ug/gm) after supplementation of laying quail hens (n=5)

Parameters		Residues after supplementation			Residues after experimental period		
		liver	kidney	Muscles	liver	kidney	Muscles
Gp1(control)		00	00	00	00	00	00
Gp2	1day	00	00	00	6.06±0.19	6.83±0.15	3.77±0.18
	3day	0.63 ±0.05	0.77±0.10	0.32±0.03	4.27±0.21	5.18±0.40	2.43±0.16
	6day	1.05 ±0.08	1.27±0.06	0.89±0.09	1.09±0.31	1.41±0.29	1.21±0.14
	15day	2.89 ±0.12	2.95±0.15	1.97±0.13	00	00	00
Gp3		00	00	00	00	00	00
Gp4	1day	00	00	00	4.11±0.26	4.30±0.25	2.87±0.30
	3day	0.55 ±0.09	0.60±0.04	0.28±0.07	3.48±0.19	3.63±0.27	1.50±0.21
	6day	0.98 ±0.12	1.08±0.15	0.76±0.10	1.28±0.28	1.39±0.19	0.49±0.06
	15day	2.76±0.14	3.09 ±0.12	1.77±0.11	00	00	00

Table 4. Effect of ochratoxin and prebiotic on egg production%, egg weight (gm) and ochratoxin residus in egg (ug/gm) of laying quail hens (n=5)

Groups		1day	3day	6day	15day
Gp1	egg production	84.52±1.22	84.70±1.40	83.96±1.04	84.12±1.48
	Egg weight	9.85±0.14	9.53±0.31	9.20±0.19	9.35±0.41
	ochratoxin residue	00	00	00	00
Gp2	egg production	80.21±1.09*	80.69±1.12*	80.46±1.11*	82.69±1.81
	Egg weight	8.90±0.13*	8.44±0.24*	8.61±0.10*	8.84±0.18
	ochratoxin residue	00	00	00	00
Gp3	egg production	88.29±1.14*	88.96±1.10*	87.23 ± 1.02*	85.06±1.66
	Egg weight	10.5±0.18*	10.29±0.12*	10.03±0.21*	10.45±0.21
	ochratoxin residue	00	00	00	00
Gp4	egg production	83.34±1.79	83.61±131	83.74±1.55	83.91±120
	Egg weight	9.68±0.21	9.74± 0.19	9.78± 0.12	9.80± 0.45
	ochratoxin residue	00	00	00	00

* Significant at P<0.05

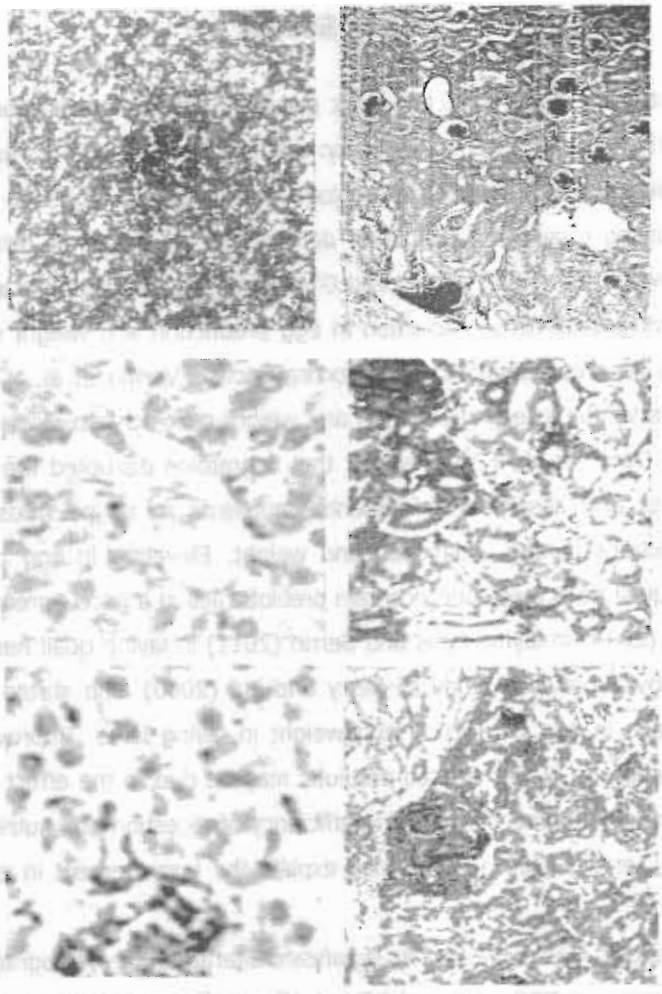

Fig. (1): Liver of laying quail hens supplemented with ochratoxin (2.5 mg/kgm ration) shows congestion of the portal blood vessels besides focal aggregation of leukocytes in the hepatic parenchyma. H&E. X120.

Fig. (2): Liver of laying quail hens supplemented with ochratoxin (2.5 mg/kgm ration) shows focal coagulative necrosis of hepatocytes. H&E. X120.

Fig. (3): Liver of laying quail hens supplemented with ochratoxin (2.5 mg/kgm ration) shows focal aggregations of mononuclear cells among hepatic parenchyma. H&E. X120.

Fig. (4): Kidney of laying quail hens supplemented with ochratoxin (2.5 mg/kgm ration) shows congestion besides leukocytic infiltration replaced the renal parenchyma. H&E. X120.

Fig. (5): Kidney of laying quail hens supplemented with ochratoxin (2.5 mg/kgm ration) shows congestion of the renal blood vessels and leukocytic aggregation replaced some renal tubules. .H&E. X120.

Fig. (6): Kidney of laying quail hens supplemented with ochratoxin (2.5 mg/kgm ration) shows leukocytic infiltration among the degenerated renal tubules. H&E. X120.

DISCUSSION

The present study showed that, the most prevalent effect of ochratoxicosis in laying quail hens was the redution in egg production and egg weight at 1st 3rd and 6th day post Ochratoxin supplementation, meanwhile prebiotic induces significant rise in egg production and weight in laying quail hens. Obtained data about the effect of ochratoxin in egg production and weight agree with Prior and O'Neil, (1981) in laying hens and Mariam, *et. al.,* (2010) in laying quail hens. Reduction in egg production and weight was associated with decrease in feed consumption due to ochratoxicosis Verma et al., (2003). Another explanation for reduction in egg production and weight post ochratoxicosis in laying quail hens come from Denli, et al,. (2008) stated that ochratoxin disrupted the activity of the digestive enzymes and absorption of essential nutrients as amino acids could mainly explain the reductions in egg production and weight. Elevation in egg production and weight in laying quail hens feed ration contain prebiotic lies in a good agreement with that of Yoruk, *et. al.,* (2004) in laying hens and Berrin (2011) in laying quail hens. Our results were supported by a previous study of Berry and Lui (2000) who stated that prebiotic induces an increase in egg production and weight in laying hens. Improvement in egg production and weight in quail hens fed prebiotic may be due to the effect of prebiotic in improving intestinal environment, increasing efficiency of digestion and nutrient absorption processes Stanley, et al., (2000) which may explain the improvement in egg production and weight.

Toxicity of ochratoxin was expressed as significant alteration in erythrogram represented by significant decrease in RBCs, Hb and PCV at 1stand 6thday post use of ochratoxin but prebiotic induce significant increase in RBCs, Hb and PCV in laying hens. Similar results were recorded in earlier study Bailey, *et. al.,* (1989) who found that ochratoxin induces significant decrease in RBCs, Hb and PCV% in broilers. Similar findings were reported in ochratoxicosis in leghorn cockerels Fakhar et.al, (2011). Whereas, Mohiuddin *et. al.,* (1993) mentioned that ochratoxicosis induced a decrease in RBCs. The above mentioned results were supported by previous studies of Mohammad, *et. al.,* (2011). The previous author mentioned that prebiotic induce significant rise in RBCs, PCV and Hb in laying hens and these results run parallel with those obtained by Onifade (1997) and Roberfroid (2000), they reported a positive correlation between dietary levels of prebiotic with the RBCs, PCV and Hb in broiler chickens

Laying quail hens fed diets contaminated with ochratoxin showed significant reduction in total protein beside significant elevation in AST, ALT, ALP, uric acid and creatinine at 1stand 6th day post the use of ochratoxin but prebiotic induces significant increase in total proteins associated with insignificant change in AST, ALT, ALP, uric acid and creatinine in

laying quail hens. Similar decrease in total proteins in the present trial has been reported during ochratoxicosis in laying hens Kalorey *et. al.,* (2005). Decline in total protein in quail hens fed on ration contained ochratoxin may be due to inhibition of hepatic protein synthesis Castegnaro and Pfohl, (2005). Elevation in AST, ALT and ALP in laying quail hens fed ration contained ochratoxin reflect the hepatic damage represented by congestion of the portal blood vessels besides focal aggregation of leukocytes in hepatic parenchyma, coagulative necrosis of hepatocytes and focal replacement of hepatic parenchyma with mononuclear cells lead to leakage of this enzymes in blood stream. Pathological findings, observed in liver of ochratoxinated laying quail hens were recorded by Kumar *et. al.,* (2004) and Zahoor, *et. al.,* (2010). Close similarity was seen between the finding and those obtained by Kalorey, et. al., (2005) they found that ochratoxin induces an increase in liver enzymes, urea and creatinine in broiler chicken. Same data was previously obtained by Huff *et. al.*, (1988) who stated that ochratoxicosis in poultry induces increases in uric acid, creatinine and ALP. Elevation in serum uric acid and creatinine in laying quail hens exposed to ochratoxin may be due to impaired kidney function due to kidney damage represented by degenerative changes in proximal and distal convoluted tubules, atrophy in a few areas of glomeruli and hypertrophy of glomerular tuft as well as presence of hyaline casts in collecting tubules Farshid and Rajan, (1995). Pathological findings, observed in kidney of chicken exposed to ochratoxin are described previously by Hoehler and Marquardt (1996). Increase in total proteins in laying quail hens fed ration contained prebiotic was in agreement with the result obtained by Mohammad, *et. al.*, (2011) in laying hens. Increased total proteins in laying hens may be due to improvement in intestinal environment which leads to improvement of digestion and absorption of nutrients, resulted in increasing the amino acids in the blood which is highly important for the protein biosynthesis (Mariam, *et. al.,* (2010). Our results came in agreement with Parks, *et. al.,* (2001),who mentioned that turkeys fed ration contains prebiotic showed insignificant changes in AST, ALT, ALP, uric acid, creatinine and these results run parallel with those obtained by Ledoux, *et. al.,* (1998) in broiler chickens.

Obtained results revealed that ochratoxin residues in examined samples of liver, kidneys, and muscles were very high during exposure and at 1st day post exposure and completely disappeared from all samples at 6thdays post administration. The previous findings are in accordance with results obtained by previous authors Denli ,et al.,(2008) they mentioned that ochratoxin is accumulated mostly in the kidneys followed by the liver but Zahoor et al.,(2012) reporting that residue of ochratoxin was significantly higher in kidneys followed by liver and lower in muscles. Our results agree with previous experiments in broilers Krogh,(1976) revealed that, there is correlation between ochratoxin concentration in feed and its residues in animal tissues. Another study by

Ringot, et al.,(2006) found that ochratoxin residues is directly dependent on the level of ochratoxin in the diet and period and it reached to high levels in the early stages of exposure and then tended to decrease in broilers organs. The obtained results nearly coincide with those reported by Prior, *et. al.,* (1980) who detected ochratoxin residues in liver, kidneys with no residues of ochratoxin A in muscles of broiler chickens fed 2 ppm ochratoxin A and Piskorska and Juszkiewicz, (1990) found that the highest ochratoxin residues in kidneys and the lowest in muscles and its still be detected in kidney, liver and muscles after 6 days after withdrawal of the 20 mg/kg ochratoxin from feed however, traces of ochratoxin were found in the muscles.

Residues of ochratoxin in eggs, Table, 3 showed that there is no ochratoxin residue in egg during and post fed laying quail hens on 2.5 mg ochratoxins / kgm ration for 30 successive days. Same results were recorded by Krogh, (1987) and Piskorska and Juszkiewicz, (1990). The previous authors failed to detect residues of ochratoxin in eggs of hens fed 0.3 and 1mg of ochratoxin /kgm ration. Similarly, Zahoor, *et. al.,* (2012) found no residues of ochratoxin in egg of leghorn hens fed on 4 mg ochratoxin/ kgm ration. In another study, ochratoxin residues in eggs of hens fed large amounts of ochratoxin (10 mg/kg ration) Juszkiewicz et al., 1982).

Macroscopically and microscopically lesions were observe in both liver and kidney in our study and recorded previously by Sakhare *et. al.,* (2007) and Sawale et al., (2009) in laying hens and also in broiler chicks by Kumar *et. al.,* (2004).

Quail hens fed diets contain ochratoxin and prebiotic together caused marked amelioration of the adverse effect of ochratoxin and induced improvement in egg production, egg weight, erythrogram, total proteins, liver enzymes and kidney functions. The same results were recorded by Stoev, (2010) and Mohammad, *et. al.,* (2005) in laying hens fed ration containing prebiotic. Our findings coincide with Mohammed ,(2006) who stated that, prebiotics bind mycotoxins in the digestive tract, allowing the mycotoxins to pass harmlessly through the digestive tract.

Finally, it could be concluded that the prebiotic are effective in ameliorating the toxic effects of ochratoxin that may be present in laying quail ration

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تاثير البريبيوتيك (نتريسيل) في تقليل التأثير الضار للأوكراتوكسين فى السمان البياض

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لمعرفة تأثير الاوكراتوكسين والبريبيوتيك على انتاج البيض وبعض التغيرات الدمويه ،البيوكيميائيه والباثولوجيه تم تقسيم 160 طائر سمان بياض بصحه جيده ظاهريا واكلينيكيا إلى اربع مجموعات متساوية وكانت المجموعة الأولى طيورسمان تركت بدون اى إضافات (مجموعة ضابطة).المجموعة الثانية طيورسمان تم تغذيتها على عليقه تحتوى على٢,٥مجم من الاوكراتوكسين /كجم من العليقة لمده شهر. المجموعة الثالثة طيور سمان تم تغذيتها على عليقه تحتوى على ١جم بريبيوتيك/ كجم علف لمدة شهر. المجموعة الرابعه طيورسمان تم تغذيتها على عليقه تحتوى على الاوكراتوكسين والبريبيوتيك بنفس الجرعه و المده السابقه . تم ذبح عدد 5 سمانه من كل مجموعه عند 1 ,3 ,6 ,15, يوم اثناء وبعد نهاية استخدام الاوكراتوكسين والبريبيوتيك وتم اخذ عينات من الكبد والكلى والعضلات لتعيين بقايا لاوكراتوكسين ودراسة تأثير الاوكراتوكسين البريبيوتيك على تلك الاعضاء باثولوجيا. كذلك تم تجميع البيض من كل مجموعة على حدة عند نفس المدد السابقه وذلك لدراسة تاثير الاوكراتوكسين والبريبيوتيك على إنتاج البيض ووزن البيضه . وكذلك تعيين بقايا لاوكراتوكسين فى البيض.وإثناء الذبح وعند ايام عند 1 و 6 و 15, يوم تم اخذ عينتين دم. الأولى أخذت على مانع للتجلط وذلك لدراسة التأثيرات على صورة الدم والثانية تمتركها لتتجلط المصل لدراسة التغيرات البيوكيميائية .

تشير النتائج أن الاوكراتوكسين أدى الى حدوث نقص معنوي في عدد كرات الدم الحمراء، تركيز الهيموجلوبين و حجم خلايا الدم المضغوطة والبروتين الكلي وانتاج البيض ووزن البيضه وكذلك نتج عنه زيادة معنوية في،انزيمات الكبد (ALT-AST ALP)وحمض اليوريك والكرياتينين.

تلاحظ أن استخدام البريبيوتيك أدي إلي إلى تلافى أضرار الأوكراتوكسين وعودة وظائف الكبد والكلى إلي المستوى الطبيعي تقريبا .كماتلاحظ ايضا وجود تغيرات باثولوجية انعكاسية فى المجموعة الرابعة تمثلت فى حدوث تورم غيمى فى بعض خلايا الكبد والكلى مع احتقان بالاوعية الدموية البابية واختفت تلك التغيرات تدريجيابينما فى المجموعة الثانية فقد تلاحظ وجود احتقانا شديدا فى الاوعية الدموية بالكبد وزيادة عدد كريات الدم البيضاء فى نسيج كلا من الكبد والكلى مع وجود نخر تخثرى فى بعض خلايا الكبد ومعظم خلايا الكلى خاصة بالنسيج الطلائى للانبيبات الكلوية وضمور فى

بعض الانبيبات الكلوية .

أثبتت الدراسة أن إعطاء البريبيوتيك بالجرعة التى تم استخدامها أحدث زيادة معنوية فـــى العـــدد الكلى لكرات الدم الحمراء، تركيز الهيموجلوبين ، حجم خلايا الدم والبروتين الكلـــي ،انتـــاج البـــيض ووزن البيضه كما أدي إلى زيادة غير معنوية فى ALP, ALT ,AST و حمض اليوريك و الكرياتينين.

وقد دلت نتائج الدراسة على أن الاوكراتوكسين له بقايا في الكبد والكلى والعضلات اثنـــاء وبعـــد استخدام الاوكراتوكسين بالعليقه وكان أعلى منسوب لبقاياه في الكلى يليها الكبد ثم العضلات ولكن وجد ان البيض لايوجد به اى بقايا للاوكراتوكسين كما ان تلك البقايا قد اختفت من الأنسجة بعد مـــرور 6 ايام من نهايه استخدام الاوكراتوكسين.كما تبين من هذة الدراسة أنه لا يوجد اى بقايا للاكروتوكســـين فى البيض سواء اثنا اعطاء الاوكراتوكسين او فى الفترة التاليه لاعطاء الاوكراتوكسين. وتم مناقشـــة وجود تلك المتبقيات فى الأنسجة وكذا المدة الزمنية اللازمة لأختفائها من تلك الأنسجة

مما سبق واستنادا إلى التغيرات فى صورة الدم وبعض الوظائف البيوكيميائية في دم السمان الذى غذى على عليقه تحتوى على الاوكراتوكسين والبريبيوتيك بالإضافة إلى إنتاج البيض في كل المجاميع يمكن القول أن استخدام البريبيوتيك يمكنه التغلب على المشاكل الناتجة من الاوكراتوكسين في الســـمان البياض. لذلك ينصح باستخدام البريبيوتك فى مزارع السمان البياض لكي يعادل تاثيرالأوكراتوكســـين فى العلائق.