Studies On The Efficiency Of Aflatoxin Control Methods In The Poultry Farms

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ABSTRACT

Aflatoxicosis represents one of the serious diseases of poultry. The objective of the present study was to evaluate the efficacy of curcumin (CM), silymarin (Sil) and Nutritox in reducing the toxic effects of aflatoxin in white pekin ducklings. One hundred ninety two one day old white pekin ducklings were equally divided randomly into 8 equal groups, which include the following: The control group was fed commercial broiler feed that were tested to be free from aflatoxin, while another expermintal groups, namely 2, 3, 4, 5, 6, 7 and 8 were containing, respectively, G2) basal diet (BD) + 700 ppb AF (aflatoxin); G3) BD + 700 ppb AF +10mg/kg feed CM (curcumin); G4) BD + 700 ppb AF + 500mg/kg of BW Sil (Silymarin); G5) BD + 700 ppb AF + 1gm/kg feed Nutritox; G6) BD + 10mg/kg feed of CM; G7) BD + 500mg/kg of B.W Sil; G8) BD + 1gm/kg feed Nutritox during study (1-21days). Results showed that, the addition of 1gm/kg feed Nutritox ameliorated to some extend the adverse effects of AF diet and improved growth performance. The addition of CM or Sil or Nutritox ameliorated the adverse effects of AF on some serum chemistry parameters [total protein, Creatinin, Aspartate aminotransferase during cetrain periods of our experiments (AST), alanin aminotransferase (ALT) and Alkaline phosphatase (ALP)]. Results revealed that the administration of CM, Sil and Nutritox in diet prevent or reduce some adverse effects of aflatoxin in ducks fed aflatoxin-conatminated diets during different periods of our experiments. Our study concluded that Nutritox can provide protection against aflatoxin more than curcumin and silymarin.

INTRODUCTION

Aflatoxin, considered to be one of the most potent fungal toxins, not only produces severe hepatotoxicity in animals but also poses a major threat to human beings. Commodities in tropical countries are susceptible to contamination by the fungi *Aspirgillus parasiticus* and *A. flavus*, which produce this toxin. Aflatoxin-contaminated feed results in lower productivity of domestic animals and poultry *(1)* The most biologically active form of AF is aflatoxinB1 (AFB1) and it is responsible for decreased performance, increase liver lesions, and immunosuppression in poultry *(2,3)* and retardation in growth and an increase in mortalities *(4,5)*.

Turmeric (*Curcuma Longa*) is a medicinal plant extensively used as home remedy for various diseases *(6)* The powdered rhizome of this plant; turmeric, is used extensively to color and flavor foods. Its yellow color is imparted primarily by curcumin *(7)* . The rhizome of turmeric has a rich history in india as food spice, food preservative and coloring agent *(8)* A recent approach to prevent aflatoxicosis in poultry is the use of antioxidants in the diet. Plant compounds such as coumarins, flavonoids, and curcuminoids have been shown to inhibit the biotransformation of AF to their epoxide metabolites, which are more genotoxic than the parent compound *(9)* Reports have shown that the curcuminoid have protective effects against AFB1 *(10)* Several studies have reported that *Curcuma Longa* is beneficial against aflatoxicosis at the level of the animal, but to date, no study has been published that reports on the beneficial effects of *Curcuma Longa* on hepatic gene expression of broiler chicks fed AF *(1, 7)*

Silymarin, a flavonolignan from *silybum marianum*, commonly known as "milk thistle", botanically related to Asteraceae family (Compositae) *(11)* The active constituents of the plant are obtained from the dried seeds and consist of 4 flavonolignans isomers which are collectively known as silymarin namely- silbin, isosilybin, silydianin and silychristin *(12)* It offers good protection in various toxic models in experimental liver disease in laboratory animals. It acts as antioxidant, anti-lipid peroxidative, anti-inflammatory and liver regenerating mechanisms. Silymarin has clinical application in alcoholic liver disease, liver cirrhosis, viral hepatitis and toxic and drugs induced liver damage *(13)*

Probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Probiotic preparations are being increasingly used in poultry diets to enhance growth rate, improve feed utilization and control intestinal infections. In poultry production dietary acids, live microfloral additives and mannanoligosaccharides in diets of chickens may help digestion by inhibiting bacterial growth and regulate pH value in intestines when incorporated into diet formulations *(14)* There are many commercial products used for detoxification including mycotoxin-binding agents holds promise for using contaminated feeds *(15)* Lactobaccillus cultures prevented absorption of aflatoxin from intestine *(16)*

The aim of these investigations was to evaluate two antimycotoxin herbs for controlling aflatoxicosis in white pekin duckling and to focus on the hepatoprotective of turmeric and silymarin (as a herbal compound) in prevention and treatment of hepatic damage induced by aflatoxin compared with Nutritox (as a detoxifying commercial product mixture).

MATERIAL AND METHODS

Experimental diet

Control diet

Duckling diets was obtained from EL-Baraka Company and were analyzed in the Mycotoxins central lab and food safety of the National Research Center to ensure that it is free from aflatoxin using thin layer chromatography.

Artificially toxicated diet

Standard toxigenic strain of *Aspergillus parasiticus* NRRL2999 (ATCC) was used for production of aflatoxin, using fresh potato dextrose agar media (PDA) *(17)* Toxicated diet was prepared in laboratory of poultry disease Dept Faculty of Vet, Mansoura University by growing *Aspergillus parasiticus* strain NRRL2999 on rice *(17)* The mouldy rice was autoclaved, dried and ground to fine powder. It was analyzed for aflatoxin content by TLC in the Mycotoxins central lab and food safety of the National Research Center. Ground rice was added to duckling basal diet at concentration of 700 ppb aflatoxin.

Drugs

1. *Curcuma Longa*: Curcumin rhizome powder was obtained from Sigma_ Aldrich, Company.
2. Silymarin (Legalex 70): It was obtained from Alexandria Company for Pharmaceuticals.
3. Nutritox: It was obtained from local market (PROFARM®).

Experimintal design and birds

One hundred ninety two one day old white pekin ducklings were equally divided randomly into 8 groups, each contains 24 birds. All birds were weighed at the beginning of the experiment weekly during our study (21days). Dietary treatments of groups are follows:

Group (1): Control diet without any additives.

Group (2): BD (Basal diet) + 700 ppb AF (Aflatoxin)

Group (3): BD + 700 ppb AF +10mg/kg feed CM (Curcumin).

Group (4): BD + 700 ppb AF + 500mg/kg of BW Sil (Silymarin).

Group (5):BD + 700 ppb AF + 1gm/kg feed Nutritox.
Group (6):BD + 10mg/kg feed of CM (Curcumin).
Group (7):BD + 500mg/kg of B.W Sil (Silymarin).
Group (8): BD + 1gm/kg feed Nutritox.

Clinicopathological examination

All experimental ducklings were daily observed for clinical signs, on day 7, 14, 21 all life birds were weighed individually and total feed intake recorded for each pen. Average feed intake was corrected for mortality when calculating feed conversion for each cage by considering the total bird per days. All sacrificed and dead birds were subjected for postmortem examination. On 21st day 6 birds from each group were slaughtered for blood collection.

Serum biochemical analysis

On day 7, 14th individual blood samples were taken from 6 birds from jagular vein without anticoagulant into a dry and clean centrifuge tubes. On day 21st, 6 birds from each group were slaughtered for blood collection. Blood was centrifuged at 3000 rpm for 15 min and serum separated and preserved at -20◈ C until submitted for biochemical analysis. Serum sample were analyzed for total protein, creatinin, aspartate aminotransferase ((AST), alanin aminotransferase (ALT) and alkaline phosphatase (ALP) *(18 – 20)*

Statistical analysis

All data were grouped and expressed as means ± standard errors of the means. Obtained data (group means for all response variable in each experiment) were analyzed by analysis of variance (two ways ANOVA) *(21)* All statement of significance are based on the 0.05 level of probability (Statistical significance was accepted at $P<0.05$).

RESULTS AND DISCUSSION

Ducks performance

One day old white pekin duckling were used in our experiments to judge the protective efficacy of curcumin, silymarin and Nutritox× against aflatoxicosis as far as the well known information about the highly susceptibility of such birds and ages to aflatoxins *(5, 22)* and because of the general role of using highly susceptible host during studying the protective efficacy of antitoxins. In the present work the cumulative mortality reach 50% in group (2) within 7 days of feeding ducklings on 700 ppb level of aflatoxin – contaminated diet. Selected dose were assumed to be 1 ppm to get the LD50 of white pekin duckling *(22)* but unfortunately retesting of the used contaminated ration proved to have 700 ppb. Mortality in duck reach 100% after 2 weeks period of feeding aflatoxin contaminated diet *(23)* . On contrary it has been reported that daily mortalities started to appear 7 days after feeding aflatoxin contaminated diet, peaked at the 12-day period of feeding contaminated diet *(22)* . Mortality in group (3) that treated with curcumin and fed AF contaminated diet appeared on 6th day and reach LD50 in the middle of 2nd week. Also in broiler chicken only one bird died in the group fed on basal diet+1.0mg/kg AFB1+444mg/kg TCMN *(10)* . Minimum mortality was recorded in group (5). Lactobacillus cultures prevent absorption of aflatoxin from chicken duodenum *(16)* . No mortality appeared in group (6), (7) and (8) fed on curcumin, silymarin and Nutritox. *Curcuma Longa* has no adverse effects on broilers and the safety of curcumin already approved *(24)* Silymarin has had a good safety record and only rare cases reported of gastrointestinal tract disturbance and allergic skin rashes *(25, 26)* . This might disagree with our results (Table 5) that prove an increase in serum creatinine levels due to feeding curcumin (G6) and silymarin (G7) for 2 weeks. This increase was significantly differing than that of the control non medicated mates by the end of 2nd weeks of its feeding but not by the end of 3rd weeks.

As shown in Tables 2 and 3, aflatoxin cause a reduction in body weight, weight gain

Table 1 · Showing mortality during experimental period in different groups

G	1st week										2nd week										3rd week									
	1	2	3	4	5	6	7	Total mortality	Cumulative mortality	% cumulative mortality	1	2	3	4	5	6	7	Total mortality	Cumulative mortality	% cumulative mortality	1	2	3	4	5	6	7	Total mortality	Cumulative mortality	% cumulative mortality
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	1	0	4	7	12	12	50	3	3	1	1	1	1□	1□	11	23	87.8	0	1	0	0	0	0	0	1	24	100
3	0	0	0	0	0	5	2	7	7	29.2	2	3	4	2	1	0	1	13	20	83.8	2	2	0	0	0	0	0	4	24	100
4	0	0	0	0	1	1	0	2	2	8.3	0	1	3	1	1	5	1	12	14	41.7	3	0	0	2	1	0	0	6	20	83.3
5	0	0	0	0	0	1	0	1	1	4.2	0	1	1	1	2	1	1	7	8	33.3	2	1	2	0	2	0	2	9	17	70.8
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NB.: Numbers were approximated to the nearest decimal point.

□ Birds died during blood sampling.

and growth performance. AFB1 200 ppb in diet significantly reduced the growth of 3 weeks old mule duckling and there were significant beneficial effects in average daily gain *(27, 28)* . Although weight gain of duckling fed Nutritox treated ration do not significantly reduced by feeding aflatoxin all over the experimental periods, yet the weight gain of duckling fed silymarin (group 4) were significantly adversely affected by aflatoxin in the first week of life but not in the 2nd and 3rd weeks and those of duckling fed curcumin (group 3) were adversely and significantly affected all over the experimental periods by feeding aflatoxin treated ration. *(29, 30)* .

The impact of dietary aflatoxin on the performance and growth rate of broilers suggesting a relationship between levels of aflatoxin in diet and growth rate. As shown in Table 3, FCR was significantly increased in aflatoxicated group and significantly decreased in group 5 treated with Nutritox. Supplementation of probiotics (Lactobacillus and *Bacillus subtillis*) in diet stimulated favorable microbial balance in gut and consequently improved FCR and growth performance in broiler *(31-33)*.

Table 2. Body weights of different group at 1st, 7th, 14th, 21st day of age

Group	Body weight (gm)		
	Day 7	Day 14	Day 21
1	158.1±4.97 [a]	266.0±7.66 [a]	340.2±5.06 [a]
2	97.5±6.49 [c]	118.5±21.5[d]	ND
3	100.0±4.31 [c]	149.8±3.35 [c]	ND
4	107.1±3.81[c]	185.0±3.80 [b]	250.0±20.04 [b]
5	106.4±3.86 [c]	200.4±3.78 [b]	266.0±11.13 [b]
6	143.8±4.91 [b]	256.08±8.71 [a]	327.5±4.24 [a]
7	171.4±5.94 [a]	269.38±6.98 [a]	338.5±12.12 [a]
8	160.2±4.89 [a]	263.9±7.05[a]	336.3±3.58 [a]

There were significant differences between groups at ($P \leq (0.05)$).
[a, b, c] values in a column with different subscript differ significantly ($P \leq (0.05)$).
[1] One bird was live by the end of the 2nd week.
NB.: Numbers were approximated to two decimal points.

Table 3. Feed intake, weight gain and FCR in different groups in 1st, 2nd and 3rd week

Group	1st week			2nd week			3rd week		
	Feed intake (g)	Body gain (g)	FCR	Feed intake (g)	Body gain (g)	FCR	Feed intake (g)	Body gain (g)	FCR
1	155.1±8.1[abc]	106.9±1.9[ab]	1.45±.05[d]	300.9±23.1[a]	107.9±5.0[a]	2.79±.09[bcd]	193.5±.64[a]	74.17±1.67[a]	2.61±.05[b]
2	121.8±24.9[bcde]	43.8±6.7[d]	2.76±.15[a]	109.98±.35[c]	36.7±.71[c]	2.99±.005[a]	ND	ND	ND
3	101.9±16.3[e]	44.6±7.5[d]	2.29±. 02[b]	144.6±1.8[b]	49.9±.71[c]	2.89±.005[abc]	ND	ND	ND
4	116.7±10.4[cde]	55.8±3.9[d]	2.09±.04[bc]	225.1±18.4[a]	77.6±6.08[b]	2.90±.010[ab]	174.5±74.4[a]	65.0±26.5[a]	2.66±.060[b]
5	108.3±6.9[de]	57.3±.64[c]	1.89±.10[c]	255.8±41.0[a]	93.8±12.7[ab]	2.72±.070[de]	135.76±48.3[a]	63.4±22.6[a]	2.14±.00[c]
6	146.3±5.1[abcd]	93.1±1.46[b]	1.57±.03[d]	231.4±37.8[a]	97.3±13.5[ab]	2.37±.060[f]	209.9±21.1[a]	71.4±6.08[a]	2.94±.045[a]
7	182.4±3.8[a]	122.4±1.8[a]	1.49±.01[d]	253.9±11.5[a]	97.9±3.67[a]	2.59±.020[e]	184.2±68.3[a]	69.2±25.4[a]	2.66±.010[b]
8	163.8±14.9[ab]	109.8±8.5[a]	1.49±.02[d]	284.0±18.2[a]	103.8±4.17[a]	2.74±.07[cde]	190.4±20.8[a]	72.3±7.29[a]	2.63◈.022[b]

There were significant differences between groups at $P \leq (0.05)$.
[a, b, c] values in a column with different subscript differ significantly ($P \leq (0.05)$)
[1] One bird was live by the end of the 2nd week.
NB.: Numbers were approximated to two decimal points.
ND: Not done.

Serum biochemistry

As shown in Table 4, feeding of aflatoxicated diet caused a significant increase in ALT, AST in 1st and 2nd week. Alteration of those enzymes during aflatoxicosis has been previously recorded *(22, 34, 35)* Aflatoxin reported to elevate serum of AST, ALT and ALP due to liver function damage, muscular trauma *(2, 27)* and hepatocellular damage in ducks *(36)* Administration of curcumin showed significant improvement in ALT by the end of the 1st week and ALT and AST by the end of 2nd weeks of aflatoxin administration Curcumin appears to reduce the aflatoxin B1 toxicity by altering the microsomal activation of AFB1 and by increasing its detoxification. The therapeutic effects of curcumin are probably mediated through antioxidant and anti-inflammatory action and modulation of hepatic xenobiotic enzymes *(37)* Silymarin cause significant improvement in ALT when compares to control in 2nd and 3rd week and on the AST in the 2nd weeks. The major activity of silymarin returned to its antioxidant properly, which makes it useful in the prevention of other organ-specific toxicities related to the induction of oxidative stress *(38)* Serum biochemical enzymes (ALT and AST) in group 5 fed on aflatoxin+Nutritox during 1st, 2nd and 3rd week of experiment revealed significant improvement in comparison to the normal picture except ALT in 1st week and in 3rd week. These findings indicate that the adverse effect of aflatoxin is reversible; while alkaline phosphatase levels do not significantly changed, yet moderate improvement in group 5 fed on Nutritox-aflatoxicated diet. Aflatoxin administration showed insignificant increase in alkaline phosphtase levels in group 2 when compared with control mates. Total protein significantly decreased by aflatoxin administration by the end of the 1st week of aflatoxin feeding and inwards. Decreasing serum total protein was significantly improved by addition of silymarin and Nutritox but this was true for one weeks of feeding the aflatoxicosed treated ration but not furthermore Silymarin produced a significant improvement in the total protein *(39)* This improvement could be due to improve protection of the cell from damage through stimulation of polymerase rRNA which protect cell membrane from the free radicals which induced damage and blockage of the uptake of toxins *(40)*.

Aflatoxin toxicity caused unsignificant increase in creatinine levels in group 2 by the end of 2nd week when compared to control mates. Similar biochemical alteration in renal function was reported by others workers *(41)* Increase serum creatinine may be attributed to nephrotoxic effect leading to renal dysfunctions *(42)* Using of silymarin and curcumin in aflatoxicated diet showed unsignificant difference when compared to control mates (non medicated control and aflatoxin medicated groups. Similar results were previously recorded *(34, 44)* .

From Table 1, one may conclude that aflatoxin is potent toxin to one day old white pekin duckling and feeding 700 ppb aflatoxin cause 100% mortalities by day 16 of age. Variable protective efficacies of curcuumin, silymarin and Nutritox against mortalities caused by aflatoxin appear clearly in Table 1. Comparing daily mortalities, weekly mortalities and cumulative mortalities of aflatoxicated ducklings (G2), mortalities of curcumin-aflatoxicated ducklings (G3), silymarin-aflatoxicated ducklings (G4), Nutritox-aflatoxicated ducklings (G5) and their control non treated mates all over the experimental periods, we concluded that there is some sort of protection afforded by the different treatments.

Although protection against mortalities was not absolutely afforded by any the treatments, yet Nutritox appears to be the superior among the tested antitoxicants. Protection against mortalities afforded by any of the tested antitoxin was much better during the 1st week than the 2nd week. This may support the well known theories that the prompt withdrawal of contaminated feeds is the best treatments plus the usage of antimycotoxins. Lacking of clear significant differences in blood parameters (serum enzymes and total proteins) may be attributed to high mortalities especially in aflatoxin-treated-non medicated groups that led to low numbers of tested samples.

Table 4 • Serum chemical enzymatic levels

G	1st week					2nd week					3rd week				
	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	CR (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	CR (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	CR (mg/dl)
1	6.13±1.25^{c}	6.48+.175^{c}	1716.3±294.9^{a}	2.87±.45^{bc}	.30±.00^{a}	20.37±1.32^{b}	23.9±11.69^{ab}	1205.7±150.9^{ab}	3.82±.59^{a}	.31±.05^{bc}	13.36±3.21^{a}	26.92±5.95^{a}	1145.5±303.5^{ab}	1.958±392^{b}	.49±.05^{a}
2	18.93±2.7^{a}	41.97+7.46^{a}	2325.5±147.0^{a}	1.07±.33^{d}	.27±.03^{ab}	28.35±.550^{a}	33.24±6.41^{a}	1939.5±311.3^{a}	.76±.31^{b}	.32±.02^{b}	ND	ND	ND	ND	ND
3	10.95±1.7^{bc}	39.13+5.98^{a}	1851.2±155.2^{a}	1.07±.20^{d}	.23±.03^{ab}	24.87±81^{ab}	29.20±2.93^{ab}	1537.2±126.9^{ab}	1.29±.28^{b}	.24±.02^{bc}	ND	ND	ND	ND	ND
4	15.68±1.87^{ab}	35.27+2.96^{ab}	2102.5±310.5^{a}	1.67±.37^{cd}	.20±00^{ab}	22.04±4.45^{b}	28.24±1.89^{ab}	1426.8±314.5^{ab}	1.31±.23^{b}	.22±.01^{c}	13.89±3.31^{a}	32.09±4.69^{b}	1191.3±168.2^{a}	.60±.09^{c}	.38±.04^{bc}
5	13.66±1.44^{ab}	19.39±10.42^{bc}	1765.0±359.1^{a}	1.73±.03^{cd}	.18±.03^{b}	19.59±2.16^{b}	22.25±4.23^{ab}	1439.5±237.2^{ab}	.99±.08^{b}	.23±.01^{bc}	16.77±3.77^{a}	34.95±3.69^{b}	1149.5±376.5^{ab}	.76±.06^{c}	.29±.02^{c}
6	6.55±1.61^{c}	8.51 ±3.58^{c}	1560.2±256.4^{a}	3.60±.27^{b}	.28±.06^{ab}	19.65±.47^{b}	28.83±6.82^{ab}	1376±86.58^{ab}	2.83±.07^{a}	.47±.05^{a}	11.21±2.16^{a}	35.82±4.0^{b}	700.7±91.12^{ab}	2.9±.20^{a}	.36±.04^{bc}
7	8.08±79^{c}	4.46±1.47^{c}	1704.5±187.5^{a}	6.08±.83^{a}	.25±03^{ab}	19.56±.05^{b}	13.33±2.14^{b}	989±118.5^{b}	2.73±.27^{a}	.48±.01^{a}	16.33±2.13^{a}	29.97±1.74^{bc}	920.7±536.6^{ab}	2.76±.27^{ab}	.47±.04^{ab}
8	6.16±.35^{c}	6.66±2.19^{c}	1775.0±454.1^{a}	3.95±48^{b}	.23±05^{ab}	19.87±.27^{b}	18.99±1.93^{ab}	877.3±394.0^{b}	3.27±.81^{a}	.45±06^{a}	13.46±1.44^{a}	20.39±1.35^{c}	628.7±365.9^{b}	2.35±.58^{ab}	.49±.07^{ab}

There were significant differences between groups at ($P \leq (0.05)$.

$^{a, b, c}$ values in a column with different subscript differ significantly ($P \leq (0.05)$.

ND: Not detremind.

NB.: Numbers were approximated to two decimal points.

Blood sampling of the 2nd week done in day 13.

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الملخص العربى

دراسات على كفاءة طرق الحماية من الأفلاتوكسين في مزارع الدواجن

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يعتبر التسمم بالأفلاتوكسين واحدا من الأمراض الخطيرة في الدواجن .وكان الهدف من هذه الدراسة هو تقييم فعالية الكركم، والسيليمارين والنتريتوكس * في الحد من الآثار السامة للأفلاتوكسين في فراخ البط البيكينى الأبيض.

مائه واثنان وتسعون فرخ من البط البيكينى عمر يوم واحد قسمت عشوائيا إلى ٨ مجموعات متساوية، والتي تشمل ما يلي :تم تغذية المجموعة الضابطة بعلف تجارى تم اختباره ليكون خالي من الأفلاتوكسين، بينما قسمت المجاميع وهي:٢, ٣, ٤, ٥, ٦, ٧ و ٨ الذي يتضمن، على التوالي :(٢) علف ملوث ب ٧٠٠ جزء في البيلون افلاتوكسين , (٣) علف ملوث ب ٧٠٠ جزء في البيلون افلاتوكسين +١٠ مجم من الكركم لكل كيلو علف, (٤) علف ملوث ب ٧٠٠ جزء البليون افلاتوكسين +٥٠٠ مجم من السيلمارين لكل كيلو وزن, (٥) علف ملوث ب ٧٠٠ جزء في البليون افلاتوكسين +١ جم من النتريتوكس لكل كيلو علف, (٦) علف تجارى خالى من الافلاتوكسين +١٠ مجم من الكركم لكل كيلو علف, (٧) علف تجارى خالى من الافلاتوكسين + ٥٠٠ مجم من السليمارين لكل كيلو وزن, (٨) علف تجارى خالى من الافلاتوكسين + ١ مجم من النتريتوكس لكل كيلو علف لمده ٢١ يوم .وأظهرت النتائج أن إضافة ا جم من النتريتوكس تحسن إلى حد ما الآثار السلبية للافلاتوكسين وتحسين أداء النمو .تحسن إضافة الكركم والسليمارين والنتريتوكس الآثار للسلبية للافلاتوكسين على بعض الانزيمات الكيميائيه لمصل الدم. كشفت النتائج أن استخدام الكركم و السليمارين و النتريتوكس في النظام الغذائي منع أو حد من الآثار السلبية للأفلاتوكسين في علف البط الملوث بالافلاتوكسين خلال فترة التجربة. وقد خلصت الدراسة في أن النتريتوكس يوفر حماية أكثر ضد الأفلاتوكسين عن الكركم والسيليمارين.