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EVALUATION OF SOME COMMERCIAL POULTRY FEED ADDITIVES USED FOR MOULD INHIBITION AND/OR MYCOTOXIN DETOXIFICATION

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SUMMARY

Four commercial mould inhibitors and antimycotoxins feed additives (Detox®, Mycotox®, Toxinil® and Nutritox®) were evaluated for their effect against fungi contaminating the poultry ration and also the possibility to detoxify mycotoxin.

Starter Corn-Soya ration proven after examination to be contaminated with fungi, aflatoxin and ochratoxin-A with an average pH 6.7 and 14 % moisture content. Nine cloth bags were sterile autoclaved then each one was filled with 10 kg. from this ration.

Each feed additive was added to the contaminated ration at equal and higher concentration than that of the manufacturer recommendations (Detox® 0.1 & 0.2%, Mycotox® 0.1 & 0.2%, Toxinil® 0.3 & 0.5% and Nutritox® 0.05 & 0.1%) then all bags were stored for 2 weeks at room temperature.

The total fungal count, pH and moisture content were determined at the end of the 1st, 7th and 14th day of the storage period, while determination of aflatoxin and ochratoxin-A was applied at the end of 7 and 14 day from each treatment.

Detox® showed the best mould inhibition results at a concentration of 0.2%, then Toxinil® at 0.5%, followed by Detox® at 0.1%. On the other hand Mycotox® was the best detoxifying agent for both aflatoxin and ochratoxin-A especially at a concentration of 0.2%, while Toxinil® at 0.5% was effective against aflatoxin only.

Those concerned with feeds and feeding in processing plants and poultry farms should recognize the differences in nature and use of both mould inhibitors and mycotoxin detoxifying agents.

INTRODUCTION

Presence of fungi and their toxic secondary metabolites (mycotoxins) in poultry rations has been

incriminated in severe economic losses in poultry production due to their immuno-suppression effects besides acute toxicity (Nahm, 1995). Mycotoxins act as carcinogen, mutagen and teratogen agents (Sashidhar, 1993). They are lowering the production performance parameters of broilers, layers and breeders (Kim et al., 2003).

Therefor, serious and hard work had been done to find a practical solution to establish preventive and control measures against these problems.

Although many and different chemical products were evaluated against fungi and their metabolites, a real challenge is still present from those parasites and the expected fungal mutation (Nahm, 1995), so it was necessary to evaluate the most common commercial antifungal products as a guidance for farm owners and in feed processing plants.

Different organic acids were defined as mould inhibitors and consequently limiting mycotoxin production in poultry feeds. Propionic, sorbic, formic, benzoic and citric acids and their salts were deeply and widely studied in many researches (Tabib et al., 1982, Smith et al., 1983 and Paster et al., 1999).

Also, different biological agents such as yeast cells, some bacterial strains and even some types of moulds were examined as mould inhibitors (Doyle et al., 1982, Stanley et al., 2002 and Mishra and Das, 2003).

Inhibitors of mould growth, such as ammonia and propionic acid, can inhibit aflatoxin production on high moisture corn (Vandergraft et al., 1975) so; it is likely to evaluate the mould inhibitors against both mould growth and mycotoxin production.

Recently the commercial products are formulated from combination of some antifungal agents like ammonium salts, organic acids, herbs and spices, yeast and others to give the maximum antifungal effect. Saleh et al., 1986, Abdel-Mallek et al., 1995 and Wu, 1997 have been evaluated some of these products.

So, the present study was planned to evaluate the effect of some available commercial products present in Egyptian-market, that used as antifungal feed additives and as detoxifying agents against mycotoxins in fungal contaminated poultry rations.

MATERIAL AND METHODS

This trial was conducted in-vitro to examine four commercial antifungal feed additives.

A- Ration Examination:

The ration batch (Starter Corn-Soya ration) used in this experiment was obtained from a ration lot stored for 15 days in a commercial broiler poultry farm. The ration was expected to be mouldy due

to its bad storage condition. The ration was examined prior to treatment with the commercial antifungal additives for determination of mycotic, mycotoxin, pH and moisture contents, and proven to be contaminated with fungi and mycotoxin (aflatoxin and ochratoxin-A).

1) Mycotic Examination:

Ten gram of each feed sample was added to 90 ml. of sterile saline, shaken for 10 minutes on shaker, then tenfold serial dilution was performed. Triple pour plates using 0.5 ml. of each dilution were obtained using about 15 ml. of melted sterile Sabouraudís dextrose agar medium (Oxoid). Inoculated plates were incubated without inversion at 250 C for 5 days. Average total fungal count was determined per gram of feed sample (DeBey et al., 1994).

2) Mycotoxin Determination:

The ration sample was examined using Series-4 Fluorometer (VICAM) based on the immunoaffinity method (Fremy and Chu, 1989 and Truchsess et al., 1991) which determined in parts per billion (PPB).

3) pH Determination:

Five grams of the ration was soaked in 50 ml. of distilled water to make a slurry was allowed to stand at room temperature for 30 minutes prior to pH measuring with digital pH meter (Rendos et al., 1975).

4) Moisture content:

Ten grams of the ration was heated in hot air oven at 1000 C for 18 hours and then determined gravimetrically (Jones et al., 1982).

B-The commercial antifungal feed additives:

Four commercial antifungal feed additives were evaluated in this study and each feed additive was used in two concentrations, which are equal and higher than that of the manufacturer recommendations, as mentioned in Table (1):

Antifungal Feed Additives	Components	Concentration Used
1) **Mycotox® premix** (Amoun Pharm. co.)	It's mainly mycotoxin control product. Each kilogram contains 50 g. of oxyquinol; 0.44 g. dichlorothymol and micronized yeast extract	0.1 and 0.2 %
2) **Detox® premix** Amoun Pharm. Co.)	It's liquid mould inhibitor product. Each liter contains 390 gram of formic acid, 120 g. acetic acid, 250 g. ammonium formate, 10 g. sorbic acid, and 5 g. ascorbic acid.	0.1 and 0.2 %
3) **Toxinil ® premix:** (Nutri-AD Intern. Belgium)	A powder mixture of organic acids, ***saccharomyces*** extract, amino acids and vitamin B complex	0.3 and 0.5 %
4) **Nutritiox® premix** (Agrarian Marketing Corporation, USA)	A powder mixture of organic acids (aspartic, lactic and citric acids), organic salts (sodium and potassium citrate) on limestone and silicon dioxide.	0.05 and 0.1 %

C- Experimental procedure:

Nine well-aerated cloth bags were sterile autoclaved then each one was filled with 10 kg. contaminated ration. The recommended concentrations of each feed additive, as in table 1, were added and mixed well with the ration. All bags were stored at room temperature with average temperature of 250°C and relative humidity 50%, while the control bag stored without treatment.

During this period of storage which lasted for 2 weeks, the total fungal count, pH and moisture contents were determined at the end of the 1st, 7th and 14th day while aflatoxin and ochratoxin-A were determined after 7 and 14 days from each treatment (Vandegraft et al., 1975 and Paster et al., 1999).

RESULTS AND DISCUSSION

Results shown in Table (2) and Figures (1, 2 &3) revealed the mould inhibition effect of feed additives and denoted that the highest reduction percentage in fungal count was obtained with the use of Detox® (0.2%) and this reduction was 98, 99.2 and 94.7 % after 1, 7 and 14 days respectively. While at 0.1% concentration it was 90% after 1 day, but this effect was reduced to 52 and 53.3% after 7 and 14 days respectively.

Toxinil® at 0.5% concentration showed the second reduction effect after Detox®, where the reduction percentage was 70, 90 and 85.3 % after 1, 7 and 14 days respectively. Toxinil® at 0.3% concentration showed lower effect, which appeared only after 7 days as 28% reduction and no effect thereafter.

Nutritox® at 0.1% concentration showed 52% reduction in fungal count after 7 days but no effect thereafter, while the lower concentration (0.05 %) showed only 20% reduction after 7 days and no effect thereafter.

Mycotox® at 0.2% concentration showed low reduction percentage of 20, 12 and 6.7% after 1, 7 and 14 days respectively, while no effect was obtained from the concentration of 0.1%.

Control group showed a reduction in the fungal count reached 75% after 7 days and 85% after 14 days of storage. This may be attributed to the shift in pH, temperature and moisture contents and the reduction in nutrient components throughout the storage period (Dixon and Hamilton 1981 and Tabib et al., 1981).

The average pH (6.7) of the ration used in this study was in the suitable limit for the action of many antifungal compounds which act mainly in acidic pH such as all organic acids (Paster et al., 1999).

Nothing was reported about the suitable pH for

Table 2: The average total fungal count of the treated ration before and after the treatment with the antifungal feed additives.

	Average total fungal count (CFU/g.)					
Before treatment	10×10^5					
After treatment	1 day	Red. %	7 days	Red. %	14 days	Red. %
Control	10×10^5	Nil	25×10^4	75	15×10^4	85
Mycotox®: 0.1%	12×10^5	Nil	25×10^4	Nil	15×10^4	Nil
0.2 %	8×10^5	20	22×10^4	12	14×10^4	6.7
Detox®: 0.1 %	10×10^4	90	12×10^4	52	70×10^3	53.3
0.2 %	20×10^3	98	20×10^2	99.2	8×10^3	94.7
Toxinil®: 0.3 %	10×10^5	Nil	18×10^4	28	30×10^4	Nil
0.5%	30×10^4	70	20×10^3	92	22×10^3	85.3
Nutritox ®: 0.05 %	10×10^5	Nil	20×10^4	20	20×10^4	Nil
0.1%	10×10^5	Nil	12×10^4	52	15×10^4	Nil

CFU/g.: colony-forming unit per gram
Red. %: Reduction %.

N. B.:
- **The pH** in all stored bags of ration sample was at an average of 6.7 throughout the 2 weeks.
- **The moisture content** in all stored bags was at an average of 14% throughout the 2 weeks.

other compounds as the ammonium salts and yeasts, etc. especially when they are formulated together in the commercial compounds.

Also, the average moisture content (14%) of the used ration sample was considered the most suitable condition for the growth of mould and yeast (Tabib et al., 1981) and consequently it was the highest challenge upon the used antifungal feed additives, which may be faced in field application.

Regarding mycotoxin detoxification, Table (3) and Figures (4 & 5) revealed that the Mycotox®, which is specifically detoxifying compound, showed the highest reduction effect upon both af-

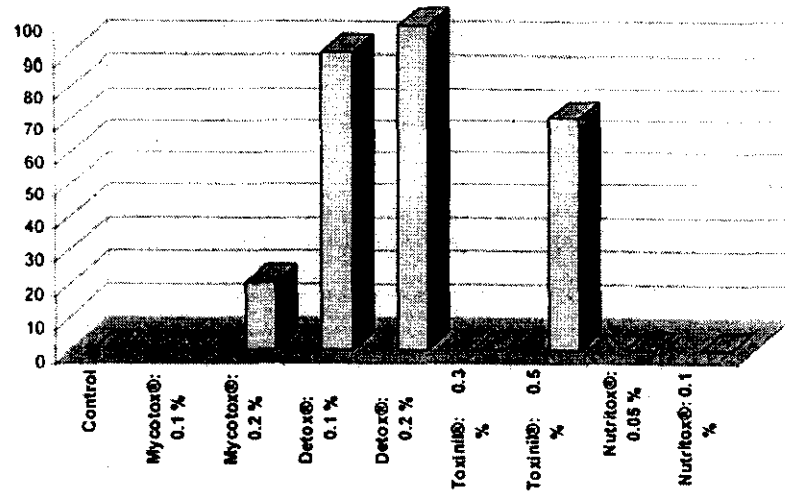

Fig. (1): Red. % in the total fungal count at the end of the 1st day after the treatment with the antifungal feed additives.

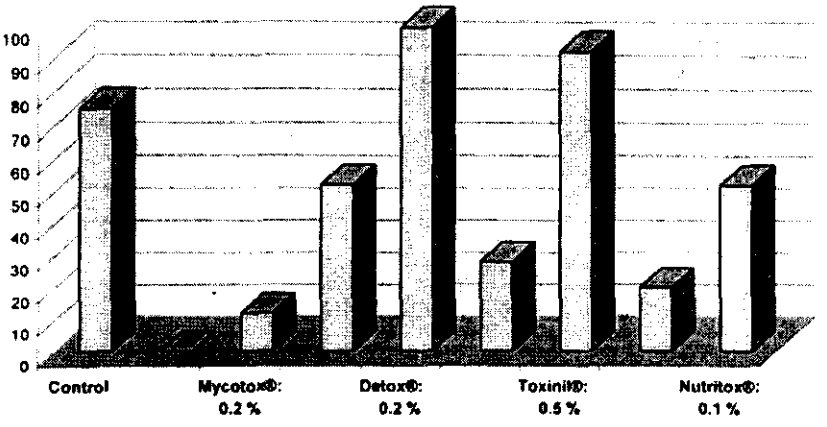

Fig. (2): Red. % in the total fungal count at the end of the 7th day after the treatment with the antifungal feed additives.

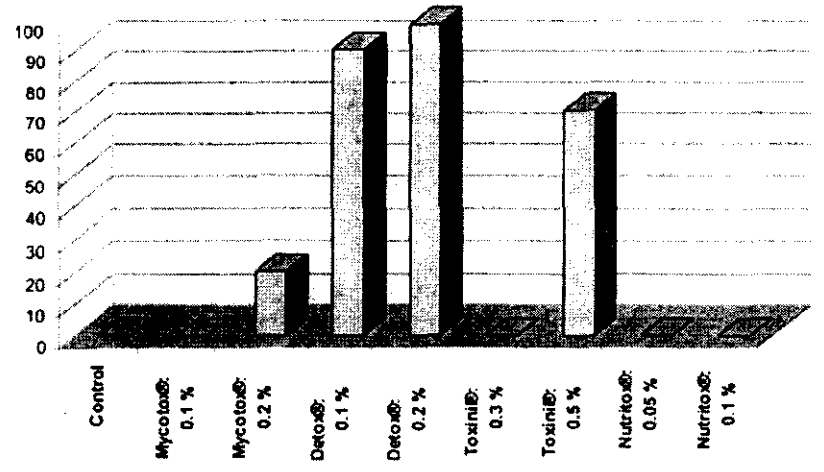

Fig. (3): Red. % in the total fungal count at the end of the 14th day after the treatment with the antifungal feed additives.

latoxin and ochratoxin-A. The concentration of 0.2% showed a reduction in aflatoxin of 42.9 and 85.7% after 7 and 14 days respectively and a reduction in ochratoxin-A of 64.3 and 85.7% after 7 and 14 days respectively. Also the lower concentration of Mycotox® (0.1%) showed a reduction of 57.1% after 14 days for aflatoxin and the same after 7 days for ochratoxin and 64.3% after 14 days.

Toxinil® was very effective against aflatoxin at a concentration of 0.5%, where the reduction reached 85.7% after 7 days and 100% after 14 days. At the lower concentration (0.3%) the reduction was 57.1% after 7 and 14 days. The effect of Toxinil® was negligible against ochratoxin-A after 7 days and reached only 7.1% after 14 days for both concentrations.

Detox® showed lower effect against mycotoxin, where the concentration of 0.2% showed a reduction in aflatoxin of 28.6% after 14 days and only 14.2% for the concentration of 0.1%. Also Detox® at both concentrations of 0.1 and 0.2 % showed a reduction in ochratoxin-A of 35.7% after 7 days and the same with 0.1% after 14 days, while at 0.2% the reduction was 50%.

Table 3: The effect of antifungal feed additives on aflatoxin and ochratoxin - A contents in the broiler ration.

	Aflatoxin (PPB)				Ochratoxin-A (PPB)			
Before treatment	7				14			
After treatment	7 days	Red. %	14 days	Red. %	7 days	Red. %	7 days	Red. %
Control	7	Nil	5	28.6	14	Nil	10	28.6
Mycotox®: 0.1%	7	Nil	3	57.1	6	57.1	5	64.3
0.2 %	4	42.9	1	85.7	5	64.3	2	85.7
Detox®: 0.1 %	7	Nil	6	14.2	9	35.7	9	35.7
0.2 %	7	Nil	5	28.6	9	35.7	7	50
Toxinil®: 0.3 %	3	57.1	3	57.1	17	Nil	13	7.1
0.5%	1	85.7	Nil	100	14	Nil	13	7.1
Nutritox® : 0.05 %	7	Nil	9	Nil	14	Nil	15	Nil
0.1%	8	Nil	8	Nil	12	14.3	14	Nil

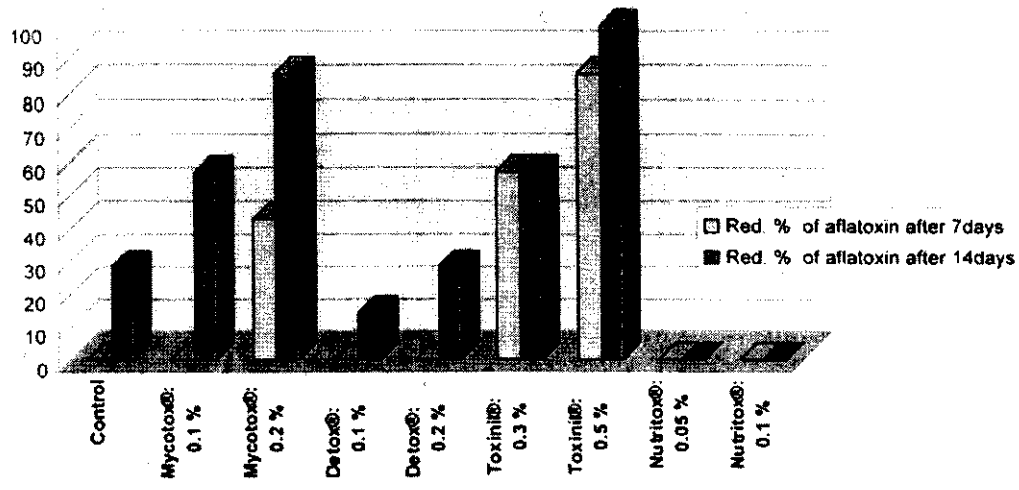

Fig. (4): Red. % in aflatoxin after treatment with antimycotoxin feed additives.

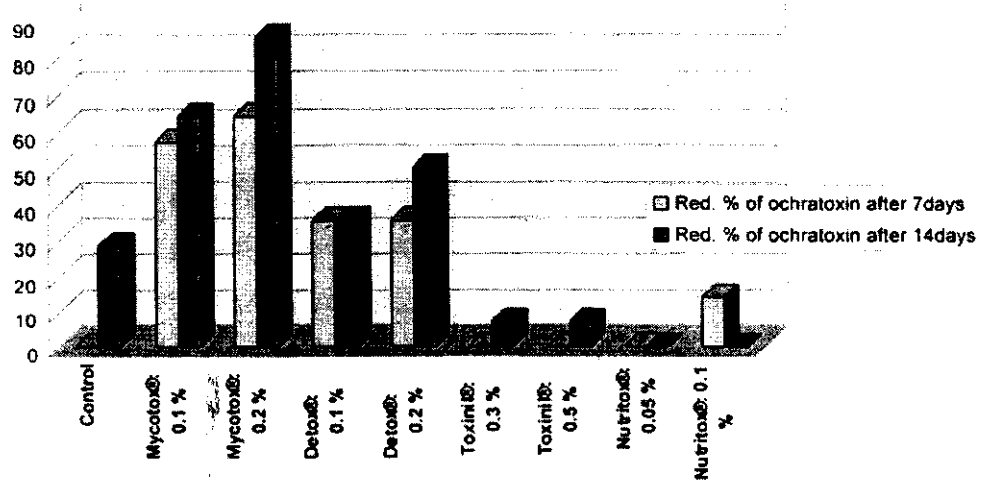

Fig. (5): Red. % in ochratoxin after treatment with antimycotoxin feed additives.

Nutritox® showed no effect upon aflatoxin and ochratoxin except insignificant reduction in ochratoxin (14.3%) after 7 days from the concentration of 0.1%.

Control group showed a reduction of 28.6% for both aflatoxin and ochratoxin-A after 14 days of feed storage and This may be attributed to the shift in pH, temperature and moisture contents and the reduction in nutrient components throughout the storage period (Tabib et al., 1981).

Finally, from the obtained results, it was concluded that the best-used commercial antifungal product was Detox® at a concentration of 0.2%, then Toxinil® at 0.5%, followed by Detox® at 0.1%.

Mycotox® was the best detoxifying product for both aflatoxin and ochratoxin-A especially at a concentration of 0.2%, while Toxinil® at 0.5% was effective against aflatoxin only.

Also, this study revealed the differences in nature and use of both mould inhibitors and mycotoxin detoxifying agents.

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