DETOXIFICATION OF AFLATOXINS-CONTAMINATED DIET BY SOME PHYSICAL AND CHEMICAL MEANS.

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ABSTRACT

In vitro comparative efficacy studies on the addition of fix-a-tox, antitox plus, tafla, autoclaving, microwave, hydrogen peroxide, ammonia solution and extraction with aqueous methanol (50%) as detoxifying agents to a diet containing 3600 ppb or 1800 ppb total aflatoxins. The 0.5% level of antitox plus, fix-a-tox and tafla gave the most constant pattern with aflatoxin reduction of 33.4, 16.7 and 44.4%, respectively at the high level of toxin but at the low level the reduction was 31.6, 15.8 and 42.1%, respectively. The autoclaving for 30 min and using microwave oven at a medium energy for 3 min gave reduction of 33.4 and 52.8% at the high level of toxin but at the low level of toxin the reduction was 31.6 and 51.6%, respectively. The effect of high temperature on the appearance, consistency and composition of a food (consequently its biological value, digestibility and utilization) raises special attention to the choice of protection as the corner stone of the aflatoxin control which is more beneficial than detoxification. Many chemicals have been tested for their ability to structurally degrade or inactivate aflatoxin, including H_2O_2 and ammoniation. These chemicals resulted in a reduction of 38.9 and 88.9% at the high level of toxin but in the low level contaminated diet, the reduction was 36.8 and 87.9%, respectively. Extraction of aflatoxin from the diet with methanol caused a reduction of 55.6% at the high level of toxin but in the low level contaminated diet, the reduction was 53.2%. However, this method for the removal of aflatoxins via solvent extraction appears to be impractical and expensive when compared with other methods.

There was no difference in the chemical composition for all different treatments, yet the treatment with ammonia increased crude protein (CP)% but the extraction caused a decrease in CP% and hence in the nutritional quality.

Aflatoxin productivity from *Aspergillus flavus* in liquid yeast media in the absence and presence of 0.5% of each of antitox plus, fix-a-tox, tafla, 1% of ammonia and 3% level of H_2O_2 was determined in the samples. Addition of any of these agents resulted in a decrease in aflatoxin productivity with the maximum effect for ammonia followed by H_2O_2, tafla, antitox plus and fix-a-toxin. Ammonia prevented the growth of *Aspergillus flavus* and this resulted in prevention of aflatoxin contamination.

Keywords: Aflatoxin - detoxication - removal - chemical & physical means - production.

INTRODUCTION

Mycotoxins are the silent enemy. Mycotoxins affect as much as 25% of the worlds' feed crops each year (Yegany *et al.*, 2002). Acute aflatoxicosis causes hepatitis, hemorrhage, immune supprension, genetic damage (carcinogenicity, teratogenicity and mutagenicity) and death (Moorthy *et al.*, 1985; Nowar *et al.*, 1987 and 1992 a&b and Pier, 1992). Growth impairment and lowering of reproductive performance are the most sensitive clinical signs of chronic aflatoxicosis (Clark *et al.*, 1980; CAST, 1989 and Nowar *et al.*, 1996). Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins (Müller, 1983; Abdelhamid *et al.*, 1985, 1986 and 1992 a&b; Abdelhamid, 1993 and Abdelhamid and Mahmoud, 1996). Aflatoxins are heat-stable and not totally

destroyed by boiling water, autoclaving and a variety of food and feed processing procedures (Pluyer *et al.*, 1987). The degradation of aflatoxins is a direct function of temperature, heating time and moisture content (Mann *et al.*, 1967). A variety of solvents were capable of extracting aflatoxins from different commodities with minimal effect on protein content or nutritional quality (Rayner *et al.*, 1977 and Goldblatt and Dollear 1979). However, these methods appear to be impractical and expensive when compared with other methods. A variety of adsorbent materials were used too including activated carbon (Decker, 1980) and clays (Masimanco *et al.* 1973 and Nowar *et al.*, 1996 & 2000). Abdelhamid and Mahmoud (1996) studied the efficiency of toxin-adsorbent-agents (florisil, aluminum oxide and fix-a-tox) on detoxification of aflatoxin. Adsorbents used herein did not lower aflatoxin level in rice grains during storage but increased the toxin concentration by storage advance, particularly in the presence of florisil, fix-a-tox and urea. Mahmoud *et al.* (1994) reported also surprising results by using such detoxifying agents (Al_2O_3, antitox plus, charcoal, fix-a-tox, and florisil). Many chemicals have been tested for their ability to structurally degrade or inactivate aflatoxins, including numerous acids, bases, aldehydes, bisulfite and oxidizing agents (Goldblatt and Dollear 1979 and Anderson 1983). Ammoniation resulted in a significant reduction in the level of aflatoxins in contaminated peanut and cottonseed meal (Dollear *et al.*, 1968; Gardner *et al.*, 1971 and Park *et al.*, 1984). Natural aflatoxin inhibitors, e.g. carotenoids and benzoazolinone compound from corn (Norton, 1998) and glucomannan from yeast cell wall can reduce also aflatoxin level and its effects (Bintvihok, 2001). Also, peptide (D4E1) may help defeat aflatoxin in cotton (STAT, 2001). Additionally, yeast (Stanley *et al.*, 1993), bacteria (El-Nezami *et al.*, 1998) and fungi (El-Sayed 1996), even atoxigenic *Aspergillus flavus* (Cotty and Bhatnagar, 1994) prevent aflatoxin contamination. However, the aim of the present study was to evaluate the effectiveness of some treatments in reducing level of aflatoxin contamination and/or production.

MATERIALS AND METHODS

Production of aflatoxins: For producing aflatoxin, the strain of *Aspergillus flavus* NRRL 3357 (from Laboratory of Mycotoxins, National Research Center, Dokki – Cairo) was grown in synthetic media, yeast extract – sucrose broth (YES) containing 2% yeast extract and 20% sucrose. The substrate was dispensed in conical flasks. The flasks were then autoclaved for 15 minutes at $121^{\circ}C$, then coold and inoculated with spores suspension and incubated for 9 days at $25 – 29^{\circ}C$. Aflatoxin concentration was determined using immunoaffinity column coupled with solution fluorometry or liquid chromatography postcolumn derivatization according to Truckes *et al.* (1991).

The media contained a mixture of aflatoxins B_1, B_2, G_1 and G_2, at a total level of 18 ppm. Half liter of the culture was added to 2.5 or 5.0 Kg feed to be contained 3600 or 1800 ppb aflatoxins. The ration used to be artificially contaminated was formulated according to Ahmed (1976). The ingredients of this ration are as following: crushed wheat 46%, shredded barley 40%, fish meal powder 9%, dried milk 3%, yeast 1% and minerals and vitamins 1%.

Detoxification methods: Different methods of aflatoxin inhibition were applied in duplication by weighing 500 g samples from the concentrate mixture mentioned before. The samples were mixed thoroughly together with aflatoxins solution and the mixture refilled into sacks (bags). Ammonia solution at a concentration of 1% was added to some treated sacks and then kept for 3 wKs. Another sacks of samples were sprayed by hydrogen peroxide solution at a level of 3% to bring the final moisture content to about 20%. The mixture was incubated in oven at 80°C for 0.5 hr using stainless steel trays. Another samples of the aflatoxicated feed mixture were extracted by methanol (50%) then filterated and air-dried. Sacks of the toxificated feed were autoclaved at 121°C for 30 minutes and 1.5 prs. Approximately 500 g of the aflatoxicated feed were thoroughly mixed with 0.5% of either antitox-plus, tafla, or fix-a-tox. The microwave oven used for heating the toxicated feed was Goldstar Model No. ER 535 MD. The frequency of the radiation emitted in this oven was 2450 MHZ. The selected setting of heating corresponding to full power (3) which provided 1300 W (1.3 KW). 250 ml beckers contained the contaminated meal (200 g) were heated using this microwave oven at a medium power for 3 mins. Proximate chemical analysis as well as calcium and phosphorus contents were determined in the treated samples according to A.O.A.C. (1990).

RESULTS

1- Efficacy of the detoxification:

Table (1) presents to what extent the used tetoxification methods were effective in lowering the contamination levels. It is clear that 1% ammonia treatment was the best one, since it reduced the initial levels of aflatoxins by 87.9 – 88.9%, depending on the initial concentration. Then followed by the extraction with 50% methanol (53.2 – 55.6%), microwave (51.6 – 52.8%), 0.5% tafla (42.1 – 44.4%), 3% hydrogen peroxide (36.8 – 38.9%), autoclaving (31.6 – 33.4%), 0.5% anti-tox-plus (31.6 – 33.4%) and at the end 0.5% fix-a-tox (15.8 – 16.7%). However, the higher aflatoxins level was associated with slightly elevated degradation rate of aflatoxins. This means that ammoniation of aflatoxins – contaminated diet was the best method among the different means used herein for decomposition of aflatoxins in the faulty feed.

2- Effect of the detoxification on chemical composition:

Data shown in Table (2) are the chemical composition percentages, which were not influenced by the contamination level or by the used means of decontamination.

Table (1): Percentages of losses from two aflatoxins concentrations by various treatments for contaminated diet.

Treatments	Aflatoxins level (ppb)	% losses	Aflatoxins level (ppb)	% losses
Contaminated diet	3600	-	1800	-
Autoclaving	2400	33.4	1300	31.6
Fix-a-tox	3000	16.7	1600	15.8
Tafla	2000	44.4	1100	42.11
Antitox plus	2400	33.4	1300	31.6
H$_2$O$_2$	2200	38.9	1200	36.8
NH$_3$	400	88.9	230	87.9
Methanol	1600	55.6	890	53.2
Microwave	1700	52.8	920	51.6

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Yet, there was some elevation in crude protein content by the ammoniation of the contaminated diets (18.7 – 19.2%) comparing with the control (without treatments), being (16.2 – 17.2%). However, the extraction with methanol appeared to reduce crude protein contents (14.3 – 14.4%) of the treated – aflatoxin contaminated diets. Hence, the last treatment may reduce the feeding value by leaching some protein from the treated diet (besides its toxicity for the presence of aflatoxins). Whereas the best detoxification method (ammoniation) improved the feeding value via N-enrichment of the treated diet.

3- Effect of the detoxicants on the aflatoxin production:

Table (3) shows the aflatoxin productivity and its inhibition by the effect of using some detoxification materials in YES – media for *Aspergillus flavus.* It was obviously that all used materials led to various inhibition rates in aflatoxin production. But the best treatment was 1% ammonia, which caused 100% inhibition of aflatoxin production. Since ammonia prevent fungal growth for its alkalinity. Hydrogen peroxide also reduced the productivity of aflatoxin by 91.8% comparing with the control. It is a strong oxidative agent for aflatoxin. However, the adsorbents (antitox plus and fix-a-tox) were less effective in reducing aflatoxin productivity, being 62.5 and 47.5%, respectively.

Table (3): Effect of various treatments on aflatoxin productivity by *Aspergillus flavus* on YES media.

Treatment	Aflatoxins (ppb)	% of inhibition
Control	160.000	-
0.5% Fix-a-tox	84.000	47.5
0.5% Antitox plus	60.000	62.5
0.5% Tafla	13.600	91.5
3% H_2O_2	13.200	91.8
1% NH_3	-	100

DISCUSSION

Aflatoxins are very toxic and carcinogenic mycotoxins. Their occurrence is outspreading in various food and feeds allover the world, particularly the developing countries, e.g. Egypt (Abdelhamid, 1981, 1983 a&b, 1985 and 1990; Kiermeir, 1985; Aziz and Youssef, 1991 and Wood, 1992). Even it was found in herb tea, medicinal plants (Halt, 1998) and tobacco, therefore aflatoxin is identified as silent killer (ASH, 1999). Thus, aflatoxin residues may be often found in animal products and human tissues (Vries *et al.*, 1990; Jonsyn *et al.*, 1995; Abdelhamid and Saleh, 1996 and Changbumrung *et al.*, 1999). Therefore, aflatoxins threaten animal and human health (Sun *et al.*, 1985; Hsieh *et al.*, 1988; Abdelhamid *et al.*, 1998 and 1999; Costantini *et al.*, 1999 and Med Nets, 2001). Hence, the prophylaxis against fungal invasion is a must to avoid the harmful effects of mycotoxicoses. But when the aflatoxin is existed, then it is necessarily to

alleviate its toxic severity. The present research is an attempt in this direction, i.e. to overcome (or inhibit) aflatoxin production and to reduce level (or detoxify) of the present aflatoxin.

Among the physical and chemical means of detoxification used herein, ammonia treatment was beneficial in reducing most of the present aflatoxin levels (by up to 88.9%). Moreover, this treatment elevated crude protein content of the contaminated diet by about 13.8% comparing with the untreated (control) diet. Additionally, ammonia treatment inhibits totally aflatoxin production in the *A. flavus* media. In accordance, Mann *et al.* (1971), Jensen *et al.* (1977) and Bagley (1979) inactivated aflatoxin in cottonseed meal and corn. Yet, Mann *et al.* (1971) found that ammonia treatments lowered nitrogen solubility and lysine availability. But Jensen *et al.* (1977) reported that ammoniation lowered feed intake. In this context, Abdelhamid *et al.* (1994) reported higher crude protein content of the treated materials with ammonia or hydrogen peroxide. Also, urea (as a source of ammonia) destroyed the aflatoxin (Abdelhamid and Mahmoud, 1996). Similar results were recorded by Abdelhamid *et al.*, (1985) concerning inhibition of aflatoxin production via using the preserving power of some additives, mainly medical herbs, spices, organic acids and fungicides. Yet, in another study (Abdelhamid *et al.*, 2002b), it was concluded that no one of the tested medicinal herbs completely overcome the effects of foodborne aflatoxicosis.

The moderate or low effects of extraction (methanol), microwave, autoclaving, oxidation (H_2O_2), and adsorbents (tafla, antitox plus, and fix-a-tox) used on lowering level of contamination with aflatoxin were reviewed. Since Decker (1980) and Abdelhamid *et al.* (1986) reported slight positive effect of charcoal in overcoming aflatoxicosis. Yet, autoclaving was effective in decontamination of aflatoxins by 92 – 100%, depending on the initial contamination level, time course of the treatment or its temperature in addition to the aflatoxin type (Abdelhamid, 1993). On the other hand, the last author added that autoclaving led to hard-sticky food with dark coloration. Therefore, the choice of protection (prevention) is preferable as the corner stone of the aflatoxin control, which is more beneficial than detoxification. However, the effect of the used adsorbents may be due to their characteristics including adsorbing water (leading to lowering water activity against the required level for fungal growth and aflatoxin production as cited by Adebaje *et al.*, 1994), which has the same effect of drying (Ozay *et al.*, 1995), fungicides or fumigation on mould inhibition (Nilipour, 1996). They also bind aflatoxin leading to reduction of its contamination level (Horvath, 1998).

In accordance with the present results; antitox plus, charcoal, fix-a-tox and florisil-as aflatoxin detoxifying agents-were of low effect. Meanwhile, addition of any of these agents resulted in an increase in aflatoxin productivity of *A. parasiticus* in sorghum (Mahmoud *et al.*, 1994). Moreover, Abdelhamid and Mahmoud (1996) concluded that the usage of such adsorbents was of no benefits during feed-storage, since their toxin-adsorbent effect is only immediately during their addition. Since many absorbents are sold based on their activity in laboratory trials (*in-vitro* tests) without actual testing in live animals (*in-vivo* tests) in the field (Anon., 1999). Anyhow, aflatoxin was found to be less adsorbed than other mycotoxins by different adsorbents and the

adsorbance of mycotoxins from aquous media is more than from corn (Shehata, 2001). Additionally, Biogen® did not detoxify aflatoxic diets (Abdelhamid *et al.*, 2002a). However, another studies (Hertrampf, 1994; Smith *et al.*, 1994 and Edrington *et* al., 1996) referred to the benefits of such materials (alumino silicates). In addition, detoxification of aflatoxin in peanut meal was completely by hydrogen peroxide at a pH of 9.5 involved heat treatment of the meal at 80ºC (Sreenivasamurthy *et al.*, 1967). Also, methanol extraction was used for removing aflatoxin from oilseeds meal (Sargent *et al.*, 1961; Feuell, 1966 and Müller, 1983). Moreover, microwave heating may reduce also other mycotoxins (DON) level to a certain extent (Horvath, 1998). In general, to choice an aflatoxin detoxification method (physical, chemical, and/or biological), it must be depended on its availability, costs, effectiveness, applicability, suitability for the contaminated material and on its side effects.

CONCLUSION

From the foregoing results, it could be concluded that ammonia treatment of aflatoxin contaminated diets strongly reduces the level of contamination and enhances crude protein level of the treated materials. The presence of ammonia can prevent fungal growth and consequently aflatoxin production. Thus, any suspicious material could be safely treated with ammonia to avoid aflatoxin occurrence.

RERERENCES

Abdelhamid, A.M. (1981). Mykotoxine in österreichischen Futtermitteln. Dissertation, Bodenkultur Universität, Wien, Österreich.

Abdelhamid, A.M. (1983a). Mykotoxin – Nachweis in Lebens – und Futtermitteln des subtropischen Klimas. 37. Tagung in Göttingen vom 21. bis 23. März, Z. Tierphysiol, Tierernährg. U. Futtermittelkde, 50: 1 – 40.

Abdelhamid, A.M. (1983b). Natural contamination of animal feedstuffs with mycotoxins. Agric. Res. Rev., 61(6): 109 – 123.

Abdelhamid, A.M. (1985). Detection of aflatoxins in Egyptian feedstuffs. Annals of Agric. Sci., Moshtohor, 23: 649 – 657.

Abdelhamid, A.M. (1990). Occurrence of some mycotoxins (aflatoxin, ochratoxin A, citrinin, zearalenon and vomitoxin) in various Egyptian feeds. Arch. Anim. Nutr., Berlin, 40: 647 – 664.

Abdelhamid, A.M. (1993). Decontamination of aflatoxins – contaminated foods by some physical means. J. Egypt. Ger. Soc. Zool., 12(A): 191 – 208.

Abdelhamid, A.M. and M.R.M. Saleh (1996). Are aflatoxin and ochratoxin endemic mycotoxins in Egypt? Proc. Conf. Foodborne Contamina-tion & Egyptian's Helath, Mansoura, Nov. 26–27, pp: 51–59.

Abdelhamid, A.M.; T.M. Dorra and H.A. Arief (1992a). Attempts to detoxicate aflatoxin – contaminated–broiler–diet. VIᵗʰ International Symposium World Association of Veterinary Laboratory Diagnosticians. June, Lyon, France.

Abdelhamid, A.M.; A.M. El-Mansoury; A.I. Osman and S.M. El-Azab (1999). Mycotoxins as causative for human food poisoning under Egyptian conditions. J. Agric. Sci., Mansoura Univ., 24: 2751 – 2757.

Abdelhamid, A.M.; I. El-Shawaf; S.A. El-Ayoty; M.M. Ali and I. Gamil (1986). Effect of low level of dietary aflatoxins on Baladi rebbits. Proc. IVth Inter. Symp. of Vet. Lab. Diag., June 2–6, Amsterdam, pp: 151–154.

Abdelhamid, A.M.; A.A.Gabr and M.M.El-Shinnawy (1994). Effect of hydrogen peroxide and urea treatment on chemical composition, cell wall constituents and *in vitro* organic matter digestibility of rice straw and maize stover. J. Agric. Sci. Mansoura Univ., 19: 3647 – 3657.

Abdelhamid, A.M.; F.F.M. Khalil; M.I. El-Barbary; V.H. Zaki and H.S. Husien (2002a). Feeding Nile tilapia on Biogen® to detoxify aflatoxic diets. Proc. 1st Ann. Sci. Conf. Anim. & Fish Prod., Mansoura, 24 & 25 Sept., pp: 207 – 230.

Abdelhamid, A.M.; F.F. Khalil and M.A. Ragab (1998). Problem of mycotoxins in fish production. Egypt. J. Nutr. and Feeds, 1: 63 – 71.

Abdelhamid, A.M. and K.I. Mahmoud (1996). Elimination or adsorption of aflatoxin from poultry feedstuffs. Proc. Conf. Foodborne Contamination and Egyptian's Health, 26 – 27 Nov., pp: 161 – 69.

Abdelhamid, A.M.; S.S. Mansy; T.M. Dorra and A.E. Sallam (1992b). Effect of dietary energy, protein and amino acids on broilers fed aflatoxin–B_1–contaminated diets. Proc. 3rd World Cong. Foodborne Infections and Intoxications, Berlin, 16 – 19 June, pp: 674 – 677.

Abdelhamid, A.M.; E.A. Sadik and E.A. Fayzalla (1985). Preserving power of some additives against fungal invasion and mycotoxin production in stored-crushed-corn containing different levels of moisture. Acta Phytopathologica Academica Scientiarum Hungaricae, 20: 309–320.

Abdelhamid, A.M.; A.E. Sallam; G.A. Abd Allah and S.H. El-Samra (2002b). Proc. 2nd Conf. Foodborne Contamination and Egyptians' Health, 23 – 24 April, El-Mansoura, pp: 99 – 121.

Adebaje, L.O.; O.A. Bamgbelu and R.A. Olowu (1994). Mould contamination and the influence of water activity and temperature on mycotoxin production by two aspergilli in melon seed. Nahrung, 38(2):209–217.

Ahmed, A.S. (1976). Breeding and housing of experimental animals. NAMRU-3, Abbassia, 3rd Ed., pp: 43-73.

Anderson, R.A. (1983). Detoxification of aflatoxin – contaminated corn. pp. 87–90. In U. Diener, R. Asquith, and J. Dickens (Eds.) Aflatoxin and *Aspergillus flavus* in corn. Southern Cooperative Series Bulletin 279, Auburn University, Auburn, Alabama.

Anon. (1999). Mycotoxin binders: research on efficacy in broilers. World Poultry – Elsevier, 15(3): 32.

A.O.A.C. (1990). Association of Official Analytical Chemists. Official Methods of Analysis. 13th Ed., Washington, D.C.

ASH (1999). Aflatoxin identified as silent killer found in tobacco. ASH Org.: http: // ash. org.

Aziz, N.H. and Y.A. Youssef (1991). Occurrence of aflatoxins and aflatoxin – producing moulds in fresh and processed meat in Egypt. Food Addit. Contam., 8(3): 321 – 331.

Bagley, E.B. (1979). Decontamination of corn containing aflatoxin by treating with ammonia. J. Amer. Oil Chem. Soc., 56: 808 – 811.

Bintvihok, A. (2001). Controlling aflatoxin danger to ducks and duck meat. World Poultry – Elsevier, 17(11): 18 – 19.

CAST (1989). Council for Agricultural Science and Technology. Mycotoxins: Economic and health risks. Task Force Report, No. 116, Ames, IOWA.

Changbumrung, S.; J. Thesasilpa; T. Hararoongroj; S. Vorasanta; K. Hongtong; Y. Chantaranipapong; S. Kusolchariya; T. Khieokhachee and C. Ariyasriwatana (1999). Aflatoxins M_1 and M_2 in breast milk. J. Nutr. Assoc. Thailand, 33: 42.

Clark, J.D.; A.V. Jain; R.C. Hatch and M.A. Haffy (1980). Experimentally induced chronic aflatoxicosis in rabbits. Am. J. Vet. Res., 41: 1841– 1845.

Costantini, A.V.; H. Wieland and L. Quick (1999). The fungalbionic book series (The Fungal/Mycotoxin Etiology of Breast Cancer, Prostate Cancer, and Atherosclerosis, and the Garden of Eden Longevity Diet, Antifungal/Antimycotoxin, Anti-Cancer, Anti-Atherosclerois), Albert Ludwigs School of Medicine, Freiburg, Germany.

Cotty, P.J. and D. Bhatnagar (1994). Variability among atoxigenic *Aspergillus flavus* strains in ability to prevent aflatoxin contamination and production of aflatoxin biosynthetic pathway enzymes. Appl. Environ. Microbiol., 60: 2248 – 2251.

Decker, W.J. (1980). Activated charcoal absorbs aflatoxin B_1. Vet. Human Toxicol, 22: 388 – 389.

Dollear, G.F.; G.E. Mann; L.P. Codifer; J.K. Gardner; S.P. Koltun and H.L.E. Vix (1968). Elimination of aflatoxins from peanut meal. J. Am. Oil Chem. Soc. 45: 862 – 865.

Edrington, T.S.; A.B. Sarr; L.F. Kubena; R.B. Harvey and T.D. Phillips (1996). Hydrated sodium calcium aluminosilicate (HSCAS), acidic HSCAS, and activated charcoal reduce urinary excretion of aflatoxin M1 in turkey poults. Lack of effect by activated charcoal on aflatoxicosis. Toxicol. Lett., 89(2): 115 – 122.

El-Nezami, H.; P. Kankaanpaa; S. Salminen and J. Ahokas (1998). Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B_1. Food Chem. Toxicol., 36(4): 321 – 326.

El-Sayed, T.I. (1996). 1st Int. Conf. Fungi: Hopes & Challenges, 2 – 5 Sept., Cairo, Abst., P: 33.

Feuell, A.J. (1966). Aflatoxin in groundnuts. Part II- Problems of detoxification. Trop. Sci., 8: 61 – 70.

Gardner, H.K.; S.P. Koltun; F.G. Dollear and E.T. Rayner (1971). Inactivation of aflatoxins in peanut and cotton seed meals by ammoniation. J. Am. Oil Chem. Soc., 48: 70 – 73.

Goldblatt, L.A. and F.G. Dollear (1979). Modifying mycotoxin contamination in feeds. Use of mold inhibitors, ammonidation, roasting. pp. 167 – 184. In: Interactions of Mycotoxins in Animal Production. National Academy of Sciences, Washington, DC.

Halt, M. (1998). Moulds and mycotoxins in herb tea and medicinal plants. Eur. J. Epidemiol., 14(3): 269 – 274.

Hertrampf, J.W. (1994). The mycotoxin hazard can be easily solved. World Poultry – Misset, 10(8): 55 – 57.

Horvath, E.M. (1998). Taking the threat out of mycotoxins. Feed Tech., 2(1): 32 – 33.

Hsieh, L.L.; S.W. Hsu; D.S. Chen and R.M. Santella (1988). Immunological detection of aflatoxin B_1-DNA adducts formed *in vivo*. Cancer Res., 48: 6328 – 6331.

Jensen, A.H.; O.L. Brekke; G.R. Frank and A.J. Peplinski (1977). Acceptance and utilization by swine of aflatoxin – contaminated corn treated with aqueous or gaseous ammonia. J. Anim. Sci., 45(1): 8 –12.

Jonsyn, F.E.; S.M. Maxwell and R.G. Hendrickse (1995). Ochratoxin A and aflatoxins in breast milk samples from Sierra Leone. Mycopathologia, 131(2): 121 – 126.

Kiermeier, F. (1985). The mycotoxin problem: results of food monitoring. Z. Lebensm. Unters. Forsh., 180(5): 389 – 393.

Mahmoud, K.I.; A.M. Abdelhamid and A. Mandour (1994). *In vitro* and *in vivo* comparative studies on the efficacy of some aflatoxin-detoxifying agents. Alex. J. Vet. Sci., 10 (1): 39 – 47.

Mann, G.E.; L.P. Jr. Codifer and F.G. Dollear (1967). Effect of heat on aflatoxins in oil seed meals. J. Agric. Fd. Chem. 15: 1090.

Mann, G.E.; H.K. Jr. Gardner; A.N. Booth and M.R. Gumbmann (1971). Aflatoxin inactivation. Chemical and biological properties of ammonia and methylamine treated cottonseed meal. J. Agric. Food Chem., 19: 1155 – 1158.

Masimanco, N.; J. Remacle and J. Ramaut (1973). Elimination of aflatoxin B_1 by absorbent clays in contaminated substrates. Ann. De Nutr. et Alimen., 23: 137 – 147.

Med Nets, Inc. (2001). Aflatoxicosis: Patient information. www.mednets.org.

Moorthy, A.S.; M. Mahendor and P. Ramarao (1985). Hepatopathology in experimental aflatoxicosis in chickens. Indian J. Anim. Sci., 55: 629–632.

Müller, H.M. (1983). Entgiftung von Mykotoxinen in Futtermitteln. Wissenschaft und Umwelt, 4: 300 – 304.

Nilipour, A.H. (1996). Keeping insects and mycotoxins out of silos. World Poultry – Misset, 12(9): 24 – 25.

Norton, R.A. (1998). Testing for natural aflatoxin inhibitors. Agric. Res. Mag. July issue. [from:hattp://www.ars.usda (4/12/2002)].

Nowar, M.S.; A.A. Abuel-Atta; A.E. Aser and A.M. El-Darawany (1992a). Aflatoxins extracts (B_1+G_1) induced changes in albino rats, some histological, teratological and reproductive studies. Egypt J. App. Sci., 7(10): 106 – 115.

Nowar, M.S.; A.M. El-Darawany and A.A. Habeed (1992b). Effect of aflatoxin ingestion on total body water (TOH^3 – Space), total body solids and on some physiological and reproductive characteristics of male albino rats. 5th Conference of Nuclear Sciences and Applications, pp. 544 – 550.

Nowar, M.S.; M.L. Abd El-Rahim, M.N. Gaafary; M.L. Tawfeek; Z. A. Ibrahim and F.R. Abdallah (2000). Aflatoxicosis in rabbits. 3- Effectiveness of Egyptian raw bentonite in prevention or diminution the detrimental effects of aflatoxins – maturally contaminated diet on reproductive performance, blood biochemistry and digestibility in rabbists. Proc. 5th Sci. Vet. Med. Conf., 12 – 14 Sept., Sharm El-Sheikh, pp: 321.

Nowar, M.S.; E.M. Hassona and M.I. Abd El-Rahim (1996). Aflatoxicosis in rabbits: 2- Prevention of aflatoxicosis in growing rabbits by addition of

tafla to aflatoxin – naturally contaminated diet. Proc. Con. Food-Borne Contamination and Egyptian's Health. Mansoura, pp: 97–111.

Nowar, M.S.; R.M. Sherif; M.G. Gbreal and A.M. El-Darawany (1987). Teratogenicity of aflatoxins (B_1 + G_1) mixture in rats and chick embryos. The Egypt. J. Environ. Mutag. and Carcinog., (In Press) cited from Nowar, M.S. and Natour, R.M. (eds.) An Overview of Mycotoxins and Mycotoxicosis in Human and Animals, Vol. I, 1st Ed., Univ. of Jordan, Amman, 1989.

Ozay, G.; N. Aran and M. Pala (1995). Influence of harvesting and drying techniques on microflora and mycotoxin contamination of figs. Nahrung, 39(2): 156 – 165.

Park, D.L.; L.S. Lee and S.A. Kolton (1984). Distribution of ammonia – related aflatoxin reaction products in cottonseed meal. J. Am. Oil Chem. Soc., 61: 1071 – 1074.

Pier, A.C. (1992). Major biological consequences of aflatoxicosis in an animal production. J. Anim. Sci., 70: 3964 – 3967.

Pluyer, H.R.; E.M. Ahmed and C.I. Wei (1987). Destruction of aflatoxins on peanuts by oven and microwave roasting. J. Food Prot., 50: 504 – 508.

Rayner, E.T.; S.P. Koltun and F.G. Dolear (1977). Solvent extraction of aflatoxins from contaminated agricultural products. J. Am. Oil Chem. Soc., 54: 242A -244A.

Sargent, K.; J. O'Kelly; R.B.A. Carnacman and R. Allcroft (1961). The assay of a toxic principle in certain groundnut meals. Vet. Rec., 73: 1219 – 1223.

Shehata, S.A.M. (2001). Detoxification of mycotoxin contaminated animal feedstuffs. Ph.D. Thesis, Zagazig Univ., Fac. Agric.

Smith, E.E.; T.D. Phillips; J.A. Ellis; R.B. Harvey; L.F. Kubena; J. Thompson and G. Newton (1994). Dietary hydrated sodium calcium aluminosilicate reduction of aflatoxin M1 residue in dairy goat milk and effects on milk production and components. J. Anim. Sci., 72: 677 – 682.

Sreenivasamurthy, V.; H.A.B. Parpia; S. Srikanta and A. Shmkar (1967). Detoxification of aflatoxin in peanut meal by hydrogen peroxide. J. Assoc. Offic. Agric. Chem., 50(2): 350 – 354.

Stanley, V.G.; R. Ojo; S. Woldesenbet; D.H. Hutchinson and L.F. Kubena (1993). The use of *Saccharomyces cerevisiae* to suppress the effects of aflatoxicosis in broiler chicks. Poultry Sci., 72: 1867–1872.

STAT (2001). Peptide may help defeat aflatoxin in cotton. STAT Pub. Com., STAT Communications Ltd. Canada.

Sun, T.T.; S.M. Wu; Y.Y. Wu and Y.R. Chu (1985). Measurement of individual aflatoxin exposure among peoples having different risk to primary hepatocellular carcinoma. Princess Takamatsu Symp., 16: 225 – 235.

Truckess, M.W.; M.E. Stack; S. Nesheim; S.W. Page; R.H. Albert; T.J. Hansen and K.F. Donahue (1991). Immunoaffinity column coupled with solution flourometry or liquid chromatography postcolumn derivatization for determination of aflatoxins in corn, peanuts and peanut butter: Collaborative study. J. AOAC., 74(1): 81 – 88.

Vries, H.R.de; S.M. Maxwell and R.G. Hendrickse (1990). Aflatoxin excretion in children with kwashiorkor or marasmic kwashiorkor – a clinical investigation. Mycopathologia, 110(1): 1 – 9.

Wood, G.E. (1992). Mycotoxins in foods and feeds in the United States. J. Anim. Sci., 70: 3941 – 3949.

Yegany,M.; G. Butcher; A.H. Nilipour; R.D. Miles and R.Karegar (2002). Feed hygiene and poultry health. World Poultry–Elsevier, 18(7): 17– 19.

إزالة سمية العلائق الملوثة بالأفلاتوكسين ببعض الطرق الطبيعية والكيماوية .
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** المعمل المركزى للأغذية والأعلاف – مركز البحوث الزراعية.

فى الدراسات المعملية لمقارنة فاعلية إضافة كل من Antitox plus أو Fix-a-tox ، الطفلة – المعاملة الحرارية تحت ضغط – المعاملة الحرارية فى أفران الميكرويف – المعاملة بفوق أكسيد الهيدروجين – المعاملة بمحلول الأمونيا – الاستخلاص بمحلول مائى من الميثانول ٥٠% كعوامل لإزالة سمية العلف المحتوى على تركيزين من الأفلاتوكسين (١٨٠٠ و ٣٦٠٠ جزء فى البليون)، وجد أن إضافة مستوى ٥ر٠% من Antitox plus أو Fix-a-tox أو الطفلة يؤدى إلى إزالة الأفلاتوكسين (على التوالى) بنسبة ٤ر٣٣، ٧ر١٦ و ٤ر٤٤% وذلك من التركيز العالى من التلوث ولكن فى التركيز المنخفض تكون الإزالة بمعدل ٦ر٣١، ٨ر١٥، ١ر٤٢% على التوالى. المعاملة الحرارية تحت ضغط لمدة ٣٠ دقيقة واستخدام أفران الميكرويف على القوة المتوسطة لمدة ٣ دقائق تؤدى إلى إزالة الأفلاتوكسين فى مستوى التلوث العالى بمعدل ٤ر٣٣ و٨ر٥٢% على التوالى. ولكن فى مستوى التلوث المنخفض يؤدى إلى إزالة ٦ر٣١ و٦ر٥١% على التوالى نظراً لما تحدثه درجة الحرارة المرتفعة من تغييرات فى مظهر وقوام وتركيب الغذاء، وبالتالى فى قيمته الحيوية وهضمه والاستفادة منه، فإن المقاومة خير من العلاج. عوامل الأكسدة مثل فوق أكسيد الهيدروجين والأمونيا تؤدى إلى إزالة ٩ر٣٨% من المستوى العالى و٨ر٣٦% من المستوى المنخفض للتلوث على التوالى. الاستخلاص للأفلاتوكسين من الأعلاف بواسطة الميثانول يسبب إزالة ٦ر٥٥% من التركيز العالى من التلوث، ولكن فى التركيز المنخفض يسبب إزالة ٢ر٥٣% مع ذلك فإن الاستخلاص بواسطة الميثانول غير تطبيقى ومكلف عندما يقارن مع الطرق الأخرى.

لا يوجد إختلاف فى التركيب الكيماوى فى المعاملات المختلفة ولكن المعاملة بالأمونيا تؤدى إلى زيادة البروتين الخام، والاستخلاص يؤدى إلى انخفاض البروتين والقيمة الغذائية. قدر إنتاج الأفلاتوكسين بواسطة فطر *Aspargillus flavus* فى البيئة السائلة فى وجود أو غياب مستوى ٥ر٠% من كل من Antitox plus وFix-a-tox، الطفلة و١% أمونيا و٣% فوق أكسيد الهيدروجين فى العينات، فوجد أن إضافة أى من هذه العوامل يؤدى إلى قلة الأفلاتوكسين المنتج، والأكثر تأثيرا فى تقليل الأفلاتوكسين المنتج هى الأمونيا يليها فوق أكسيد الهيدروجين، والطفلة وAntitox plus وFix-a-tox فالأمونيا تمنع نمو فطر *Aspargillus flavus* مما يؤدى إلى منع التلوث بالأفلاتوكسين. وعلى ذلك فمعاملة أى مادة علف (موضع شك) بالأمونيا يقى من آثار الأفلاتوكسين السامة.

Table (2): Effect of various treatments on chemical composition (%) of the contaminated – diet with two aflatoxin concentrations.

Treatments	Mositure	Dry matter	Crude protein	Crude fiber	Ether extract	Ash	NFE	Calcium	Phosph-orus
High contaminated diet (H)	10.2	89.8	17.2	4.7	2.78	8.8	56.32	1.97	0.72
Low contaminated diet (L)	11.1	88.9	16.2	4.3	3.02	9.2	56.48	2.09	0.73
H + Autoclaving	11.8	88.2	17.2	4.6	3.05	9.1	54.25	2.01	0.71
L + Autoclaving	11.9	88.1	16.8	4.7	2.88	9.4	54.32	1.85	0.70
H + antitox plus	10.9	89.1	16.8	4.6	2.74	8.6	56.36	1.98	0.71
L + antitox plus	11.1	88.1	16.8	4.7	2.89	8.8	55.71	2.15	0.74
H + Fix-a-toxin	11.3	88.7	16.8	4.3	3.29	8.7	55.61	2.03	0.69
L + Fix-a-toxin	10.9	89.1	16.8	4.5	3.10	8.8	55.90	2.02	0.67
H + tafla	11.4	88.6	16.7	4.6	2.95	8.8	55.55	1.94	0.64
L + tafla	11.2	88.8	17.1	4.8	2.68	9.2	55.02	1.98	0.62
H + H_2O_2	13.9	86.1	16.4	4.4	2.91	8.7	53.69	2.02	0.66
L + H_2O_2	14.3	85.7	16.7	4.9	3.15	8.2	52.75	1.98	0.63
H + NH_3	12.2	87.8	18.7	4.8	3.01	8.3	53.99	2.02	0.72
L + NH_3	12.1	87.9	19.2	4.8	2.89	8.3	53.71	1.98	0.65
H + Methanol	15.5	83.5	14.3	4.6	2.69	8.5	53.91	1.99	0.66
L + Methanol	15.6	83.4	14.4	4.6	2.65	8.4	54.35	1.88	0.62
H + Microwave	11.4	88.6	16.4	4.9	2.66	9.1	55.54	1.90	0.60
L + Microwave	11.2	88.8	16.8	4.8	3.17	8.7	55.33	1.95	0.69