Full Report

ibc Interdisciplinary Bio Central

Open Access, Open Review Journal

Field Study on the Mycotoxin Binding Effects of Clay in *Oreochromis niloticus* Feeds and Their Impacts on the Performance as Well as the Health Status throughout the Culture Season

Mohamed Abdelaziz[1,*], Wael Anwer[2] and Abeer Hamada Abdelrazek[2]

[1]Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, Giza 11221, Egypt
[2]Department Veterinary hygiene and management, Faculty of Veterinary Medicine, Cairo University, Giza 11221, Egypt

Subject areas: Environmental bioscience and technology/Bioenergy, Biological frontiers (General Biology), General

Author contribution: M.A., sample collection, clinical examination, health assessment and data interpretation; W.A., mycotoxin supply, environmental water parameters assessment and data interpretation; A.H.A., sample collection, field trips, environmental water quality assessments, fish rearing and care.

***Correspondence** and requests for materials should be addressed to M.A. (mabdelaziz1973@yahoo.com).

Reviewer: Mohamed Moustafa Mohamed, Cairo University, Egypt; Alaa Eldin Eissa, Cairo University, Egypt

Editor: Sun Shim Choi, Kangwon National University, Republic of Korea

Received October 22, 2010;
Accepted October 26, 2010;
Published November 12, 2010

Citation: Abdelaziz, M., et al. Field Study on the Mycotoxin Binding Effects of Clay in *Oreochromis niloticus* Feeds and Their Impacts on the Performance as Well as the Health Status throughout the Culture Season. IBC 2010, 2:10, 1-5
doi: 10.4051/ibc.2010.2.4.0010

Competing interest: All authors declare no financial or personal conflict that could inappropriately bias their experiments or writing.

SYNOPSIS

Total aflatoxin and ochratoxin were detected in 3 naturally contaminated fish feed samples using immune-affinity method. The results revealed that the average levels of aflatoxins in the 3 examined samples were (15, 22 and 12 µg/kg) respectively while the average levels of ochratoxins were (15, 6 and 6 µg/kg). The results of determination of the effects of clay as a mycotoxin binder on the health status and performance of *Oreochromis niloticus* in comparing with a control group revealed that the survival rate in control group was 81% after the end of the culture season. The results also revealed that the survival rate in group 2 which received clay treated feed was 86%. The results of regular parasitological examination revealed the identification of trichodina as external protozoa in *Oreochromis niloticus* from both ponds but without manifestation of disease signs. The results of bacteriological examination revealed the isolation and identification of *Pseudomonas flouresence* from some moribund *Oreochromis niloticus*. Higher performance parameters were recorded in group 2 that received feeds treated with clay which reflected in the total production which reaches 1646.47 kg while in the control pond, the total production was1308.36 kg.

Keywords: mycotoxins, binding agents, clay, fish, *Oreochromis niloticus*, health status

Introduction

The contamination of animal, fish and human feeds with mycotoxins represent a worldwide problem constituting a real threat to the health of livestock for animals, aquaculture industry and human by the continuing intermittent occurrence in their feeds[1,2]. Mycotoxin is a secondary toxic-metabolite that is produced from a mycotoxic mould. A mould species can produce several types of mycotoxin. Therefore, any mouldy sample may contain numerous mould species; hence it may be contaminated with different mycotoxins. Thus, when a mycotoxin is detected, man should suspect that other types are also present in a contaminated feed[3,4].

The molds that produce mycotoxins tend to grow under warm and moist conditions, which is the same condition that predominates in the tropics where most aquaculture is practiced. Most commercial feed millers are scrupulous when it comes to checking raw materials and feeds for aflatoxin. The heat and pressure of pelleting and extrusion do not destroy appreciable amounts of mycotoxins. Also, blending contaminated feed ingredients with "clean" ingredients in order to reduce the concentration of mycotoxins in the final feed is not recommended. If the contaminated ingredient contains viable mould or spores, the mycotoxin concentration in the feed will likely increase when conditions favor mould growth.

The unreasonable global rise of animal derived proteins prices have enforced the farmers to use less expensive vegetable proteins like those of plant origin. Thus, the importance of mycotoxin contamination may increase since feed ingredients of plant origin, including byproducts, tend to have greater incidences of mycotoxin contamination[5]. At the same time, in the tropics many farmers store feeds in inferior conditions where feeds may become wet during transportation due to heavy rains and further deteriorated by rat and insect infestation.

Veterinarians and producers of terrestrial animals are familiar with the effects of mycotoxins such as aflatoxin, zearalenone, T-2, ochratoxin and deoxynivalenol (DON or vomitoxin) on livestock, while the impact on aquatic species has not been extensively studied. In aquatic animals, although there is scarcity in available information, yet there are some evidences that mycotoxins could be associated with reduced performance for many aquaculture species[6-8].

Numerous researchers have reported that mycotoxins can act synergistically so that the negative effects of two mycotoxins are worse than the effects of single toxin[1,9]. The negative effects of mycotoxins including inhibition of DNA, RNA and protein synthesis, genotoxic, proteolytic, chromatide plaster, depression of ATPase, hormonotoxic**,** estrogenic, sexual, free radical and active oxygen producing, carcinogenic**,** immunotoxic, neurotoxic, hepatotoxic, nephrotoxic, digestive system toxins, and dermal toxic effects[10-13]. As a results of these negative effects, mycotoxins can cause a wide variety of adverse clinical signs among fishes depending on the nature and concentration of the mycotoxin, duration of exposure, the fish species/age, nutritional and health status at the time of exposure to contaminated feed[14-16]. Thus mycotoxins are well known to negatively affect production, growth and immune system function[17].

Table 1. Levels of aflatoxins and ochratoxins in examined *Oreochromis niloticus* feed samples

Sample numbers	Fish feed sample	ug/kg
1	Aflatoxin level (µg/kg) Ochratoxin level (µg/kg)	15 15
2	Aflatoxin level (µg/kg) Ochratoxin level (µg/kg)	22 6
3	Aflatoxin level (µg/kg) Ochratoxin level (µg/kg)	12 6

Therefore, the aim of the current study is to determine the levels of aflatoxines and ochratoxins in fish feeds samples and to conduct a field study on the effects of clay on the health status and performance of *Oreochromis niloticus* during a culture season.

Results

Levels of detected mycotoxins in fish feeds samples

The results of detection of total aflatoxin and ochratoxin in naturally contaminated fish feeds are shown in Table 1. Results revealed that the average levels of aflatoxins in the 3 examined samples were (15, 22 and 12 ug/kg) respectively while the average levels of ochratoxins were (15, 6 and 6 ug/kg) respectively.

Effects of clay as a mycotoxin binder on the health status and performance of *Oreochromis niloticus*

The results of determination of the effects of clay as a mycotoxin binder on the health status and performance of *Oreochromis niloticus* in comparing with a control group as shown in Table 2 revealed that the survival rate in control group was 81% after the end of the culture season, which means that the total mortalities along the season were 1330 representing 19% of the total cultured *Oreochromis niloticus* in the control pond. The results also revealed that the survival rate in group 2 which received clay treated feed was 86% which means that the total mortalities along the culture season were 980 fish, representing 14% of the total cultured *Oreochromis niloticus* in the treated pond. Number of mortalities has occurred in both groups shortly after adaptation of fish in the 2 ponds, but the exact number in this period not exactly determined due to the small size of fish. Also mortalities of very few fish noticed in both ponds every 2 or 3 days or even daily and was recorded accurately. The mortalities in most cases did not exceed 5 fishes in the pond received clay treated feed while in the control pond it usually exceeds this number. Some died fish showing signs of disease while some died fish exhibit the appearance of being healthy.

Parasitology

The results of regular parasitological examination of fish from both pond revealed the identification of trichodina as external

Table 2. Survival rate, growth performance, total production of control *Oreochromis niloticus* compared to those fed on feeds treated with clay

	Group1 (control)	Group 2 (treated group)
W.G. (gm)	230.80 ± 9.16	[a]273.50 ± 8.30
Survival rate	81%	86%
Total production	1308.36 kg	1646.47 kg
SGR (%)	1.53 ± 0.05	[a]1.82 ± 0.08*
FCR	2.29 ± 0.52	[a]1.82 ± 0.10
PER	54.20 ± 4.80	[a]131.65 ± 4.25*

Group(1) ; *Oreochromisniloticus* control pond.
Group(2) ; *Oreochromisniloticus* received clay treated feed.
Values represent means ± standard deviations.
* Significantly different from control P< 0.05.
Means with different alphabetical letters are significantly different P< 0.05.

protozoa in examined *Oreochromis niloticus* from both ponds but without manifestation of disease signs.

Bacteriology

The results of bacteriological examination revealed the isolation and identification of *Pseudomonas flouresence* from some moribund *Oreochromis niloticus* showing external skin lesions, the average size of affected fish was over 150 gm in both ponds. The isolated bacteria was gram negative short motile bacilli on microscopical examination, also on RS media the isolated bacteria was identified as dark green colonies, at the same time the biochemical identification revealed positive oxidase test, positive oxidation and negative fermentation in O\F test, positive for catalase and negative for citrate and H2S production. The rapid exchange of water from ponds associated with stop feeding for 2 days, leads to correction of this stipulation.

Growth performance

The results of growth performance parameters revealed that the weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio were 230.80 ± 9.16, 1.53 ± 0.05, 2.29 ± 0.52 and 54.20 ± 4.80 respectively in the control group while in the group 2 that received feeds treated with clay as a mycotoxin binder the results were ^{a}273.5 0 ± 8.30, ^{a}1.82 ± 0.08*, ^{a}1.82 ± 0.10 and ^{a}131.65 ± 4.25* respectively Table 2. The higher parameters in group 2 reflected in the total production which reach 1646.47 kg while in the control pond, the total production was 1308.36 kg.

Discussion

Aflatoxins are group of naturally occurring isocoumarine compounds that poses an extreme threat to the health of livestock by their continuing intermittent occurrence in both feeds and foods[18]. The detection of total aflatoxin and ochratoxin in naturally contaminated fish feed samples revealed the presence of aflatoxins in the 3 examined samples at levels (15, 22 and 12 (µg/kg)) respectively while the average levels of ochratoxins were (15, 6 and 6) respectively. The results revealed the presence of high levels of mycotoxins in naturally examined *Oreochromis niloticus* feed samples, which agree with El-Azab et al.[5].

The higher occurrence of mycotoxins in feeds may be aggravated by the fact that there are no strict regulations that impose limits on the concentration of mycotoxins in crops. Low quality storage standards (humidity, rats and insects infestation) of fish feeds in the Egyptian aquaculture is the most reliable predisposing factors for the continual growth of mould with consequent production of mycotoxins. This fact is well fulfilled in our case, where examined samples possessed higher levels of aflatoxins and ochrotoxins.

Also the results are in complete accordance with Lim et al.[19] and Vekiru et al.[2] who reported that the contamination of animal feed with mycotoxins represents a worldwide problem for the animal industry. The mycotoxin contamination of feeds and raw materials, especially those of plant origin, is recognized as an increasing problem and the range of problems associated with mycotoxins is becoming more evident[2]. At the same time, the increasing trend for replacement of expensive animal-derived proteins, such as fish meal, meat and bone meal, with less expensive vegetable proteins, may be a factor in the recent time for increasing the importance of mycotoxin contamination in *Oreochromis niloticus* feeds, since feed ingredients of plant origin, including byproducts, tend to have greater incidences of mycotoxin contamination. A large percentage of the fish feed used around the world as well as in Egypt contains corn as a major ingredient. Corn is likely to contain high concentrations of mycotoxins, especially aflatoxin[5,19].

The results also revealed the presence of aflatoxins and ochratoxins in both powdered and extruded pellets. This could be due to the extreme stability of such mycotoxins. The heat and pressure of pelleting and extrusion do not destroy appreciable amounts of mycotoxins.

The detoxification of mycotoxins in feeds cereals has been examined using physiochemical, chemical, and biological processes[20]. Binders have been used to neutralize the effects of mycotoxins by preventing their absorption from the digestive tract. The most common binders are clays, such as bentonite[21]. The results also coincide with Sean et al.[22] who reported that the addition of calcium bentonite significantly improved larval growth over filtered water alone in multiple experiments, increasing the growth of larval cultures by as much as 33% at a dose of 5 ppm day. These results reflect the positive effect of using clay.

The effectiveness of bentonite was also reported by Vekiru et al.[2], who indicated that the most applied method for protection against aflatoxicosis is the utilization of clay minerals.

Some reported information explained that although clays such as bentonite are of the most common binders because they are inexpensive and widely available, but they have some disadvantages that they bind only a narrow range of mycotoxins-mainly aflatoxin- and are usually only effective at high concentrations[23]. Also Clays are indigestible and insoluble in water and do not contribute to the nutritional value of the feed. At the same time Inrecirculating aquaculture systems, clay may clog or damage water filtration systems and fine clay particles may damage the gills of fish. Also, some sources of clay contain measurable concentrations of naturally occurring dioxin, a potent carcinogen that accumulates in the fat tissue of fish and raises issues related to food safety. The constructive and affirmative effects of using clay also explained by Ellis et al.[24] that 2% bentonite contained in trout diets contaminated with 20 µg/kg AFB1 significantly reduces the amount of AFB1 absorbed from the digestive system following ingestion of contaminated diets.

The results of determination of the effects of clay as a mycotoxin binder on the health condition and performance of *Oreochromis niloticus* revealed that higher survival rate was recorded in group 2 which fed on feeds treated with clay in comparing with the control group. This means that the total mortalities recorded were lower among the group fed on feeds treated with clay as a mycotoxin binder. The beneficial effect of bentonite on fish have been reported by Schazmayr et al.[20] and Sean et al.[22] that the use of clay interfere with the toxic effect of mycotoxines which may be reflected on the higher survival rate in comparing with the control group. Moreover, the higher performance parameters including, the weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio in *Oreochromis niloticus* fed on feed treated with clay in comparing with the control group reflect the negative effect of aflatoxins and ochratoxins on these parameters, also it explained the positive effect of using clay as a mycotoxin binder in prevention of the drawbacks resulted from the presence of these mycotoxins. The higher performance parameters reflected on the higher total production in this group which reach 1646.47 kg while in the control pond, the total production was 1308.36 kg.

These results agreed with that reported by Sean et al.[22] that the addition of calcium bentonite significantly improved larval growth, also significant improvement in seed growth compared with SPF water with no clay addition was observed. The results also agreed with Schazmayr et al.[20] that when domestic animals ate the feed polluted with mycotoxins, various symptoms of poisoning are shown. Therefore, the detoxication on the mycotoxin is very important.

The mode of action of aflatoxins and ochratoxins may explain the negative effects which occurred in the group fed on feed without clay. It includes inhibition of DNA, RNA and protein synthesis (Aflatoxin, Ochratoxin-A), genotoxic (Aflatoxin), cellulartoxic–free-radical and active oxygen producing (Aflatoxin), carcinogenic (Aflatoxin, Ochratoxin-A), circulatory system (Aflatoxin), hepatotoxic (Aflatoxin), nephrotoxic (Aflatoxin, Ochratoxin)[10-12]. This toxic effect reflects the manifestations of low performance parameters, low survival rate, low production rate which recorded along the culture season. The recorded results regarding parasitological and bacteriological examinations revealed that no variations between the involved groups. Many factors affecting the toxicity of mycotoxins including the mycotoxin itself, level of contamination, time of exposure, route of application, presence of other mycotoxins, the fish species, sex and age of the exposed organism (hormonal effect), and clinical status of the exposed organism (hepatic enzymes status)[25]. The economic losses associated with aflatoxicosis from the low performance parameters, total production and low survival rates agree with the results recorded by Goldblatt[26], Lovell[27], Zhang et al.[28], Omar et al.[29], Tuan et al.[16]. Also regarding ochratoxicosis, Manning et al.[30] and Srour[31] showed that increasing OCTA levels in the diet resulted in decreasing growth performance and feed utilization parameters, Significant reductions in body weight gain were observed.

In conclusion, the obtained results in this study revealed that aflatoxins and ochratoxins could be associated with low survival rate, reduced performance of *Oreochromis niloticus* and to some extent affecting the general health condition of this species rendering *Oreochromis niloticus* susceptible to diseases with a negative drawback on the economy of culture of this species. Auspiciously, the high levels of aflatoxins and ochratoxins in examined natural *Oreochromis niloticus* feed samples and the negative effects associated with the existence of aflatoxins and ochratoxins explained the necessary for the feed manufacturer to secure mycotoxin-free sources and test all in-coming ingredients for the presence of mycotoxins, maintaining proper storage conditions for feeds and raw materials, and using a good mycotoxin binder like clay which applied in this study and give a good results, to adsorb the widest possible range of different mycotoxins that may be present.

Materials and Methods

Fish

A total of 40,000 frys of *Oreochromis niloticus* with the same weight range (1-3 gm) were held in two earthen ponds with an equal area of 2150 m2. The 1st pond received feeds treated with clay as a mycotoxin binder, while the second pond received feeds without clay as a control pond. The rate of feeding varied according to the size of fish and was analogous in both ponds till the end of the culture season according to De silva and Anderson[32]. The water parameters were adjusted according to American public health association 1989.

Ration

Naturally contaminated fish feed samples were examined for the presence of total aflatoxin and ochratoxin levels. At the same time the levels of these specific mycotoxins were detected after adding the different doses of clay.

Chemicals

The mycotoxin binding material (Clay: calcium bentonite).

Determination of the levels of aflatoxins and ochratoxins in fish feeds samples

Total aflatoxin and ochratoxin was detected in 3 naturally contaminated fish feed samples using immune-affinity method which is applicable for mycotoxins that have fluorescence[33]. Series-4 Fluorometer (VICAM) was used in this procedure which is summarized as follows:

Sample extraction

Total amounts of 50 gram sample, 5 gram NaCl, 100 ml methanol (80%) were blended at high speed (1 min.), filtered with fluted filter paper. A total amount of 10 ml extract was diluted with 40 ml distilled water and filtered with glass microfibre filter paper.

Column chromatography

10 ml (1 gm sample) of filtered extract was passed through AflaTest-p and OchraTest affinity columns with a rate of 1-2 drops/second. Column washed twice with 10 ml distilled water (at first with wash buffer in case of ochratoxin). The aflatoxin was eluted with 1 ml HPLC methanol to which 1 ml of freshly prepared aflatoxin developer was added spontaneously. The ochratoxin was eluted with 1.5 ml. OchraTest Eluting solution (without developer). Reading of total aflatoxin or ochratoxin was obtained after 60 seconds as part per billion (ppb).

Determination of the effects of clay on the performance and health condition of *Oreochromis niloticus*

This field study was applied on 7000 *Oreochromis niloticus* semi intensively cultured on an earth pond sized 2150 m2, in comparison to a control pond containing the same number of fish, fed on the same fish feed with the same feeding rate but without a mycotoxin binder. Throughout the culture season the general health condition, mortalities.

Parasitological examination

Regular parasitological examination was performed on samples from both ponds according to Lucky[34].

Bacteriological examination

Samples were taken from the kidney and from external skin lesions of moribund *Oreochromis niloticus* and examined according to Austin and Austin[35] in a trial to isolate a bacterial causative agent. At the same time the performance parameters including body weight gain (W.G.), specific growth rate (SGR%), feed conversion ratio (FCR) and protein efficiency ratio (PER) were observed and calculated as follows:

Body weight gain (W.G.)

Total weight was determined to the nearest gram according to Annet[36]. Specific growth rate (SGR%): Specific growth rate (SGR%) was calculated as the percentage increase in weight per fish per day as suggested by Pouomonge and Mbonglang[37].

Feed conversion ratio (FCR)

Feed conversion ratio was determined according to De silva and Anderson[32].

Protein efficiency ratio (PER)

Protein efficiency ratio (PER) was determined according to De silva and Anderson[32].

Acknowledgements

We are grateful to Dr. Mahdy Asar, Professor of Higher Institute of Cooperative Agriculture for his endless support in sample collection during the entire study.

References

1. Abdelhamid, A.M. (1999). Pollution of fish with heavy metals and mycotoxins. 1st Meeting “Water pollution, its effects on fish and

its relation to human health". Ismailia, May 5 (In Arabic), 4 p.

2. Vekiru, E., Fruhauf, S., Sahin, M., Ottner, F., Schatzmayr, G. and Krska, R. (2007). Investigation of various adsorbents for their ability to bind aflatoxin $B_{1,1}$. *Mycotoxin research* 23, 27-33.
3. Solfrizzo, M., Visconti, A., Avantaggiato, G., Torres, A., Chulze, S. (2001). *In vitro* and *in vivo* studies to assess the effectiveness of cholestyramine as a binding agent for fumonisins. *Mycopathologia* 151, 147-153.
4. Abdelhamid, A.M., Abdel- Khalek, .A.E., Mehrm, A.I., Khalil and F.F. (2004). An attempt to alleviate aflatoxicosis on Nile tilapia fish by dietary supplementations with chicken-hatchery by-products (egg shells) and shrimp processing wastes (shrimp shells). 1-On fish performance and feed and nutrients utilization. *J. Agric. Sci. Mansoura Univ.* 29, 6157-6173.
5. El-Azab, S.M., Abdelhamid, A., Shalaby, H.A., Mehrim, A. & Ibrahim, A.H. (2009). Study OfAflatoxin B1 As A Risk Factor That Impair The Reproductive Performance In Females- Egypt. *The Internet Journal of Toxicology*. Volume 6 Number.
6. Aruke, A., Grotmol, T., Haugen, T.B., Knudsen, F.R. and GoksØyr, A. (1999). Fish model for assessing the *in vivo* estrogenic potency of the mycotoxin zearalenone and its metabolites. *Sci Total Environ* 236, 153-161.
7. Bailey, G.S., Dashwood, R., Loveland, P.M., Pereira, C. and Hendricks, J.D. (1998). Molecular dosimetry in fish: quantitative target organ DNA adduction and hepatocarcinogenicity for four aflatoxins by two exposure routes in rainbow trout. *Mutat Res* 399, 233-244.
8. Manning, B.B., Li, M.H., Robinson,E.H., Gaunt, P.S.,Camus A.C., and Rottinghaus G.E. (2003). Response of channel catfish to diets containing T-2 toxin. *J. Aquatic Animal Health* 15, 229-238.
9. Carlson, D.B., Williams, D.E., Spitsbergen, J.M., Ross, P.F., Bacon, C.W., Meredith, F.I. and Riley, R.T. (2001). Fumonisin B1 promotes aflatoxin B1 and N-methyl-N'-nitro-nitrosoguanidine-initiated liver tumors in rainbow trout. *Toxicol Appl Pharmacol* 172, 29-36.
10. Armbrecht, B.H. (1972). Aflatoxin residues in food and feed derived from plant and animal source. *Residue Rev.* 41, 13-54.
11. Lee, D.J., R.O., Sinnhuber, J.H., Wales and G.B. Putnam (1978). Effect of dietary protein on the response of rainbow trout (Salmo gairdneri) to aflatoxin B1. *J Natl Cancer Inst* 60, 317-320.
12. 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. Science* 10, 39-47.
13. Ottinger, C.A. and S.L. Kaattari (2000). Long-term immune dysfuntion in rainbow trout (*Oncorhynchusmykiss*) exposed as embryos to aflatoxinB1. *Fish & Shellfish Immun* 10, 101-106.
14. Horvath, E.M. (1998). Taking the threat out of mycotoxins. *Feed Tech* 2, 32-33.
15. Marzouk, M.S., M.M. Bashandi, R. El-Banna, M. Moustafa and M.A. Eissa (1994). Hematological studies on *Oreochromisniloticus* exposed to chronic dietary aflatoxicosis. Egypt. *J. Comp. Pathol. Clin.Pathol.* 7, 497-504.
16. Tuan, N.A., Manning, B.B., Lovell, R.T. and Rottinghaus, G.E. (2002). Response of Nile tilapia (*Oreochromisniloticus*) fed diets containing different concentrations of moniliformin or fumonisin B1. *Aquaculture* 217, 515-528.
17. Chavez-Sanchez, M.C., Martinez-Palacios, C.A., Osorio–Mareno I., Palacios, C.A.M. and Moreno, I.O. (1994). Pathological effects of feeding young *Oreochromisniloticus* diets supplemented with different levels of aflatoxin B1. *Aquaculture* 127, 49-60.
18. Pietri, A., T. Bertuzzi, G. Piva, E.M. Binder, D. Schatzmayr and I. Rodrigues. (2009). Aflatoxin transfer from naturally Ccontaminated feed to milk of dairy cows and the efficacy of a mycotoxin deactivating product. *Int. J. Dairy Sci.* 4.
19. Lim, H-A., Ng, W-K., Lim, S-L. and Ibrahim, C.O. (2001). Contamination of palm kernel meal with Aspergillusflavus affects its nutritive value in pelleted feed for tilapia, *Oreochromismossambicus*. *Aquaculture Research* 32, 895-905.
20. Schazmayr, G., Biomin Ian Gmbh, Herzogenburg, (2004). Types and characteristics of mycotoxin and and the countermeasures. Damage of domestic animals due to the mycotoxin of feed and its prevention. *Sust. Livestock Prod. Human Welf.* 58, 1087-1092.
21. Phillips, T.D., Clement, B.A., Kubena, L.F., Harvey, R.B. (1990). Detection and detoxification of aflatoxins: prevention of aflatoxicosis and aflatoxin residues with hydrated sodium calcium aluminosilicate. *J. Veterinary and human toxicology Vet Hum Toxicol*, Suppl 32, 15-19.
22. Sean, E., Matson, Christopher, J., Langdon and Sanford Evansn (2006). Specific pathogen free culture of the Pacific oyster (*Crassostreagigas*) in a breeding research program: Effect of water treatment on growth and survival. *Aquaculture* 253, 475-484.
23. Winfree, R., A. and Anilaallred. (1992). BentoniteReduces, Measurable Aflatoxin B1 in Fish Feed The Progressive Fish-Culturist 54, 157-162.
24. Ellis, R.W., Clements, M., Tibbetts, A. and Winfree, R. (2000). Reduction of the bioavailability of 20 µg/kg aflatoxin in trout feed containing clay. *Aquaculture* 183, 179-188.
25. Abdelhamid, A.M. (2000). Fungi and Fungal Toxins. DarAlnashr for Universities–Cairo, Deposit No. 13738/97 (InArabic).
26. Goldblatt, L.A. (1976). Significance of aflatoxin in foods. Proc. 80th Annual Conference of the Association of Food and Drug Officials, Atlanta, Georgia June 22, 191-201.
27. Lovell, R.T. (1992). Mycotoxins: Hazardous to farmed fish. Feed International March, 22-28.
28. Zhang, Q., Suorsa-Super, K. and Curtis, L.R. (1992). Temperature-modulated aflatoxinB1 hepatic disposition and formation and persistence of DNA adducts in rainbow trout. *Toxic.& Appl. Pharmac.* 113, 253-259.
29. Omar, E., T. Srour and A. Nour. (1996). Effect of aflatoxin contaminated feeds on some freshwater fishes. Proc. Conf. Food borne Contamination & Egyptian's Health, Mansoura. Nov.26-27, 71-84.
30. Manning, B.B., R.M. Ulloa, M.H.Li, E.H. Robinson and G.E. Rottinghaus. (2003). Ochratoxin A fed to channel catfish (*Ictaluruspunctatus*) causes reduced growth and lesions of hepatopancreatic tissue. *Aquaculture* 219.
31. Srour, T.M. (2004). Effect of ochratoxin-A with or without Biogen® on growth performance, feed utilization and carcass composition of Nile tilapia (*Oreochromisniloticus*) fingerlings. *J. Agric. Sci. Mansoura Univ.* 29, 51-61.
32. De silva, S.S. and Anderson, T.A. (1995). "Fish Nutrition in Aquaculture." Ed., Chapman and Hall, 2-6 Boundary Row, London SEI 8 FIN, UK.
33. Trucksess, M.W., Stack, M.E., Nesheim, S., Page, S.W., Albert, R.H., Hansen, J.T. and Donahue, K.F. (1991). Immunoaffinity column coupled with solution fluorometry of LC post column derivatization for aflatoxins in corn, peanuts and peanut butter: Collaborative study. J.A.O.A.C. 74:81.
34. Lucky, Z. (1977). "Methods for the diagnosis of fish diseases." Amerind Publ. Co. Pv Ltd., New York, 1stEd.
35. Austin, B. and Austin, D.A. (2007). Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. Chichester: Praxis Publishing Ltd.
36. Annet, C.S. (1985). "A model to facilitate optimal aquaculture production by quantitatively relating fish growth to feed and other environmental resources." Ph.D. Thesis, Michigan State University, USA.
37. Pouomonge, V. and Mbonglang, J. (1993). "Effect of feeding rate on the growth of tilapia (*Oreochromis niloticus*) in earthen ponds." Bamidegh 45, 147-153.