



Journal of
**Fisheries and
Aquatic Science**

ISSN 1816-4927



Academic
Journals Inc.

www.academicjournals.com

Effect of Biogen® and Myco-Ad® on the Growth Performance of Common Carp (*Cyprinus carpio*) Fed a Mycotoxin Contaminated Aquafeed

¹H.M. Agouz and ²W. Anwer

¹Central Laboratory of Agriculture Research, Abbassa, Sharkia, Agricultural Research Center, Egypt

²Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt

Corresponding Author: H.M. Agouz, Central Laboratory of Agriculture Research, Abbassa, Sharkia, Agricultural Research Center, Egypt

ABSTRACT

A commercial probiotic (Biogen®) was tested against a commercial mycotoxin binder (Myco-Ad®) for their effects on survival, growth, body composition and hematological picture of common carp (*cyprinus carpio*) fed a naturally contaminated aquafeed with aflatoxin and ochratoxin. A total number of 150 apparently healthy fingerlings common carp *cyprinus carpio* were divided into 5 triplicate groups. G-1 (control) received naturally ration found contaminated with aflatoxin (22 ppb) and ochratoxin (15 ppb). The other four groups supplemented with Biogen® at a rate of 0.2 and 0.4% (G-2 and G-3) and Myco-Ad® at a rate of 0.15 and 0.25% (G-4 and G-5). Results showed a significant reduction ($p \leq 0.05$) in aflatoxin recorded in aquafeeds of G-2 and G-3, while ochratoxin level showed a significant reduction in G-3. The four groups received Biogen® and Myco-Ad® showed a significant improve in the final weight, feed intake, FCR, survival rate, protein efficiency, body composition represented an increase in crude protein and reduction of ether extract and also improve hematological picture represented in erythrocyte counts, hematocrite and hemoglobin. It can be concluded that aflatoxin and ochratoxin contamination of fish diets can cause many drastic effects on performance parameters, feed utilization, hematological picture and body composition of common carp. Hence, there is always a demand for risk assessment regarding mycotoxins especially with the moving to plant protein sources in the aquafeeds. Commercial probiotic Biogen® at a level of 0.4 and 0.2% has a good improvement effect among common carp fed mycotoxin contaminated aquafeed, followed by the commercial mycotoxin binder Myco-Ad® at a level of 0.25 and 0.15%.

Key words: Mycotoxin, common carp, aquaculture, biogen, mycoad

INTRODUCTION

The first documented incidences of aflatoxicosis affecting fish health occurred in the 1960s in trout hatcheries where, rainbow trout were fed a pelleted feed prepared with cottonseed meal contaminated with aflatoxins, developed liver tumors (Motalebi *et al.*, 2008). Interest in the toxic effects on cultured warm-water fishes, such as tilapia and channel catfish, has increased as diets for these species are now being formulated to contain more plant and less animal ingredients. This

increases the potential for development of aflatoxicosis in these species because, as noted earlier, plant ingredients have a higher potential than animal ingredients for contamination with aflatoxins (Royes and Yanong, 2008).

Mycotoxins cause a wide variety of adverse clinical signs among fishes depending on the nature and concentration of mycotoxins present, duration of exposure, the fish species, its age, nutritional and health status at the time of exposure to contaminated feed (Tuan *et al.*, 2002). Jantrarotai and Lovell (1990) and Lovell (1992) showed that rainbow trout are extremely sensitive to AFB₁, while channel catfish are much less responsive. Even the production systems of aquaculture found affect the tolerance levels for tilapia. In green water and flow-through systems, the presence of aflatoxins at 25 to 30 parts per billion (ppb) in the water decreased growth without any noticeable signs of mortality. However, in cage culture, concentrations of aflatoxins above 5 ppb in the water caused an increase in mortality rates (Royes and Yanong, 2008).

Mycotoxins have been shown to negatively affect production, growth and immune system function among different aquaculture species (Lee *et al.*, 1978; Mahmoud *et al.*, 1994; Bautista *et al.*, 1994; Marzouk *et al.*, 1994; Chavez-Sanchez *et al.*, 1994; Abdelhamid *et al.*, 1998; Bailey *et al.*, 1998; Horvath, 1998; Aruke *et al.*, 1999; Ottinger and Kaattari, 2000; Lim and Webster, 2001; Manning *et al.*, 2003). Also, Carlson *et al.* (2001) mentioned that mycotoxins act synergistically so that the negative effects of two mycotoxins are worse than the effects of each individually.

Probiotics are pure cultures of one or more living microorganisms given in feed that proliferates in the host gastrointestinal (GI) tract. They ensure that the host maintains a beneficial microbial population in the GI tract (Linge, 2005). The research of probiotics for aquatic animals is increasing with the demand for environment-friendly aquaculture (Wang *et al.*, 2008). Most probiotics have been undertaken by isolating and selecting strains from aquatic environment (Gatesoupe, 1999). Also, probiotics have found use in aquaculture as a means of disease control, supplementing or even in some cases replacing the use of antimicrobial compounds (Irianto and Austin, 2002; Sahu *et al.*, 2008).

Many physical, chemical and biological techniques to neutralize mycotoxins have been developed and were reported in the literature throughout the years (Doyle *et al.*, 1982; Samarajeewa *et al.*, 1990). Binders have been used to neutralise the effects of mycotoxins by preventing their absorption from the animal's digestive tract through bind toxins by adhesion or by electrostatic charge or cation exchange capacity. The effectiveness of smectite clays (HSCAS) as adsorbents of aflatoxin has been investigated and found successful for many farm animals over the past 20 years (Grim, 1962; Bluthgen and Schwertfeger, 2000; CAST, 2003; Dixon *et al.*, 2008).

Therefore, the aim of this paper is to study the effect of feeding a naturally fish diets found contaminated with aflatoxin and ochratoxin on the performance and health status of common carp (*Cyprinus carpio*) fingerlings and a trial to control such effect through supplementation of Biogen[®] (probiotic-immunostimulant) and Myco-Ad[®] (smectite clay, HSCAS for mycotoxin binding) in this contaminated diet.

MATERIALS AND METHODS

This study was carried out in the Central Laboratory of Agriculture Research, Abbassa, Sharkia governorate, Agricultural Research center, Ministry of Agriculture, Egypt during summer season of 2009.

Experimental fish and management: A total number of 150 apparently healthy fingerlings of common carp *Cyprinus carpio* obtained from the hatchery of Agriculture Research at Abbassa, Sharkia, Egypt. The fish maintained in glass aquaria (each of 70×50×40 cm) filled with dechlorinated tap water which continuously aerated. They divided into 5 triplicate groups (10 fingerlings/aquarium) and named G-1 for control group and from G-2 to G-5 for the other four experimental groups. They were acclimatized to the laboratory conditions for 2 weeks before starting the experiment. The water temperature was kept at 25±2°C throughout the experiment. About half of the water was changed daily in all the experimental aquaria. The average weight of fingerlings was 15 gram/fish at the start of the experiment. The fingerlings weighted every 2 weeks and fed the experimental diets at 3% of the total biomass along the period of the experiment (90 days). The feeding rate was adjusted according to the last fish weight.

Aquafeeds and feeding regimes: A naturally ration found contaminated with aflatoxin (22 ppb) and ochratoxin (15 ppb) divided into five experimental diets. The first part used as a control (G-1). The other four parts supplemented with Biogen® (China-Way Corp; Taiwan) at a rate of 0.2% (G-2) and 0.4% (G-3) and Myco-Ad® (Special Nutrients, Inc. USA) at a rate of 0.15% (G-4) and 0.25% (G-5). The five experimental diets formulated to contain about 30% cp and 4700 GE Kcal kg⁻¹ (Table 1). Each diet mechanically mixed, pressure pelleted by using meat mincer, air dried at room temperature, broken into small pieces, sieved to obtain appropriate size and stored at -5°C. The experimental fishes received the tested diets twice daily at 8 a.m. and 2 p.m.

Biogen®: A synthetic probiotic immunostimulant product from China Way Corp; Taiwan. It contains allicin (garlic extract) not less than 0.247 micromil g⁻¹, *Bacillus subtilis* nato 6×10⁷ cells g⁻¹, high-unit hydrolytic enzymes 3690 units g⁻¹ (proteolytic, lipolytic, amylolytic and cell separating enzymes), germanium (Ginseng extract 41.98 ppm) and organic selenium.

Myco-Ad®: Commercial smectite clay composed of an activated, broad spectrum, hydrated, sodium/calcium aluminosilicate (HSCAS) from Special Nutrients, Inc., USA specially formulated to adsorb major mycotoxins.

Determination of the levels of aflatoxins and ochratoxins in aquafeed samples: A stored aquafeed, suspected to be contaminated with mycotoxins, was subjected to determine the presence of aflatoxin and ochratoxin, after feed supplementation with Biogen and Myco-Ad, using immunoaffinity method which is applicable for mycotoxins that have fluorescence (Trucksess *et al.*, 1991). Series-4 Fluorometer (VICAM) was used in this procedure. Reading of total aflatoxin or ochratoxin was obtained as part per billion (ppb = µg kg⁻¹).

Water quality: Water parameters (temperature, pH, salinity and dissolved oxygen) were measured twice daily (10 a.m. and 10 p.m.). Dissolved oxygen measured by oxygen meter as mg L⁻¹. (yellow spring Instrument Co. model 57). Water samples were collected weekly from aquaria to detect total ammonia (ionized and non-ionized) and total hardness. Ammonia measured by Direct-Nesslerization method, while Total hardness measured by the EDTA-tetrimetric method (APHA, *et al.*, 1989).

Table 1: Composition and chemical analysis of the experimental aquafeeds

Composition of the Aquafeeds	Control (G-1)	Biogen® 0.2% (G-2)	Biogen® 0.4% (G-3)	Myco-Ad® 0.15% (G-4)	Myco-Ad® 0.25% (G-5)
Yellow corn meal (7.5 CP %)	44	44	44	44	44
Soybean meal (44 CP %)	23	23	23	23	23
Fish meal (65 CP %)	26	26	26	26	26
Cotton seed oil	5	5	5	5	5
Vitamin and Minerals	2	2	2	2	2
Total	100	100	100	100	100
Chemical analysis:					
Dry Matter (DM %)	84.32	83.75	82.71	83.41	83.52
Crude Protein (CP %)	30.32	30.65	30.72	30.35	30.18
Ether Extract (EE)	9.30	8.78	8.20	9.54	9.33
Crude Fiber (CF %)	5.47	5.37	5.34	5.57	5.32
Ash (%)	7.88	7.32	7.02	7.93	8.31
NFE ²	47.03	47.38	48.72	46.61	46.86
Gross energy ³ (kcal kg ⁻¹)	4765.00	4763.00	4767.00	4753.00	4745.00

Performance parameters:

- **Body weight gain (W.G.):** Total weight determined to the nearest gram according to (Annet, 1985)
- **Specific Growth Rate (SGR%/d):** It was calculated as suggested by Pouomonge and Mbonglang (1993)
- **Feed Conversion Ratio (FCR):** FCR determined according to DeSilva and Anderson (1995)[feed intake (g)/body weight gain (g)]
- **Protein Efficiency Ratio (PER):** PER determined according to DeSilva and Anderson (1995)

Hematological investigation: At the end of the experiment blood samples were collected from the fish caudal peduncle of the different groups. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of:

- Total erythrocytes (RBCs) and total leucocytes (WBCs) counted on an A₀ Bright-Line Haemocytometer model (Neubauer improved, Precicolor HBG, Germany) according to Dacie and Lewis (1995)
- The packed cell volume carried out in small hematocrite graduated tubes using Hematocrite centrifuge at 3000 rpm for 15 min
- Hemoglobin (Hb) estimated by Boehringer munnheim kit according to Wintrobe (1965)

Statistical analysis: Data listed, computed and analyzed using Analysis of Variance (ANOVA) and differences between means (Duncan, 1955). Multiple range test was done to determine differences between treatment (mean at significance level of (p<0.05). Standard errors were also estimated. All analyses were run on the computer using the SAS program.

RESULTS

The examined aquafeed samples as shown in Table 2, found contaminated with a mean value of 22 ppb for aflatoxin and 15 ppb for ochratoxin. A significant reduction (p<0.05) in aflatoxin found in G-2 and G-3 groups, while ochratoxin reduced in G-3. No significant reduction in both

Table 2: Mean values of mycotoxins detected in the examined aquafeed samples

Aquafeed samples (n = 10)	Aflatoxin ($\mu\text{g kg}^{-1}$)	Ochratoxin ($\mu\text{g kg}^{-1}$)
G-1 (Control)	22 ^a	15 ^a
G-2 (Biogen® 0.2%)	12 ^b	14 ^a
G-3 (Biogen® 0.4%)	9 ^c	6 ^b
G-4 (Mycos-Ad® 0.15%)	21 ^a	13 ^a
G-5 (Mycos-Ad® 0.25%)	21 ^a	15 ^a

a-c: Means in the same column with different superscripts are significantly ($p \leq 0.05$) different

Table 3: Water quality parameters

Aquaria groups	DO (mg L^{-1})	Salinity (%)	pH	Temp. ($^{\circ}\text{C}$)	Total ammonia (mg L^{-1})	T.H. (mg L^{-1})
G-1	5.6±0.81	0.50 ^b ±0.10	7.8±0.11	26.0±0.99	0.06 ^c ±0.00	125 ^a ±15
G-2	5.31±1.11	1.4 ^a ±0.07	7.7±1.21	26.22±2.21	1.00 ^a ±0.001	440 ^a ±25
G-3	5.56±1.05	1.4 ^a ±0.06	7.3±1.0	26.35±1.91	1.00 ^a ±0.00	440 ^a ±27
G-4	5.50±0.91	1.5 ^a ±0.10	7.0±0.55	26.22±1.15	0.08 ^b ±0.00	270 ^b ±40
G-5	5.70±0.17	1.5 ^a ±0.11	7.0±0.61	26.12±1.00	0.08 ^b ±0.01	290 ^b ±35

a-c: Means in the same column with different superscripts are significantly ($p \leq 0.05$) different, Values are presented as Mean±SE

Table 4: Growth performance and feed utilization of common carp fingerlings

	G-1	G-2	G-3	G-4	G-5
Initial weight (g/fish)	15.30±0.17	15.29±0.04	15.33±0.01	15.40±0.15	15.29±0.11
Final weight (g/fish)	48.25±2.01 ^e	55.08±2.12 ^b	58.37±2.10 ^b	51.71±1.98 ^d	53.55±1.08 ^c
Total weight gain (g/fish)	32.95±1.00 ^c	39.79±1.39 ^b	43.04±1.79 ^a	36.31±1.10 ^d	38.26±1.55 ^c
SGR (%/d)	1.36±0.22 ^c	1.44±0.09 ^a	1.48±0.11 ^a	1.40±0.18 ^b	1.42±0.16 ^b
Feed intake (g/fish)	83.36±1.00 ^a	56.10±2.35 ^d	71.87±1.72 ^b	67.89±1.55 ^c	71.92±1.19 ^b
FCR	2.53±0.11 ^a	1.41±0.07 ^d	1.67±0.03 ^c	1.87±0.12 ^b	1.88±0.13 ^b
PER	1.518±0.09 ^b	1.574±0.09 ^b	2.157±0.11 ^a	1.645±0.10 ^b	1.857±1.03 ^a
Survival rate%	88	95.45.00	96.47.00	92.83.00	93.77.00

a-d: Means in the same row with different superscripts are significantly ($p \leq 0.05$) different, Values are presented as Mean±SE

Table 5: Body composition at the end of the experiment for common carp fingerlings

	DM (%)	CP (%)	EE (%)	ASH (%)
Initial body composition	30.21	53.72	18.42	15.41
Final body composition				
G-1	33.11±0.57	51.02±0.33 ^c	32.15±0.05 ^a	17.83±0.26
G-2	32.34±0.37	55.13±0.14 ^a	28.12±0.97 ^c	17.75±1.23
G-3	33.07±0.22	56.78±0.09 ^a	27.98±0.58 ^c	17.10±0.92
G-4	33.00±0.87	53.11±0.56 ^b	29.50±0.19 ^b	17.25±0.37
G-5	32.15±0.74	53.00±0.79 ^b	29.00±0.09 ^b	18.00±0.15

a-c: Means in the same column with different superscripts are significantly ($p \leq 0.05$) different, Values are presented as Mean±SE

Table 6: Hematological parameters from common carp fingerlings

Parameters	RBC ($10^6/\text{mm}^3$)	WBC ($10^3/\text{mm}^3$)	Hematocrite	HB (g/100 mL)
G-1	1.1±0.01 ^c	12.00±0.75 ^a	11.23±1.05 ^c	5.10±0.44 ^c
G-2	1.4±0.001 ^a	9.11±0.21 ^b	16.97±2.10 ^a	7.21±0.26 ^a
G-3	1.5±0.001 ^a	8.71±0.10 ^c	18.00±1.81 ^a	8.10±0.19 ^a
G-4	1.30±0.001 ^b	10.0±0.23 ^b	14.12±0.23 ^b	6.31±0.35 ^b
G-5	1.2±0.001 ^b	9.51±0.57 ^b	14.11±0.73 ^b	6.37±0.39 ^b

a-c: Means in the same column with different superscripts are significantly ($p \leq 0.05$) different, Values are presented as Mean±SE

aflatoxin and ochratoxin recorded in G-4 and G-5 groups. Table 3 illustrated that dissolved oxygen, pH and temperature estimated in all aquaria showed nearly the same readings along the 90 days of the experiment. Salinity increased in all treated groups (from G-2 to G-5). Ammonia was highest in G-2 and 3 (1 mg L^{-1}), followed by 0.08 mg L^{-1} in G-4 and 5. Also, total hardness showed significant increase in G-2 and 3 (about 440 mg L^{-1}), followed by G-4 and 5 (270 and 290 mg L^{-1}).

Results in Table 4, showed a significant improvement in the final weight of cultured fish in G-2 and G-3 (58.37 and $55.08 \text{ g fish}^{-1}$) and followed by G-5 and G-4 (53.55 and 51.7 g fish^{-1}). This improvement in final weight reflected on the total weight gain and specific growth rate which improved by the same supplements. A significant reduction in feed intake and consequently in FCR recorded in G-3 and G-4, followed by G-2 and G-5. Protein efficiency significantly improved in G-3 and G-5, Followed by G-4 and G-2. Survival rate improved in the 4 groups of supplemented feed in comparison with G-1. In Table 5, all treated groups (from G-2 to G-5) showed improvement in final body composition as the increase in crude protein from about 51% in control to the maximum value (56.78) in G-3. Ether extract reduced in fish bodies of treated groups.

Results in Table 6, showed improvement in erythrocyte counts, hematocrite and hemoglobin in all treated groups (from G-2 to G-5), white blood cells which rose as an immune response in control group, showed a significant reduction in all treated groups (from G-2 to G-5).

DISCUSSION

The natural contamination of aquafeeds with aflatoxin (22 ppb) and ochratoxin (15 ppb) indicate the demand for risk assessment regarding mycotoxins especially with the moving to plant protein sources in aquafeeds (Ali *et al.*, 1998; Lim *et al.*, 2001; Tacon, 2004).

In Egypt, feeds may expose to bad hygienic condition, during transportation and storage from wetting condition, insects infestation besides the long period of storage leading to the feed becoming mouldy and contaminated with mycotoxins.

Although, the little research studies on mycotoxins and mycotoxicoses in cultured fish and still absence of definite regulation for mycotoxin levels of aquafeeds (single or multi-mycotoxin contamination), there is studies on the effect of mycotoxins on aquaculture which ascertained the deleterious effect on fish health, immunity and consequently on their performance (Bailey *et al.*, 1998; Aruke *et al.*, 1999; Lim and Webster, 2001; Sahoo and Mukherjee, 2001; Tuan *et al.*, 2002, 2003; Abdelhamid *et al.*, 2004a, b; 2002b, c; Salem, 2002; Manning *et al.*, 2003).

The significant reduction of aflatoxin in G-2 and G-3 and also the reduction of ochratoxin in G-3, may be attributed to the presence of garlic extract (allicin) and the presence of *Bacillus subtilis* in component of Biogen[®], both are known that they have the characteristics of mould inhibition and mycotoxin detoxification (Azzouz and Bullerman, 1982; Yin and Cheng, 1998; Galvano *et al.*, 2001; Petchkongkaew, 2007). This finding disagrees with Abdelhamid *et al.* (2002b) who found that dietary Biogen[®] supplementation was not useful in AFB₁ detoxification.

The values of water parameters recorded in this study are within the acceptable ranges recommended for fish culture (APHA *et al.*, 1989; Boyd and Tucker, 1993; Chapman, 2000; Abdelhamid, 2009). The total concentration of all ions in the water is its salinity. The increase in salinity in Biogen[®] and Myco-Ad[®] groups may be attributed to the content of feed additives from minerals and salts. The increase in ammonia in Biogen[®] and Myco-Ad[®] groups may be attributed to the increase in feed intake and fish growth rates, which consequently reflected on the increase of the nitrogenous wastes.

An acceptable range for free calcium in culture waters is 25-100 mg L⁻¹ or 63-250 mg L⁻¹ CaCO₃ hardness (Wurts, 1989). Calcium has an important role in the biological processes of fish. Fish can absorb calcium directly from the water or food. The increase in total hardness in Biogen® and Myco-Ad® groups may be attributed to the content of feed additives from minerals and salts.

Biogen® at the recommended level of 0.4% and even the half of that level (0.2%) improved the performance parameters and feed utilization of the common carp fingerlings which are being stressed from feeding on mycotoxin contaminated aquafeed. The explanation of this finding may be due to that the commercial probiotic Biogen® consists of *Bacillus licheniformis* and *Bacillus subtilis*. These spore-forming bacteria which survive the pelletization process, can enhance the metabolism and energy of fish body cells, raise the efficiency of feed utilization and balance the secretion of various secretory glands. Moreover, it increases the vitality of cells by supplying oxygen to whole body, improves the immune responses, helps to excrete heavy metals, inhibits aflatoxin and maintains the normal endocrine system. Biogen® has bactericidal effects and increases the palatability of feed, promotes the secretion of digestive fluids and stimulates the appetite (EL-Dakar *et al.*, 2007; Diab *et al.*, 2008; Eid and Mohamed, 2008; Mehrim, 2010).

Also, Myco-Ad® comes after Biogen® in the improvement of performance parameters and feed utilization of the cultured common carp fed mycotoxin contaminated ration. This result may be attributed to the composition of Myco-Ad® which is hydrated sodium calcium aluminosilicates (HSCAS) which capable to the alleviation of mycotoxicosis through adsorption capacity of mycotoxins (Barrer, 1989; Mumpton, 1999). Phillips *et al.* (1990) interpreted the binding mechanism as the formation of a complex by the β-carbonyl system of the aflatoxin with 'uncoordinated edge site' aluminium ions. Thus, HSCAS can be used as an 'inorganic sponge' sequestering aflatoxins in the gastro-intestinal tract of farm animals. Regarding the applicability of aluminosilicates for the binding of mycotoxins, it can be concluded that they are very effective in preventing aflatoxicosis, but their efficacy against zearalenone, ochratoxin and trichothecenes is limited (Huwig *et al.*, 2001). This finding disagrees with Abdelhamid *et al.* (2002a) who mentioned that mycotoxin adsorbents did not significantly reduce the aflatoxicity.

Survival rate in control group was lower (88%) compared with the other groups supplemented with Biogen® and Myco-Ad® (about 96 and 93%, respectively). The sensitivity to mycotoxins varies and the exact susceptibility of different fish species could not be determined up till now as described by Lovell (1992) who found that warm water fish such as channel catfish are reported to be less sensitive to aflatoxin than rainbow trout.

In practical terms, the improvement in performance and feed utilization parameters means that the use of probiotic, Biogen® and the mycotoxin binder, Myco-Ad® in case of feeding carp fish a mycotoxin contaminated ration can decrease the amount of feed necessary for animal growth which could result in reductions of production cost.

The improvement in body composition of carp fish in groups supplemented with Biogen® as found by Khattab *et al.* (2004b) and also groups supplied with Myco-Ad® in the ration contaminated with mycotoxin, is a significant evidence of the improvement in general health condition of the cultured fish. These positive effects in body composition of experimental fish may be due to the dietary supplementation with Biogen® which caused the good growth performance, enhance the metabolism and energy of fish body cells and raise the efficiency of feeds (Mehrim, 2001). Also, these results are in close agreement with Srour (2004), EL-Haroun *et al.* (2006) and Mohamed *et al.* (2007) for tilapia and EL-Haroun (2007) for catfish. Moreover, Eid and

Mohamed (2008) found that no statistical differences were observed in whole body moisture, crude protein, ether extract and ash of mono-sex *O. niloticus* fingerlings fed diets containing different levels of commercial feed additives (Biogen® and Pronifer®).

Regarding the results from G-4 and G-5, the improvement in body composition is a good indicator for the efficiency of Myco-Ad® containing HSCAS against aflatoxin and ochratoxin to alleviate body from their effect. This finding agree with Zaki *et al.* (2008) who mentioned that dietary HSCAS clay act as an aflatoxin enterosorbant that tightly and selectively binds the toxin in the gastrointestinal tract of fish, so that diminishing the clinical signs of aflatoxicosis.

Improvement in erythrocyte count, hematocrite and hemoglobin in groups supplemented with Biogen® agreed with the findings of Khattab *et al.* (2004a), EL-Gohary *et al.* (2005) and Mehrim (2010). White blood cells which raised as an immune response in control group showed a significant reduction in treated groups with Biogen® and Myco-Ad® and this finding disagree with Marzouk *et al.* (2008) who found increases in RBCs count, Hb value, PCV%, WBCs count and differential of leukocytic count in the two fish groups fed the diets supplemented with probiotics (dead *Saccharomyces cerevisiae* yeast and both of live *Bacillus subtilis* and *Saccharomyces cerevisiae*). On the other hand, Abdelhamid *et al.* (2002b) found that Biogen® reduced blood hemoglobin of aflatoxicated *O. niloticus* fish.

CONCLUSION

From the foregoing results it could be concluded that aflatoxin and ochratoxin contamination of fish diets can cause many drastic effects on performance parameters, feed utilization, hematological picture and body composition of common carp. Hence, there is always a demand for risk assessment regarding mycotoxins especially with the moving to plant protein sources in the aquafeeds. Commercial probiotic Biogen® at a level of 0.4 and 0.2% has a good improvement effect among common carp fed mycotoxin contaminated aquafeed, followed by the commercial mycotoxin binder Myco-Ad® at a level of 0.25 and 0.15% which also can alleviate the effect of mycotoxin. The detoxification of aflatoxin and ochratoxin recorded after the addition of Biogen® need more research to investigate this result deeply.

REFERENCES

- APHA., AWWA. and WPCF., 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edn., American Public Health Association, Washington, DC., pp: 423-427.
- Abdelhamid, A.M., F.F. Khalil and M.A. Ragab, 1998. Problem of mycotoxins in fish production. Egypt. J. Nut. Feeds, 1: 63-71.
- Abdelhamid, A.M., A.M. Ahmed and K.M. El-Meleigy, 2002a. Detoxification of aflatoxins-contaminated diet by some physical and chemical means. J. Agric. Sci. Mansoura Univ., 27: 8213-8224.
- Abdelhamid, A.M., F.F. Khalil, M.I. El-Barbary, V.H. Zaki and H.S. Hussein, 2002b. Feeding Nile tilapia on Biogene to detoxify aflatoxic diets. Proceedings of the 1st Conference on Animal and Fish Production, Sept. 24-25, Mansoura, pp: 207-230.
- Abdelhamid, A.M., F.I. Magouz, M.F.E. Salem, A.A. Mohamed and M.K. Mohsen, 2002c. Effect of graded levels of aflatoxin B1 on growth performance and biochemical, chromosomal and histological behaviour of Nile tilapia *Oreochromis niloticus*. Proceeding of the 1st Conference Animal and Fish Production, Sept. 24-25, Mansoura, pp: 231-250.

- Abdelhamid, A.M., A.E. Abdel-Khalek, A.I. Mehrim and F.F. Khalil, 2004a. 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). 2-On clinical, blood and histological parameters. *J. Agric. Sci. Mansoura Univ.*, 29: 6175-6196.
- Abdelhamid, A.M., A.I. Mehrim and F.F. Khalil, 2004b. Detoxification of aflatoxin- contaminated diet of tilapia fish using dietary supplementation with egg shell Betafin, clay or silica. *J. Agric. Sci. Mansoura Univ.*, 29: 3163-3174.
- Abdelhamid, A.M., 2009. *Fundamentals of Fish Production and Culture*. New Universal Office, Alexandria.
- Ali, N., Y.A. Sardjono and T. Yoshizawa, 1998. Natural co-occurrence of aflatoxins and *Fusarium mycotoxins* (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia. *Food Addit. Contam.*, 15: 377-384.
- 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.
- Aruke, A., T. Grotmol, T.B. Haugen, F.R. Knudsen and A. Goksoyr, 1999. Fish model for assessing the *in vivo* estrogenic potency of the mycotoxin zearalenone and its metabolites. *Sci. Total Environ.*, 236: 153-161.
- Azzouz, M.A. and L.B. Bullerman, 1982. Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. *J. Food Prot.*, 45: 1298-1301.
- Bailey, G.S., R. Dashwood, P.M. Loveland, C. Pereira and J.D. Hendricks, 1998. Molecular dosimetry in fish: Quantitative target organ DNA adduction and hepatocarcinogenicity for four aflatoxins by two exposure routes in rainbow trout. *Mutati. Res.*, 399: 223-244.
- Barrer, R.M., 1989. Shape-selective sorbents based on clay minerals-a review. *Clays Clay Miner.*, 37: 385-395.
- Bautista, M.N., C.R. Lavilla-Pitogo, P.F. Subosa and E.T. Begino, 1994. Aflatoxin B1 contamination of shrimp feeds and its effect on growth and hepatopancreas and pre-adult *Penaeus monodon*. *J. Sci. Food Agri.*, 65: 5-11.
- Blüthgen, A. and M. Schwertfeger, 2000. Excretion of aflatoxin M1 in milk of lactating cows after simultaneous Zufütterung adsorptive additives and aflatoxin B1 *in vivo* and *in vitro* experiments. *Kiel Me Econ. Res. Rep.*, 52: 145-164.
- Boyd, C.E. and C.S. Tucker, 1993. *Water Quality and Pond Soil Analysis for Aquaculture*. Auburn University, Alabama, ISBN-13: 978-0817307219, pp: 183.
- CAST (Council for Agricultural Science and Technology), 2003. *Mycotoxins: Risks in plant, animal and human systems*. Ames, IA, 2003. Task Force Report, pp: 139.
- Carlson, D.B., D.E. Williams, J.M. Spitsbergen, P.F. Ross, C.W. Bacon, F.I. Meredith and R.T. Riley, 2001. Fumonisin B1 promotes aflatoxin B1 and n-methyl-n-nitro-nitrosoguanidine-initiated liver tumors in rainbow trout. *Toxicol. Applied Pharmacol.*, 172: 29-36.
- Chapman, F.A., 2000. *Farm-Raised Channel Catfish*. Department of Fisheries and Aquatic Sciences, Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.
- Chavez-Sanchez, M.C., C.A. Martinez-Palacios, I. Osorio-Mareno, C.A.M. Palacios and I.O. Moreno, 1994. Pathological effects of feeding young *Oreochromis niloticus* diets supplemented with different levels of aflatoxin. *Aquaculture*, 127: 49-61.

- Dacie, J.V. and S.M. Lewis, 1995. Practical Haematology. 8th Edn., Churchill Livingstone, Edinburgh, Scotland.
- DeSilva, S.S. and T.A. Anderson, 1995. Fish Nutrition in Aquaculture. Chapman and Hall, London, pp: 319.
- Diab, A.S., S.M. Aly, G. John, Y. Abde-Hadi and M.F. Mohammed, 2008. Effect of garlic, black seed and Biogen as immunostimulants on the growth and survival of Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae) and their response to artificial infection with *Pseudomonas fluorescens*. Afr. J. Aquatic Sci., 33: 63-68.
- Dixon, J.B., I. Kannevischer, M.G.T. Arvide and A.L.B. Velazquez, 2008. Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: An introductory plan. Applied Clay Sci., 40: 201-208.
- Doyle, M.P., R.S. Applebaum, R.E. Brackett and E.H. Marth, 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. J. Food Protect., 45: 964-971.
- Duncan, D.B., 1955. Multiple range and multiple F-tests. Biometrics, 11: 1-42.
- Eid, A. and K.A. Mohamed, 2008. Effect of using probiotic as growth promoter in commercial diets for monosex Nile tilapia (*Oreochromis niloticus*) fingerlings. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, Oct. 12-14, Cairo, Egypt, pp: 241-253.
- El-Dakar, A.Y., S.M. Shalaby and I.P. Saoud, 2007. Assessing the use of a dietary probiotic/prebiotic as an enhancer of spinefoot rabbitfish *Siganus rivulatus* survival and growth. Aquac. Nut., 13: 407-412.
- El-Gohary, M.S., S.G. Mohamed, R.H. Khalil, S. EL-Banna and M.K. Soliman, 2005. Immunosuppressive effects of metrifonate on *Oreochromis niloticus*. Egypt. J. Aquatic Res., 31: 448-458.
- El-Haroun, E.R., 2007. Improved growth rate and feed utilization in farmed African catfish *Clarias gariepinus* (Burchell 1822) through a growth promoter Biogen® supplementation. J. Fish. Aquatic Sci., 2: 319-327.
- El-Haroun, E.R., A.M.A.S. Goda and M.A.K. Chowdhury, 2006. Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquac. Res., 37: 1473-1480.
- Galvano, F., A. Piva, A. Ritieni and G. Galvano, 2001. Dietary strategies to counteract the effects of mycotoxins: A review. J. Food Prot., 64: 120-131.
- Gatesoupe, F.J., 1999. Review, the use of probiotics in aquaculture. Aquaculture, 180: 147-165.
- Grim, P.E., 1962. Applied Clay Mineralogy. McGraw-Hill, New York, PP: 422.
- Horvath, E.M., 1998. Taking the threat out of mycotoxins. Feed Tech., 2: 32-33.
- Huwig, A., S. Freimund, O. Kappeli and H. Dutler, 2001. Mycotoxin detoxication of animal feed by different adsorbents. Toxicol. Lett., 122: 179-188.
- Irianto, A. and B. Austin, 2002. Probiotics in aquaculture. J. Fish Dis., 25: 633-642.
- Jantrarotai, W. and R.T. Lovell, 1990. Subchronic toxicity of dietary aflatoxin B₁ to channel catfish. J. Aquatic Anim. Health, 2: 248-254.
- Khattab, Y.A.E., A.M.E. Shalaby, S.M. Sharaf, H.I. El-Marakby and E.H. Rizkalla, 2004a. The physiological changes and growth performance of the Nile tilapia *Oreochromis niloticus* after feeding with Biogen® as growth promoter. Egypt, J. Aquat. Biol. Fish., 8: 145-158.

- Khattab, Y.A.E., A. Mohsen and M.H. Ahmed, 2004b. Effect of protein level and stocking density on growth performance, survival rate, feed utilization and body composition of Nile tilapia fry (*Oreochromis niloticus* L.). Proceedings of the 6th International Symposium on Tilapia in Aquaculture, 2004, Roxas Boulevard, Manila, Philippines, pp: 264-276.
- 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.
- Lim, C. and C.D. Webster, 2001. Nutrition and Fish Health. 1 Edn., CRC Press, New York.
- Lim, H.A., W.K. Ng, S.L. Lim and C.O. Ibrahim, 2001. Contamination of palm kernel meal with *Aspergillus flavus* which affects its nutritive value in pelleted feed for Tilapia, *Oreochromis mosambicus*. Aquac. Res., 32: 895-905.
- Linge, P., 2005. The use of probiotics and yeast derivatives in India. World Poultry, 21: 12-15.
- Lovell, R.T., 1992. Mycotoxins: Hazardous to farmed fish. Feed Int., 13: 24-28.
- Mahmoud, K.I., A.M. Abdel-Hamid and A. Mandour, 1994. *In vitro* and *in vivo* comparative studies on the efficacy of some aflatoxin-detoxifying agents. Alex. J. Vet. Sci., 10: 39-47.
- Manning, B.B., R.M. Ulloa, M.H. Li, E.H. Robinson and G.E. Rottinghaus, 2003. Ochratoxin A fed to channel catfish (*Ictalurus punctatus*) causes reduced growth and lesions of hepatopancreatic tissue. Aquaculture, 219: 739-750.
- Marzouk, M.S., M.M. Bashandi, R. El-Banna, M. Moustafa and M.A. Eissa, 1994. Hematological studies on *Oreochromis niloticus* exposed to chronic dietary aflatoxicosis. Egypt. J. Comp. Pathol. Clin. Pathol., 7: 497-504.
- Marzouk, M.S., M.M. Moustafa and N.M. Mohamed, 2008. Evaluation of immunomodulatory effects of some probiotics on cultured *Oreochromis niloticus*. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, Oct. 12-14, Cairo, Egypt, pp: 1043-1058.
- Mehrim, A.I., 2010. Effect of dietary supplementation of biogen® (Commercial probiotic) on monosex Nile tilapia *Oreochromis niloticus* under different stocking densities. J. Fish. Aquatic Sci., 4: 261-273.
- Mehrim, A.I.M., 2001. Effect of some chemical pollutants on growth performance, feed and nutrient utilization of tilapia. M.Sc. Thesis, Faculty of Agriculture Saba Basha, Alexandria University.
- Mohamed, K.A., B. Abdel Fattah and A.M.S. Eid, 2007. Evaluation of using some feed additives on growth performance and feed utilization of monosex Nile tilapia (*Oreochromis niloticus*) fingerlings. Agric. Res. J. Suez Canal Univ., 7: 49-54.
- Motalebi, A.A., K. Ardalani and S. Jamili, 2008. Effect of temperature on the produced aflatoxins in the rainbow trout feed in West Azerbaijan province. J. Fish. Aquatic Sci., 3: 392-397.
- Mumpton, F.A., 1999. *La roca magica*: Uses of natural zeolites in agriculture and industry. Proc. Natl. Acad. Sci., 96: 3463-3470.
- Ottinger, C.A. and S.L. Kaattari, 2000. Long-term immune dysfunction in rainbow trout (*Oncorhynchus mykiss*) exposed as embryos to aflatoxin B1. Fish Shellfish Immunol., 10: 101-106.
- Petchkongkaew, A., 2007. Reduction of mycotoxin contamination level by *bacillus* spp. isolated from thai fermented Soybean Product (Thua-Nao). Ph.D. Thesis, Suranaree University of Technology.
- Phillips, T.D., B.A. Clement, L.F. Kubena and R.B. Harvey, 1990. Detection and detoxification of aflatoxins: Prevention of aflatoxicosis and aflatoxin residues with hydrated sodium calcium aluminosilicate. Vet. Hum. Tox., 32: 15-19.

- Pouomonge, V. and J. Mbonglang, 1993. Effect of feeding rate on the growth of tilapia (*Oreochromis niloticus*) in earthen ponds. *Bamidegh*, 45: 147-153.
- Royes, J.A.B. and R.P.E. Yanong, 2008. Molds in fish feeds and aflatoxicosis. Florida Cooperative Extension Service. <http://en.engormix.com/MA-mycotoxins/articles/molds-fish-feeds-aflatoxicosis-t863/p0.htm>.
- Sahoo, P.K. and S.C. Mukherjee, 2001. Immunosuppressive effects of aflatoxin B1 in Indian major carp (*Labeo rohita*). *Comp. Immunol. Microbi. Infect. Dis.*, 24: 143-149.
- Sahu, M.K., N.S. Swarnakumar, K. Sivakumar, T. Thangaradjou and L. Kannan, 2008. Probiotics in aquaculture: Importance and future perspectives. *Indian J. Microbiol.*, 48: 299-308.
- Salem, M.F.E., 2002. Effect of dietary graded levels of aflatoxin B1 on growth performance and chromosomal behaviour of Nile tilapia *Oreochromis niloticus*. Ph.D. Thesis, Kafr El-Sheikh, Tanta Univ.
- Samarajeewa, V., A.C. Sen, M.D. Cohen and C.I. Wey, 1990. Detoxification of aflatoxins in foods and feeds by physical and chemical methods. *J. Food Protect.*, 53: 489-501.
- Srour, T.M., 2004. Effect of ochratoxin-A with or without Biogen® on growth performance, feed utilization and carcass composition of Nile tilapia (*Oreochromis niloticus*) fingerlings. *J. Agric. Sci. Mansoura Univ.*, 29: 51-61.
- Tacon, A.G.J., 2004. Fish Meal and Fish Oil use in Aquaculture: Global Overview and Prospects for Substitution. In: *Nutritional Biotechnology in the Feed and Food Industries*, Lyons, T.P. and K.A. Jacques (Eds.). Nottingham University Press, Nottingham, UK., pp: 433-448.
- Trucksess, M.W., M.E. Stack, S. Nesheim, S.W. Page, R.H. Albert, J.T. Hansen and K.F. Donahue, 1991. Immunoaffinity column coupled with solution fluorometry of LC post column derivatization for aflatoxins in corn, peanuts and peanut butter: Collaborative study. *J. Assoc. Off. Anal. Chem.*, 74: 81-88.
- Tuan, N.A., B.B. Manning, R.T. Lovell and G.E. Rottinghaus, 2003. Response of Nile tilapia (*Oreochromis niloticus*) fed diets containing different concentrations of moniliformin or fumonisin B1. *Aquaculture*, 217: 515-528.
- Tuan, N.A., J.M. Grizzle, R.T. Lovell, B.B. Manning and G.E. Rottinghaus, 2002. Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B₁. *Aquaculture*, 212: 311-319.
- Wang, Y.B., Z. Tian, J. Yao and W. Li, 2008. Effect of probiotics, *Enterococcus faecium*, on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Aquaculture*, 277: 203-207.
- Wintrobe, M.M., 1965. *Clinical Hematology*. 4th Edn., Lea and Febiger, Philadelphia, USA.
- Wurts, W.A., 1989. Understanding water hardness. *World Aquacult.*, 24: 18-18.
- Yin, M.C. and W.S. Cheng, 1998. Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices. *J. Food Protect.*, 61: 123-125.
- Zaki, M.S., N.E. Sharaf, H. Rashad, S.O. Mostafa and O.M. Fawzi, 2008. Diminution of aflatoxicosis in *Tilapia nilotica* fish by dietary supplementation with fix in toxin and *Nigella sativa* oil. *Am. Eurasian J. Agric. Environ. Sci.*, 3: 211-215.