

**Studies on Zearalenone toxic effects on health status of
Oreochromis niloticus fish with special reference to
chelating methods**

Thesis

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Abbreviations

AFP: Alphafetoprotein
ARE: Antioxidants response elements
CAT: Catalase
CRP: C-reactive protein
D.O: Dissolved Oxygen meter
GI tract: Gastrointestinal tract
GSH: Glutathione
Gpx: Glutathione Peroxidase
GSR: Glutathione Reductases
GST: Glutathione-S-Transferase
IGF-I: Insulin-like growth factor I
IgM: Immunoglobulin M
IM: Inner mitochondrial membrane
IP: intraperitoneal
LPO: Lipid Peroxidation
MDA: Malondialdehyde
NBT: Nitro blue tetrazolium
NH₃-N: Unionized ammonia
NO₂-N: Nitrites
RBCs count: Red blood cells
ROS: Reactive oxygen species
S. GOT: serum glutamic oxaloacetic transaminase
S.ALT: serum alanine amino transferase
S.AST: serum aspartate amino transferase
S.GPT: serum glutamic pyruvic transaminase
SOD: Superoxide Dismutase
TP: total protein

TNF- α : Tumor Necrosis Factor Alpha

V. anguillarum: *Vibrio anguillarum*

WBCs count: White blood cells

ZEA: Zearalenone

ZEN: Zearalenone

VII- SUMMARY

Zearalenone is a non-steroidal oestrogenic mycotoxin biosynthesized through a polyketide pathway by a variety of *Fusarium* fungi which are common soil fungi. Zearalenone is implicated in reproductive disorders of farm animals and in hyperestrogenic syndroms in humans. Binders have been used to neutralize the effects of Zearalenone by preventing their absorption from the digestive tract. The most common binders are clays, such as bentonite.

1) In our study, a total number of 120 apparently healthy *O. niloticus* of average body weight (30 ± 5 g) private fish farms at El.Behera Governorate were used to determine of LC_{50} of zearalenone. Another 120 apparently healthy *O. niloticus* were used to determine chronic toxicity of zearalenone. Fish were transported alive to the laboratory of the department of poultry and fish diseases, Faculty of Veterinary Medicine, Alexandria university.

2) Lethal dose fifty (LC_{50}) of Zearalenone in *O. niloticus* was injected intraperitoneally (start at a dose of 10 mg / kg of body weight) dissolved in corn oil as a carrier solution, or corn oil alone (control sample). The ZEN injected by different concentrations (0, 10, 20, 30, 40, 50 mg / kg of body weight) during 96 h There was a simultaneous control group (no ZEN additions), keeping all other conditions alike. The concentration of dilution ZEN caused 50% mortality in fish for 96 h was taken as the LC_{50} value.

3) Chronic toxicity test were established by injected intraperitoneally of 1/10 LC_{50} of ZEN (2.9 mg / kg B. W) with a trail of reducing impacts of ZEN by addition of Ortamin Feed Zeolite ® by 1% in ration for 4 weeks. At the end of the experiment the all groups were challenged intraperitoneally (IP) with the pathogenic *V. anguillarum*. Investigation concerning the challenge infection test was performed to study the cumulative impact of Zearalenone. By the end of the Zearalenone exposure duration (4weeks), 10 fish from each group were injected intraperitoneally (IP) with the pathogenic *V. anguillarum* (0.2 ml of 10^6 CFU/ml). Clinical examination and postmortem (PM) examination of experimentally intoxicated fishes was performed. Blood and serum samples were collected at 1st, 2nd, 3rd and 4th weeks during the experimental period Determination of serum aspartate amino transferase (s.AST); serum alanine amino transferase (S.ALT); Serum Protein (Albumin and α 1-globulin (%), α 2-

globulin (%), β -globulin (%) and γ -globulin (%); Alphafetoprotein (AFP) Concentration ;Tumor Necrosis Factor Alpha TNF- α : Creatinine; percent of ammonia; determination of antioxidants enzyme activities in serum (Catalase activity assay; Superoxide dismutase activity assay; Glutathione peroxidase activity assay and Lipid Peroxidation. Serum immune parameters assay (Serum lysozyme activity assay: bactericidal activity and Pentaxins (C-reactive protein). Determination of estradiol and testosterone as well as determination of residue of zearalenone in cartilage and musculature of fish was carried out. Histopathological studies: gills, spleens, liver and kidneys including both the anterior and posterior parts were harvested from both Zearalenone and Zearalenone plus Ortamin Feed Zeolite ® supplemented groups at 2nd and 4th weeks has been studied and **the results revealed that:-**

- a) **LC₅₀ of Zearalenone (ZEN)** in *O. niloticus* was 29 mg / kg B. W; so the 1/10 dose of LC₅₀ of ZEN in *O.niloticus* to induce chronic toxicity was 2.9 mg / kg B. W.
- b) **The clinical manifestation** during the 1/10 dose of LC₅₀ of ZEN in *O.niloticus* (2.9 mg / kg B.W) showed scattered darkness colourations spots on the dorsal region (1st weeks); sever hemorrhagic explosion on the operculum and in the eye (2nd weeks); slight distension of the abdomen and hemorrhagic patches on the dorsal region (3rd weeks) and large darkness patches area on the dorsal region with cataract of the eye (4th weeks).On the other hand the most important postmortem lesions were showing sever large hemorrhagic patches on the surface of the liver and cyanosis colour of the kidney and ovary appeared severely congested (4th weeks)) and the more pronounced lesion were reported in the kidney and intestine where, slight congestion of the kidney and intestine field with mucoid bloody tinged: sever congestion of the kidney and intestine field with mucoid tinged with bloody.
- c) **Changes in antioxidant enzyme activities of serum as biomarkers of chronic exposure of ZEN and /or Ortamin Feed Zeolite ® in *O.niloticus*:** GPX activity varied among the experimental groups in the serum of *O.niloticus*. GPX activity in group exposed to 2.9 mg / kg B.W ZEN for 4th weeks was lower comparing to both feeding group on Ortamin Feed Zeolite ® and control group respectively. In group feed on Ortamin Feed Zeolite ® increased its activity of GPx while a

decrease was observed after 2nd weeks of ZEN exposure. The serum of *O. niloticus* GPx activity was significantly decreased in ZEN exposed group at 4th weeks of exposure compared with the control, and without any significant changes in Ortamin Feed Zeolite ®supplemented groups. In groups feed on Ortamin Feed Zeolite ® and at the same times exposed to 2.9 mg / kg B.W of ZEN for 4th weeks the CAT of serum of *O. niloticus* catalase activity were gradually decreased compared to the control and treated groups . Levels of SOD activity in the serum of *O.niloticus* after chronic exposure to ZEN was gradually decreased compared to the control and treated groups. The Lipid peroxidase activity at the ZEN exposed group was found higher than at the Ortamin Feed Zeolite ® supplemented groups.

d) Effect of ZEN and /or Ortamin Feed Zeolite ® on AST; ALT; Albumin; different fractionation of globulin; creatinine and percent of Ammonia of *O.niloticus* the present results indicated that there were increases in the activities of AST/GOT in the serum of *O.niloticus* exposed to ZEN. But levels of the activities of AST in the serum of Ortamin Feed Zeolite ® supplemented group decreased significantly in comparison to the control group. But levels of the activities of ALT/GPT in the serum of Ortamin Feed Zeolite ® supplemented group decreased significantly in comparison to the control group. In relation to control group increased the serum levels of ALT at 2nd and 4th weeks of exposure period of 2.9 mg / kg B.W of ZEN. Ortamin Feed Zeolite ® alone has no effect on the measured of levels of ALT, fortunately it returned the increased levels of ALT to their normal values in ZEN and /or Ortamin Feed Zeolite ® treated fish. Also, revealed a decrease in different fractionation of globulin in serum of *O. niloticus* exposed to 2.9 mg / kg B.W Zearalenone for 4th weeks. We also observed a strong linear relationship between ZEN periods of exposure and the biochemical parameters in the serum. However, these biochemical endpoints are potential biomarkers for ZEN exposure in *O. niloticus*. In addition there were slight significant increases in levels of different fractionation of globulin in treated Ortamin Feed Zeolite ® *O.niloticus* compared with *O.niloticus* intoxicated and the control group. Different fractionation of globulin in group exposed to 2.9 mg / kg B.W ZEN for 4th weeks was lower comparing to both feeding group on Ortamin Feed

Zeolite ® and control group respectively. A significant increase of creatinine in serum of *O.niloticus* occurred at ZEN exposure of *O.niloticus* to 2.9 mg / kg B.W for 4th weeks. A significant reduction of creatinine in serum of *O.niloticus* was found at Ortamin Feed Zeolite ® supplement group alone. ZEN intoxication results in increase of ammonia percentage in serum of *O.niloticus*.

- e) **Effect of ZEN and /or Ortamin Feed Zeolite ® on Alphafetoprotein (AFP) concentration (ng/ml) and Tumor Necrosis Factor Alpha TNF- α (pg/ml) in serum of *O.niloticus*.** The AFP and TNF- α at the exposed groups to ZEN 2.9 mg / kg B.W in the serum of *O.niloticus* were found higher than at the Ortamin Feed Zeolite ® supplemented groups. The levels of AFP and TNF- α in the serum of *O.niloticus* returned to normal level in ZEN and /or Ortamin Feed Zeolite ® supplemented group.
- f) **Effect of ZEN and /or Ortamin Feed Zeolite ® on Non-specific immune parameters (Lysozyme activity and Bactericidal activity) in *O. niloticus*** shown that slightly decrease in lysozyme activity and bactericidal activity levels in serum of *O. niloticus* exposed to 2.9 mg / kg B.W ZEN for 4th weeks. In addition there were significant little increases in levels of the levels of LA and BA in treated Ortamin Feed Zeolite ® *O.niloticus* compared with intoxicated and the control group.
- g) **Effect of ZEN and /or Ortamin Feed Zeolite ® on C-reactive protein in the serum of *O.niloticus*** ZEN intoxication results in increase of C-reactive protein in serum of *O.niloticus*. The increase in C-reactive protein in serum of *O.niloticus* at dose of 2.9 mg / kg B.W for 4th weeks in *O.niloticus* under studied caused by ZEN.
- h) **Effect of ZEN and /or Ortamin Feed Zeolite ® on hormones at initial of maturation ng/ml in the serum of *O.niloticus*** results indicated that there were decreases in the levels of 17 α -estradiol and methyl testosterone in the serum of *O.niloticus* exposed to ZEN. But levels of the levels of 17 α -estradiol and methyl testosterone in the serum of Ortamin Feed Zeolite ® supplemented group slightly increased significantly in comparison to the control group.
- i) **Effect of Ortamin Feed Zeolite ® on residues of ZEN in cartilage and musculature of *O.niloticus* by quantitative thin layer chromatography.** The decrease of residues of ZEN in cartilage and

musculature was much notified at 4th weeks during supplement of Ortamin Feed Zeolite ® by 1% in ration even in present of ZEN in *O.niloticus*.

- j) Results of challenge infection test** revealed that the RLP (%) was increased by addition of Ortamin Feed Zeolite ® where reached to 50%.
- k) Results of histopathological changes associated with ZEN and /or Ortamin Feed Zeolite ® in *O.niloticus*** were appeared as; haemorrhage of the capillaries in the inter-lamellar space in gills and liver from ZEN toxicated groups after 2nd weeks shows diffuse necrosis of hepatocytes. Liver from ZEN and /or Ortamin Feed Zeolite ® groups has moderate diffuse necrosis of hepatocytes. Liver from Zearalenone toxicated groups after 4th weeks has signs of inflammation and lipid accumulations within liver tissue. Anterior Kidney from ZEN toxicated groups after 2nd weeks has hyaline droplet; degeneration of tubular epithelial cells and activation of Melanomacrophage centers. Anterior Kidney from Zearalenone groups after 4th weeks has disorganized renal parenchyma, glomerular compression and atrophy. Anterior Kidney from Zearalenone and /or Ortamin Feed Zeolite ® groups has renal parenchyma of fairly homogeneous appearance, renal corpuscles and nephron tubules.