



Reduction of Aflatoxin in grey mullet By Ginseng Extract and probiotic

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ABSTRACT

Aflatoxins are the major toxic metabolites of fungi which are able to induce chronic liver damages. The antioxidant and hepatoprotective effects of Ginseng extract and probiotic on Aflatoxin were investigated. Aflatoxicosis causes significant increase in liver enzyme SGOT and SGPT, Alkaline phosphatase activity and an increase in the level of cholesterol total lipid, decrease the level of total protein and hemoglobin and P.C.V. Moreover the liver exhibited some clinicopathological changes and decreased body weight. Both Ginseng extract and probiotic reduced the development of hepatotoxicity by Aflatoxin. Probiotic showed more improvement of all enzymes of kidney and liver, and also total lipid and cholesterol were reduced and body weight increased.

Keywords: Aflatoxin toxicity, probiotic, Ginseng extract, grey mullet

INTRODUCTION

The aflatoxins are a group of structurally related toxic compounds produced by certain strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Under favorable conditions of temperature and humidity, these fungi grow on certain foods and feeds, resulting in the production of aflatoxins, which can enter into the human food chain directly through foods of plant origin (cereal grains), indirectly through foods of animal origin (kidney, liver, milk, eggs) [1].

The most pronounced contamination has been encountered in tree nuts, peanuts, and other oilseeds, including corn and cottonseed. The major aflatoxins of concern are designated B1, B2, G1, and G2. These toxins are usually found together in various foods and feeds in various proportions [2]; however, aflatoxin B1 is usually predominant and is the most toxic. Aflatoxin M is a major metabolic product of aflatoxin B1 in animals and is usually excreted in the milk and urine of dairy cattle and other mammalian species that have consumed aflatoxin-contaminated food [3]. These poisons are completely heat stable, so neither cooking nor freezing destroys the toxin. They remain on the food indefinitely. Aflatoxins grow on grains and legumes mostly during storage, so the grains and legumes must be stored correctly to limit this problem [4]. Aflatoxins produce acute necrosis, cirrhosis, and carcinoma of the liver in a number of animal species; no animal species is resistant to the acute toxic effects of aflatoxins; hence it is logical to assume that humans may be similarly affected. Aflatoxin B1 is a very potent carcinogen in many species, including nonhuman primates, birds, fish, and rodents. In each species, the liver is the primary target organ of acute injury [5-7].

The term probiotic was firstly used to denominate microorganisms that have effects on other microorganisms [8]. Probiotics can provide some solutions to this problem through different mechanisms or properties such as the production of inhibitory compounds such as bacteriocins, competition for adhesion sites with opportunistic or pathogen microorganisms, competition for nutrients with other bacteria or an improvement of the immune status (e.g. increase of production of immunoglobulins, acid phosphatase, antimicrobial peptides, improvement of cellular activities, etc.) [9-16]. Use of microbial probiotics to promote health maintenance and disease prevention and control is now widely accepted as the new ecofriendly alternative measures for sustainable aquaculture [17]

Panax ginseng C.A. Mayer is an herbal root that has been used for more than 2000 years throughout Far Eastern countries including China, Japan and Korea. Its beneficial effects have been analyzed by extensive preclinical and epidemiological studies [18]. Recently, 20-O-(β -D-glucopyranosyl)-20(S)-protopanaxadiol (IH-901), a novel ginseng saponin metabolite, formed from ginsenosides Rb1, Rb2 was isolated and purified after giving ginseng extract p.o. to humans and animals [18]. IH-901 has been shown to enhance the efficacy of anticancer drugs in cancer cell lines previously resistant to several anticancer drugs [19].

Aim of present work

This study was conducted to evaluate the effect of Aflatoxin and ginseng with probiotic on some nutritional status and clinicopathological changes in grey mullet in toxicated with Aflatoxin and treated with ginseng and probiotic

MATERIALS AND METHODS

Experimental conditions: 60 grey mullet were obtained from Abbassa and were acclimatized to laboratory conditions. They were kept in glass aquaria supplied with dechlorinated tap water at a rate of one liter for each cm of fish body. They were fed commercial fish diet were supplied by Aflatoxin contaminated ration with corn 80ug toxin/kg ration, as shown in Table (1). A total number of 60 grey mullet were used in this experiment: 20 Fish each group, 20 grey mullet control, 20 fed Aflatoxin and 20 treated with probiotic and ginseng

The third group Aflatoxin contaminated ration + 0.2 ginseng + probiotic in ration fish were fed by hand twice daily and feed consumption in all groups was recorded daily, also mortality and body weight due to Aflatoxin were recorded.

The probiotic bacterium, *Lactobacillus rhamnosus* (ATCC 53103) was cultured in MRS broth at 26.8C for 48 h, centrifuged and washed with sterile PBS 2 times. Bacterial pellets were measured in PBS and their densities were determined. Under sterile conditions, the bacteria were manually incorporated into commercial dry pellets at rates of 10⁸ and 10¹⁰ CFU/g in feed for low and high LAB diets, respectively. Fish fed only commercial dry pellets served as a control. Fish were fed approximately 0.8% of body weight once a day. The probiotic groups ingested an average of 3.8 x 10⁶ and 3.8 x 10⁸ cells day⁻¹.

Samples: Serums were collected 3 times at 3 months interval and sera were frozen at -20. Tested kits supplied from biomérieux, France were used for determination of the activity of serum glutamic pyruvic transaminase and glutamic oxalocetic transaminase as described by Reitman and Frankel [20], serum creatinine was determined according to Silversmit, [21]. Enzymatic determination of urea was done according to King [22].

Blood hemoglobin was assessed by cyame hemoglobin method Hematocrit value was carried out by using microhematacrit capillary tubes centri fuded at 2000P.M. for 5min according to the method of Drabkin [23] serum cholesterol according to the method Fiegg(24), total lipids according to the method of Siesta [25], and statistical analysis according to the method of Gad and Weil [26].

Table(1). Ingredients and proximate chemical composition of diets used in the experiments

Ingredient	Control		Proximate chemical composition
Hah meal	30	Crude protein Pg%	35.87
Meas meal	8	M.E/kg	2297.21
Bone meal	1	Ether extract g%	2.78
Soya bean	5	Crude fiber g%	3.91
Skimmed milk	3	Ash g%	8.735
Wheat bran	20	Calcium mg&	2.069
Wheat flour	20	Lysine mg%	2.105
Yeast	10	Methionine mg%	0.562
Codliver oil	1		
Mineral and premix	2		

Mineral and vit. Premix per/kg of pellet food

Vit. A 8000 IU, vit. D 900 IU, vit. E 2 IU, vit. K4mg, B2 3.6mg, niacin 20mg, choline chloride 160mg, pantothenic acid 7mg, pyridoxine 0.2mg, vit. B12, 5ug, Mn 70mg, Zn 60mg, Fe 20mg, Cu 2mg, Co 0.2mg.

N.B.: we added 80Ug polluted corn with Aflatoxin B1, in this ration.

RESULTS

Aflatoxicosis produced a significant decrease in body weight if compared with control group as shown in Table2. statistical analysis revealed effect of Aflatoxin, B1 on erythrogram. There is a significant decrease in P.C.V. Hemoglobin (P<0.01) as shown in Table2. there is a significant decrease in mean of total protein and a significant increase in SGOT, SGPT, Urea, creatinine, total lipid, cholesterol and alkaline phosphatase (P<0.01).

Post treatment with ginseng and probiotic for 3 months. All this parameters return to normal level as shown in Table3 and 4 if compared with control group.

Table(2). (Effect of Aflatoxin after 1-2 months on clinicopathological changes in grey mullet after treatment with ginseng and probiotic

Parameters	Control N=(20)	Aflatoxin 1month N=(20)	Aflatoxin + ginseng + probiotic N=20	Control N=20	2months group N=20	Aflatoxin+ ginseng +probiotic N=20
AST U/L	62±0.13	132±0.05**	102±0.04	83±1.07	119±2.2**	94.6±0.07
ALT U/L	15±0.52	25±0.60**	19±0.74	16±0.62	30±0.79**	20±0.16
Urea mg/dl	2.60±0.20	3.6±0.53**	5.0±0.17*	2.5±0.70	4.3±0.91**	3.2±0.10
Creatinine mg/dl	0.61±1.4	0.6±0.13**	0.85±0.24	0.80±0.16	1.2±0.40**	0.80±0.20
Total protein mg/dl	46±0.17	3.5±0.72**	4.0±0.50	5.0±0.12	3.0±0.11**	4.5±0.50
Total lipidscholesterol mg/dl	87±0.95	140±0.20**	100±0.20*	95±0.10	180±1.20**	100±0.70
Cholesterol	170±0.72	202±2.0**	190±0.29*	180±0.54	240±3.4**	190±2.0
Alkaline phosphates mg/dl	15.9±0.27	26.8±0.23**	21±0.16	17.7±0.16	33.8±0.17**	20±0.22
Hemoglobin mg/dl	7.0±0.20	4.4±0.64**	6.1±1.00	7.6±0.19	4.0±0.70**	6.3±1.5
P.C.V%	36±0.43	31±0.15	330±0.05	42±0.51	26±0.12**	35±0.17

P<0.01

Table(3). Effect of Aflatoxin after 3 months on clinicopathological changes in grey mullet after treatment with ginseng and probiotic

Parameters	Control 3 months	Aflatoxin 3 months	Aflatoxin plus ginseng + probiotic 3 months
AST U/L	80±0.10	130±5.2**	81±0.25
ALT U/L	172±0.10	23±0.36**	17.3±0.05
Urea mg/dl	2.08±0.20	5.0±0.08**	2.04±0.19
Creatinine mg/dl	0.80±0.40	1.3±0.44**	0.80±0.3
Total protein mg/dl	5.4±0.14	3.0±0.25**	5.0±0.70
Total lipid mg/dl	90±0.70	181±1.14**	90±0.80
Cholesterol mg/dl	180±0.84	244±2.1**	172±0.63
Alkaline phosphatase U/L	17.8±0.17	35.4±0.71**	17.2±0.22
Hemoglobin %	8.8±0.45	4.0±0.71**	8.4±0.32
P.C.V. %	36±0.20	20±0.10**	38.3±0.14

P<0.01

Table(4). Effect of Aflatoxin on body weight of grey mullet during the course of experiment

Group	1month	2months	3months
Control 20 fish	64±0.23	95±0.15*	120±0.52
Aflatoxin group (20fish)	90±0.10	80±0.2*	72±0.10
Aflatoxint + gensing + Nigella sative (20fish)	84±0.05	100±0.72*	130±0.61

*P<0.01

DISCUSSION

Aflatoxins are hepatotoxins [27-28] and also impair immunity which ultimately led to increased susceptibility to disease [29]. The present work demonstrated a severe necrosis in liver of catfish. The liver is the primary site of metabolism of ingested Aflatoxin. [30] the pathological changes of liver observed in the present investigations may be due to primary site of metabolism o ingested Aflatoxins as well as the primary laceratian laceratian of residues and lesions. Similar finding reported by Newperne [31]. The increase of enzyme Urea, creatinine. These changes due to necrosis of kidneys reported by Pier [27]. The lipid metabolism was altered during Aflatoxicosis as judged by increase of total lipid content. In the present experiment, here is a highly elevation of total lipid and cholesterol in serum which agree with Slppej, et al. [32], Sisk et al. [33]. It is obvious that administration of ginseng and probiotic reduced the Aflatoxin in liver, kidney, of infected fish and may protect liver from free radical reactions due to Aflatoxin, also total lipid, cholesterol return to normal level [29]

The present study showed a significant decrease in P.C.V., HB concentration in the affected fish that was proportionally correlated with the severity of Aflatoxicosis. This result is in accordance with Robert [34]. El-Bouhy et al., [35]. They found similar results in broilers chickens common carp Fish and this indicates that the toxin causes a deleterious effect on the hemopoetic system.

Regarding the biochemical serum analysis, the noticed decreased in T.P. may be attributed to the improved protein synthesis as a result of liver function due to Aflatoxicosis. [36 -37]. The increase in ALT and AST activities recorded by Jassar and Balwant [38], Rasmassen et al. [39], Sisk et al., [33], due to liver affection in case of Aflatoxicosis the elevation of ALP activity comes in consistence with mentioned by Jassar and Balwant [38], Svobodava et al. [40], in chicken due to degenerative changes in the liver causing leakage of enzymes into serum and cause the highest concentration of alkaline phosphates. The great increase of alkaline phosphates activity due to damage of liver. The detection of Aflatoxin in the liver tissues explain the liver degeneration. Similar results were described by Kubena et al. [41], who used ginseng for preventing the absorption of Aflatoxins from gastrointestinal tract.

In conclusion, the metabolism of Aflatoxin result in the alteration of various metabolic process within hepatocytes which leads to severe serum biochemical alterations and serious pathological changes which affect fish production but treatment with ginseng and probiotic give an excellent of results.

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