INFELUENCE OF SYNTHETIC 4-METHYL-7 HYDROXY COUMARIN ON MINIMIZING THE TOXICITY OF AFLATOXIN B_1 IN NILE TILAPIA FISH DIETS.

S.A. Shehata[1] and S.M. Mohamed[2]

[1]*Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.*

[2]*Central Laboratory for Aquaculture Research, Abbassa, Abo-Hamad, Agriculture Research Center, Egypt.*

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SUMMARY

A total number of 180 Nile tilapia (*Oreochromis niloticus*) fish were used in a six experimental groups in a 3 x 2 factorial design to evaluate the efficiency of 4-methyl-7 hydroxy coumarin in reducing the toxicity of aflatoxin B_1 in fingerlings fish diets (initial weight 13 g). The experimental groups were commercial basal diet served as control (C); C+ 0.25% coumarin ; C+ 0.5% coumarin; aflatoxin B_1 (3 mg/kg diet) , C+ aflatoxin B_1 + 0.25% coumarin ; C+ aflatoxin B_1 +0.5% coumarin. There were 3 replicate aquariums of 10 fish Nile tilapia per aquarium for each experimental group. Fish were fed at a rate of 4% of the total body weight for 3 months experimental period. Aflatoxin B_1 contaminated diet significantly ($P<0.05$) decreased body weight gain, feed conversion, mortality rate and blood total protein, albumin, globulin, aspertate aminotransferase (AST) and alanine aminotransferase (ALT). Addition of coumarin to contaminated diets improved ($P<0.05$) significantly growth performance, feed conversion, blood parameters measured and mortality rate. Most results indicated that addition of 0.25% coumarin to fish diets contaminated with aflatoxin was safe in practice to minimize the aflatoxin B_1 toxicity.

Keywords: *fish; aflatoxin; coumarin; growth; blood; mortality.*

INTRODUCTION

Aflatoxins are mycotoxins produced as secondary metabolites by *Aspergillus flavus* and *A. parasiticus* (Deng *et al.*, 2010). Aflatoxin contaminated-diets lead to many hazard effects on human and animals (death; reduce the production and reproduction; mutagenic, carcinogenic and teratogenic effects and immunotoxicity) (Shehata, 2002). Up until the present, the eradication of aflatoxins contamination in agriculture products has been detected, especially in drought, hot and humid regions. One of the effective methods to overcome the toxic and carcinogenic effects of aflatoxins is to enhance aflatoxin metabolism toward its detoxification in humans or animals (Tulayakul *et al.*, 2007).

More than 1300 coumarins have been identified from natural sources especially green plants (Hoult and Paya 1996). Coumarins are antioxidants, contain the parent nucleus of benzo-α -pyrone and occur in plants like Tonka beans, Sweet clover, Wood ruff, Cassia leaf (Lake *et al.*, 1989). Also, it was in the variety of plants families like Loganiaceae (Bhattacharyya *et al.*, 2008), Orchidaceae, Leguminaceae, Rutaceae, Umbelliferae and Labiatae (Vyas *et al.*, 2009). The synthetic coumarin (4-methyl-7 hydroxy coumarin) derived from resorcinol and ethyl aceto-acetate in presence of concentrated sulphuric acid is structurally close to scopoletin, beaing a coumarin derivative. Naturally derived and synthetic coumarins have been used in treatment of oedemas (Clasley Smith, 1993), anti cancer (Battacharyya *et al.*, 2009), antibacterial (Khan *et al.*, 2005 and Devienne *et al.*, 2005), anticoagulants, anti-thrombotic and vasodilatory (Houlf and Paya, 1996), anti-mutagenic (Pillai *et al.*, 1999) and anti-tumorigenic (Maucher *et al.*, 1994 and Prince *et al.*, 2009).

Coumarin was used for reduction aflatoxin B_1 toxicity in pigs (Tulayakul *et al.*, 2007) and rats (Kelly *et al.*, 2000).

This work was carried out to study the effect of minimizing aflatoxin B_1 by 4-methyl- 7 hydroxy coumarin in Nile tilapia fish through coumarin.

MATERIALS AND METHODS

The experimental work was carried out in the Aquaculture Research Lab., Abbassa, Abo-Hamad, and Animal Production Dept., Faculty of Agriculture, Zagazig Univ., Egypt. Six experimental groups in a 3 x 2 factorial design were used to evaluate the effeciency of coumarin in reducing the toxicity of aflatoxin B_1 in fingerlings Nile tilapia (*Oreochromis niloticus*) fish diets. The experimental groups were as follow: 1) basal diet (control, C); 2) C+0.25% coumarin; 3) C+0.5% coumarin; 4) C+aflatoxin B_1 (3 mg / kg diet); 5) C+aflatoxin B_1 + 0.25% coumarin; 6) C+aflatoxin B_1 + 0.5% coumarin. A total number of 180 fish (average body weight 13 g) were used in 3 replicate glass aquaria (per group) of 10 fish per aquarium. The dimensions of each aquarium were 150 x 150 x 50 cm, these aquaria were supplied with dechlorinated tap water up to 80% of its height and continuous aeration was adapted by using an air pump and air stones. Fish wastes were filtered by siphon method each day and the rearing water was completely changed every 3 days. Mean water temperature was 27°C ± 2°C.

Coumarin (7-hydroxy-4-methyl coumarin) was prepared according to method of Furniss *et al.*, (1978) which is summarized as follow: place 1 liter of concentrated sulphuric acid in a 3 liter necked flask. Immerse the flask in an ice bath, add a solution of 100 g (0.91 mol) of resorcinol in 134 g (130.5 ml, 1.03 ml) of ethyl aceto-acetate drop wise and stirring 2 hrs., keep the reaction mixture at room temperature for about 18 hrs., then pour it with vigorous stirring into a mixture of crushed ice and water, collect the crude yield [yield = 155 g, 97% concentration], recrystallization in ethanol 95% the air dried. The dried coumarin was added to a ground commercial diet which was pelleted again. *Aspergillus flavus* MD 341 was used for production of AFB_1 on liquid media (2% yeast extract and 20% sucrose). The media contain AFB_1 alone. The AFB_1 concentration was determined according to the method of AOAC (1990). The media containing AFB_1 was sprayed on pelleted diet to obtain required AFB_1 level (3 mg/kg diet).

The fish were fed 2 times a day (0900 and 1600 h.) at a rate of 4% of the total body weight (at two equal meals). Commerical diet composed of fish meal, soybean meal meat meal, yellow corn, bone meal and a mixture of vitamins and minerals. The chemical composition of diet (Table 1) was adopted according to AOAC (1990). The fish were weighted every 1 month for 3 months.

Table (1): Chemical composition (%) of the commercial basal diet.

Item	Proximate analysis						
	DM	OM	CP	CF	EE	NFE	Ash
As fed	93	75.33	28.64	5.95	4.77	35.97	17.67
On dry matter basis	100	81.00	30.80	6.40	5.13	38.67	19.00

At the end of the experiment, blood samples were taken from the caudal vain of 12 fish for each treatment (4 fish / replicate). Blood plasma was separated and stored at -20 °C to analysis. Plasma total protein, albumin, aspertate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed by using commercial kits from Diamond Diagnostics Company, Egypt.

Data of the experiment were statistically analyzed using the General Linear Model Program of SAS (1996). Significant differences between treatment means were tested by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISSCUSION

Growth performance:

Data presented in Table (2) show that aflatoxin B_1 contaminated diet decreased ($P<0.05$) growth performance (live body weight, body weight gain, specific and relative growth rate). These results agree with the findings of Shehata *et al.* (2003) on Nile tilapia (*Oreochromis niloticus*). They reported that aflatoxin B_1 (9 mg/ kg diet for 8 weeks) caused significantly ($P<0.05$) loss in live body weight. Also, Deng *et al.*, (2010) reported that 1.641 mg aflatoxin B_1/ kg diet for 20 weeks reduced significantly growth performance of tilapia. Decrease of body weight gain by aflatoxin may be due to disturbance of one or more basic metabolism processes (carbohydrate, lipid or protein metabolism) in the liver and loss of appetite (Cheeke and Shull, 1985).

Table (2): Effect of aflatoxin B_1 and coumarin and their interaction on fish performance.

Item	Live body weight (g)				Body weight gain (g)							Survival rate (%)
	Initial	1st month	2nd month	3rd month	1st month	2nd month	3rd month	Total period	Daily	*SGR	**RGR	
Aflatoxin effect:	NS	**	**	**	**	**	**	**	**	**	**	**
Control	13.00 ± 0.0	19.66^a ± 0.06	28.11^a ± 0.13	40.63^a ± 0..17	6.66^a ± 0.06	8.45^a ± 0.07	12.52^a ± 0.10	27.63^a ± 0.17	0.31^a ± 0.003	1.27^a ± 0.003	212.54^a ± 1.29	93.33^a ± 1.67
Aflatoxin	13.00 ± 0.0	18.69^b ± 0.10	26.39^b ± 0.48	36.45^b ± 1.05	5.69^b ± 0.10	7.70^b ± 0.39	10.06^b ± 0.57	23.45^b ± 1.05	0.26^b ± 0.01	1.15^b ± 0.003	180.38^b ± 8.06	57.78^b ± 3.24
Coumarin effect:	ns	**	**	**	**	**	**	**	**	**	**	ns
0.0	13.00 ± 0.0	18.89^b ± 0.26	26.04^b ± 0.71	36.21^b ± 1.76	5.89^b ± 0.27	7.15^c ± 0.45	10.17^b ± 1.04	23.21^b ± 1.76	0.26^b ± 0.02	1.14^b ± 0.05	178.54^b ± 13.50	71.67^b ± 10.13
0.25%	13.00 ± 0.0	19.36^a ± 0.19	27.83^a ± 0.25	39.68^a ± 0.56	6.36^a ± 0.19	8.47^b ± 0.06	11.85^a ± 0.31	26.68^a ± 0.56	0.30^a ± 0.008	1.25^a ± 0.02	205.23^a ± 4.32	78.33^a ± 7.03
0.50%	13.00 ± 0.0	19.29^a ± 0.20	27.87^a ± 0.20	39.74^a ± 0.54	6.29^a± 0.20	8.58^a ± 0.008	11.87^a ± 0.35	26.75^a ± 0.54	0.30^a ± 0.004	1.25^a ± 0.02	205.69^a ± 4.14	76.67^a ± 8.02
Interaction effect:	ns	*	*	**	**	**	**	**	**	**	**	ns
Control x 0.0 coumarin	13.00 ± 0.0	19.47 ± 0.09	27.62 ± 0.12	40.12 ± 0.16	6.47 ± 0.09	8.15 ± 0.003	12.50 ± 0.06	27.12 ± 0.16	0.30 ± 0.00	1.26 ± 0.006	208.62 ± 1.24	93.33 ± 3.34
Control x 0.25%	13.00 ± 0.0	19.78 ± 0.003	28.39 ± 0.01	40.89 ± 0.15	6.78 ± 0.006	8.61 ± 0.003	12.50 ± 0.21	27.89 ± 0.21	0.31 ± 0.00	1.28 ± 0.006	214.54 ± 1.59	93.33 ± 3.34
Control x 0.5%	13.00 ± 0.0	19.74 ± 0.03	28.33 ± 0.04	40.87 ± 0.029	6.74 ± 0.029	8.59 ± 0.01	12.53 ± 0.29	27.86 ± 0.29	0.31 ± 0.006	1.28 ± 0.006	214.38 ± 2.21	93.33 ± 3.34
Aflatoxin x 0.0	13.00 ± 0.0	18.30 ± 0.03	24.45 ± 0.03	32.29 ± 0.14	5.30 ± 0.03	6.15 ± 0.006	7.84 ± 0.12	19.29 ± 0.14	0.21 ± 0.006	1.02 ± 0.006	148.38 ± 1.09	50.00 ± 3.34
Aflatoxin x 0.25%	13.00 ± 0.0	18.93 ± 0.07	27.27 ± 0.09	38.47 ± 0.26	5.93 ± 0.07	8.34 ± 0.03	11.20 ± 0.13	25.47 ± 0.26	0.28 ± 0.006	1.21 ± 0.01	195.92 ± 1.96	63.33 ± 3.35
Aflatoxin x 0.5%	13.00 ± 0.0	18.84 ± 0.03	27.46 ± 0.03	38.60 ± 0.26	5.84 ± 0.02	8.62 ± 0.01	11.14 ± 0.22	25.60 ± 0.26	0.28 ± 0.006	1.22 ± 0.01	196.92 ± 2.25	60.00 ± 5.78

Means in the same column bearing different letters differ significantly (P≤0.05 or 0.01). NS= not significant at P≤0.05.

**SGR (specific growth rate) =r√ final weight/ initial weight – 1 x 100, where t= period of exp.*

*** RGR (relative growth rate) =100 (final weight – initial weight) / initial weight.*

Table (3): Effect of aflatoxin B_1 and coumarin and their interaction on feed intake and feed conversion of fish.

Item	Monthly feed intake (g)				Feed conversion (feed/gain)			
	1st month	2nd month	3rd month	Total period	1st month	2nd month	3rd month	Total period
Aflatoxin effect:	NS	**	**	**	**	**	**	**
Control	15.60	$23.59^{a} \pm 0.02$	$33.73^{a} \pm 0.02$	$72.92^{a} \pm 0.03$	$2.34^{b} \pm 0.02$	$2.79^{b} \pm 0.02$	$2.69^{b} \pm 0.03$	$2.64^{b} \pm 0.06$
Aflatoxin	15.60	$22.43^{b} \pm 0.03$	$31.43^{b} \pm 0.13$	$69.46^{b} \pm 0.20$	$2.74^{a} \pm 0.03$	$2.91^{a} \pm 0.13$	$3.15^{a} \pm 0.2$	$2.96^{a} \pm 0.37$
Coumarin effect:	ns	**	**	**	**	**	**	**
0.0	15.60	$22.67^{b} \pm 0.11$	$31.25^{c} \pm 0.18$	$69.52^{b} \pm 0.42$	$2.65^{a} \pm 0.11$	$3.17^{a} \pm 0.18$	$3.07^{a} \pm 0.42$	$3.00^{a} \pm 0.72$
0.25%	15.60	$23.23^{a} \pm 0.08$	$33.40^{b} \pm 0.02$	$72.23^{a} \pm 0.13$	$2.45^{b} \pm 0.08$	$2.74^{b} \pm 0.02$	$2.82^{b} \pm 0.13$	$2.71^{b} \pm 0.23$
0.50%	15.60	$23.15^{a} \pm 0.08$	$33.44^{a} \pm 0.03$	$72.19^{a} \pm 0.14$	$2.48^{b} \pm 0.08$	$2.70^{c} \pm 0.003$	$2.82^{b} \pm 0.14$	$2.70^{b} \pm 0.22$
Interaction effect:	ns	**	**	**	**	**	**	**
Control x 0.0 coumarin	15.60	23.36 ± 0.002	33.14 ± 0.03	72.10 ± 0.09	2.41 ± 0.05	2.87 ± 0.002	2.65 ± 0.03	2.66 ± 0.09
Control x 0.25%	15.60	23.74 ± 0.002	34.07 ± 0.12	73.41 ± 0.12	2.30 ± 0.003	2.76 ± 0.002	2.73 ± 0.12	2.63 ± 0.12
Control x 0.5%	15.60	23.69 ± 0.006	34.00 ± 0.17	73.29 ± 0.17	2.31 ± 0.02	2.76 ± 0.006	2.71 ± 0.17	2.63 ± 0.17
Aflatoxin x 0.0	15.60	21.96 ± 0.003	29.34 ± 0.07	66.90 ± 0.08	2.94 ± 0.02	3.57 ± 0.003	3.74 ± 0.07	3.47 ± 0.08
Aflatoxin x 0.25%	15.60	22.72 ± 0.02	32.72 ± 0.08	71.04 ± 0.15	2.63 ± 0.04	2.72 ± 0.02	2.92 ± 0.08	2.79 ± 0.15
Aflatoxin x 0.5%	15.60	22.61 ± 0.006	32.95 ± 0.13	71.16 ± 0.15	2.67 ± 0.01	2.62 ± 0.006	2.96 ± 0.13	2.78 ± 0.15

Means in the same column bearing different letters differ significantly ($P \leq 0.05$ or 0.01). NS= not significant at $P \leq 0.05$.

Also, it might be due to detoxification process in the body utilizing glutathione enzymes. Glutathione is the intracellular antioxidant (Deng et al., 2010) and partly composed of methionine and cystein, hence this detoxification process depletes the metabolic availability of methionine leading to poor growth and feed efficiency (Devegowda *et al.*, 1998).

Adding coumarin (0.25 and 0.5 %) to diets significantly improved ($P<0.05$) growth performance. The interaction showed that coumarin had a positive effect on growth perfprmance when fed contaminated diets. The beneficial effect of coumarin may be due to: 1) Reduction AFB_1-DNA adducts formation by both liver and intestinal microsomes (coumarin enhanced aflatoxicol formation therefore decrease AFB_1-DNA adduct, because direct interaction of aflatoxicol-epoxide with DNA is minor compared with AFB_1-epoxide (Loveland, 1987). 2) enhancement of glutathione S-transferase (GST) activity in the intestine to conjugate AFB_1. 3) Suppression of P450 enzyme activity in the liver and the enhancement of GST in the intestine (Tulayakul *et al.*, 2007). 4) Improving liver function (Gilani and Janbaz, 1993) and body health (Maucher *et al.*, 1994, Hoult and Paya, 1996, Pillai *et al.*, 1999, Devienne *et al.*, 2005). 5) Increasing the digestibility of crude protein and ether extract (Ko *et al.*, 2006).

Feed intake and feed conversion:

Aflatoxin B_1 decreased ($P<0.05$) feed intake and feed conversion (Table 3). These results agree with those reported by Shehata, (2003) on *Oreochromis niloticus* fish. Decreasing feed intake is due to reduction fish body weight gain, while reduction of feed efficiency may be due to decreasing metabolism of nutrients by aflatoxin (Cheeke and Shull, 1985) and impaired organs functions (El-Said, 1997 and Shehata *et al.*, 2009a & b). Adding coumarin to diets significantly improved feed utilization (Table 3), this may be due to reduction of aflatoxin effect on body function (Loveland, 1987), improve digestion and absorption of protein and fat (Ko *et al.*, 2006). Coumarin stimulates the secretion of bile salts and lipolytic enzymes in the small intestine (Hahn, 1966). The interaction effect showed an improvement in feed utilization with contaminated diets.

Blood parameters:

Some plasma constituents of fish are shown in Table (4). Total protein, albumin and globulin concentrations were significantly decreased due to aflatoxin effect. These results agree with those reported by Hussein *et al.* (2000) and Shehata *et al.*, (2009 a & b) on *Oreochromis niloticus*. The decrease in total protein and albumin may be attributed to aflatoxin interaction with protein synthesis and cellular integrity in liver (Srivastava, 1984). The activity of AST and ALT enzymes significantly decreased in fish fed aflatoxin B_1 contaminated diet. These results agreed with the findings of Abd El-Baki *et al.* (2002) and Shehata *et al.*, (2003). Reduction of AST and ALT may be due to toxic hepatosis (Abdelhamid and Dorra, 1993). Addition of coumarin significantly improved blood parameters measured. These results may be due to the effect of coumarin on detoxification of aflatoxin (Kelly *et al.*, 2000 and Tulayakul *et al.*, 2007) and improve body health (Clasley Smith, 1993, Hoult and Paya, 1996, Devienne *et al.*, 2005, Battacharyya *et al.*, 2009 and Prince *et al.*, 2009). Interaction effect showed better responses with contaminated diets.

Survival rate:

The Survival rate (Table 2) was significantly decreased in fish fed aflatoxin B_1 contaminated diet where the mortality rate was 42.22% in comparison with 6.67% for control. These results agreed with those reported by El-Banna *et al.* (1993) who reported that mortality rate was 16.7% in fish fed aflatoxin B_1contaminated diet (0.2 mg/kg) for 10 weeks. Also, Shehata *et al.* (2009 a & b) reported that 3 mg aflatoxin B_1/kg feed caused 50 and 53.3% mortality in *Oreochromis niloticus* after 8 weeks. The incidence of death may be due to the disturbance of organs function, since the aflatoxicosis caused liver neoplasm, nechrosis of hepatocytes and degenerative changes in pancreatic and kidney tissues of rainbow trout (Halver, 1967). Also, Lovell, (1991) repoeted that aflatoxin caused damage of liver and other organs, thereby caused poor growth, anemia, impaired blood clotting, senstivity to burising, decreased immune responsiveness and increased mortality. Addition of 0.25 and 0.5% coumarin significantly reduced mortality rate which were 21.67 and 23.33 %, respectively in comparison with 42.22% in fish fed aflatoxin B_1 only.

The decrease of mortality rate by coumarin addition may be due to improve organs body functions (Gilani and Jambaz, 1993); induction carcinogen-detoxifing enzymes (GST) and/or NAD (P) H quinone oxidoreductase (NQO1) (Prince *et al.*, 2009); prevent the formation of free radicals by induction of NQO1 enzyme (Jaiswal, 2000) and inhibitied aflatoxin B_1 initiated hepatic prenoplastic foci and decreased the number and size of AFB_1 induced tumor of rats (Kelly *et al.*, 2002). Interaction showed that the effect of coumarin was obvious with contaminated diets.

Table (4): Effect of aflatoxin B_1 and coumarin and their interaction on plasma constituents of fish.

Item	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AST (u/l)	ALT (u/l)
Aflatoxin effect:	**	**	**	**	**
Control	$4.48^a \pm 0.10$	$3.09^a \pm 0.05$	$1.39^a \pm 0.04$	$29.67^a \pm 0.76$	$20.33^a \pm 0.56$
Aflatoxin	$3.89^b \pm 0.17$	$2.77^b \pm 0.16$	$1.12^b \pm 0.03$	$27.54^b \pm 1.43$	$15.78^b \pm 0.64$
Coumarin effect:	**	**	ns	**	**
0.0	$3.77^b \pm 0.24$	$2.58^b \pm 0.20$	1.19 ± 0.05	$26.75^c \pm 2.05$	$16.75^c \pm 1.47$
0.25%	$4.37^a \pm 0.13$	$3.11^a \pm 0.04$	1.26 ± 0.06	$29.32^a \pm 0.70$	$18.09^b \pm 0.44$
0.50%	$4.40^a \pm 0.13$	$3.12^a \pm 0.05$	1.28 ± 0.10	$29.75^a \pm 1.09$	$19.33^a \pm 1.36$
Interaction effect:	**	**	**	**	**
Control x 0.0 coumarin	4.30 ± 0.05	3.00 ± 0.20	1.30 ± 0.05	31.00 ± 2.50	20.00 ± 0.30
Control x 0.25%	4.48 ± 0.42	3.12 ± 0.12	1.36 ± 0.14	30.50 ± 1.30	18.67 ± 0.67
Control x 0.5%	4.65 ± 0.25	3.16 ± 0.16	1.49 ± 0.11	27.50 ± 1.50	22.33 ± 0.73
Aflatoxin x 0.0	3.24 ± 0.24	2.15 ± 0.15	1.09 ± 0.09	22.50 ± 1.70	13.50 ± 0.70
Aflatoxin x 0.25%	4.27 ± 0.17	3.10 ± 0.10	1.17 ± 0.07	28.13 ± 1.23	17.50 ± 1.20
Aflatoxin x 0.5%	4.15 ± 0.15	3.07 ± 0.07	1.08 ± 0.12	32.00 ± 0.60	16.33 ± 0.57

Means in the same column bearing different letters differ significantly ($P \leq 0.05$ or 0.01). ns= not significant at $P \leq 0.05$.

Results indicated that addition of coumarin specially 0.25% to fish aflatoxin contaminated diet was safe and practical to minimize the aflatoxin B_1 toxicity.

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تقليل سمية الأفلاتوكسين B_1 فى علائق سمك البلطى النيلى بواسطة 4 ميثايل- 7 هيدروكسى كومارين المحضر صناعيا.

صبرى عبد الحافظ شحاتة[1] ، محمد صلاح محمد[2]

[1] قسم الانتاج الحيوانى – كلية الزراعة – جامعة الزقازيق – مصر.

[2] المعمل المركزى لبحوث الثروة السمكية – العباسة- أبوحماد – مركز البحوث الزراعية- مصر.

أجريت تجربة عاملية 3 على 180 سمكة بلطى نيلى لدراسة مقدرة 4 ميثايل – 7 هيدروكسى كومارين المحضر صناعيا فى تقليل سمية الأفلاتوكسين B_1 فى علائق الاصبعيات (الوزن المبدئى 13 جم) وكانت المجموعات التجريبية كما يلى:

عليقة تجارية بدون اضافات (كونترول) ، 0.25 % كومارين ، 0.50 % كومارين ، أفلاتوكسين B_1 (3 ملحم / كجم عليقة) ، أفلاتوكسين B_1 + 0.25 % كومارين ، أفلاتوكسين B_1 + 0.50 % كومارين. واشتملت كل مجموعة على 3 أحواض زجاجية بكل حوض 10 سمكات وتم تغذية السمك بمعدل 4 % من وزن الجسم واستمرت التجربة 3 شهور.

انخفض معنويا معدل النمو، كفاءة التحويل الغذائى والبروتين الكلى فى الدم ، الألبيومين ، الجلوبيولين ، الانزيمات الناقلة لمجموعات الأمين (AST, ALT) بالأفلاتوكسين B_1 وعلاوة على ذلك ارتفع معدل النفوق معنويا بالأفلاتوكسين.

اضافة الكومارين للعلائق الملوثة والغير ملوثة حسن معنويا قياسات النمو، كفاءة التحويل الغذائى، مكونات الدم التى تم قياسها ومعدل النفوق.

معظم النتائج تشير الى أن اضافة الكومارين بمعدل 0.25 % لعلائق السمك الملوثة بالأفلاتوكسين طريقة فعالة ، امنة وعملية لتقليل سمية الأفلاتوكسين.