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Health & Welfare

Dietary gossypol benefits limited in improving ESC resistance in catfish

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Gossypol adversely affected growth performance, other physiological conditions



Although gossypol improved some health parameters in channel catfish, its use in diets also had negative effects on growth performance.

Due to its high protein content, availability, low cost and palatability, cottonseed meal is a good alternative protein source for fish. The amount of cottonseed meal that can be included in fish diets depends mainly on its levels of free gossypol and available lysine.

The concentration of free gossypol in cottonseed meal is affected by environmental factors, cotton species, and meal-processing conditions. Gossypol naturally exists in cotton plants as a mixture of (+) and (-) optical isomers. When supplied in large amounts in diets, free gossypol is toxic to fish.

However, gossypol has also been shown to possess antifertility, antitumor, antiparasitic, antiviral and anti-microbial properties. Recent research at the Aquatic Animal Health Research Unit in Auburn, Alabama, USA, also suggested gossypol from gossypolacetic acid can have some beneficial effect in improving the resistance of channel catfish to enteric septicemia of catfish.

Dietary gossypol study

A casein gelatin-based diet containing 34 percent crude protein and 3,200 kcal digestible energy per kilograms was supplemented with 0, 300, 600, 900, 1,200, and 1,500 milligrams per kilograms of gossypol from gossypolacetic acid at the expense of celufil. Twice daily for 12 weeks, each diet was fed to apparent satiation to channel catfish of 6.2 g average weight stocked at 50 fish per aquarium in four replicate aquariums.

The flow-through aquariums were supplied with dechlorinated city water heated to 26 to 27 degrees-C and continuously aerated using air stones. Photoperiod was maintained on a 12 hours light, 12 hours dark schedule. Fish in each aquarium were group weighed and counted at two-week intervals.

At the end of week 12, blood samples from four fish per aquarium were collected for determination of hematology, serum protein, and immunological response. Livers were removed and stored at minus-80 degrees-C for gossypol analysis. Seven fish per aquarium were also sampled and stored at minus-20 degrees-C for whole-body proximate composition analysis.

Another five fish per aquarium were intraperitoneally injected with squalene as an attractant for macrophages. Six to seven days later, peritoneal exudate cells were collected for determination of macrophage chemotaxis. Twenty fish per aquarium were challenged by immersion in *E. ictaluri*, with mortality recorded twice daily for 14 days. Blood samples were collected from four fish per aquarium prior to and 15 days after challenge to determine antibody titer.

Mixed gossypol effects

Final weight gain, feed intake and feed efficiency linearly decreased with increasing concentrations of dietary gossypol (Table 1). Fish fed the highest level of dietary gossypol refused to accept the diet. Fish fed the diet containing 900 mg gossypol per kilogram diet ceased to grow at week 6, whereas those fed the two higher levels began to lose weight.

Lim, Mean performance of channel catfish fed diets containing various levels of gossypol, Table 1

Dietary Level of Gossypol (mg/kg)	Weight Gain (g)	Feed Intake (g)	Feed Efficiency Ratio	Gossypol Intake (mg/g wt. gain)	Survival (%)
0	32.4 ^a	37.2 ^a	0.94 ^a	–	92
300	28.9 ^b	34.9 ^a	0.88 ^{ab}	0.36 ^d	97
600	18.7 ^c	23.7 ^b	0.85 ^b	0.76 ^{cd}	94
900	7.1 ^d	12.7 ^c	0.61 ^c	1.62 ^c	94
1,200	3.1 ^e	8.0 ^d	0.43 ^d	3.13 ^b	96
1,500	1.4 ^e	7.2 ^d	0.21 ^e	8.22 ^a	96
Pooled SEM	1.00	1.00	0.02	0.33	1.9

Table 1. Mean performance of channel catfish fed diets containing various levels of gossypol for 12 weeks. Four replicates per treatment. Means in same column with different superscripts significantly different ($P < 0.05$).

By week 8, the weight gain of fish fed the 300-mg gossypol diet was significantly lower than that of the control fish. Feed intake and feed efficiency were a reflection of weight gain. Gossypol intake linearly increased with increasing levels of dietary gossypol.

Survival was not affected by dietary levels of gossypol. Whole body moisture increased, while lipid decreased with increasing dietary gossypol concentrations. Body protein significantly decreased in fish fed the 1,500-mg gossypol diet, but did not differ among the fish fed the other diets. Body ash did not differ in fish fed diets containing 0-900 mg gossypol per kilogram, but was significantly higher in fish fed higher levels of gossypol.

Gossypol concentration in the animals' livers was linearly related ($R^2 = 0.99$, $P < 0.001$) to dietary levels of gossypol (Table 2). The (+) isomer of gossypol was predominantly retained in liver tissue regardless of dietary levels of gossypol. The ratio of (+) to (-) gossypol isomers in the livers was relatively constant.

Lim, Isomers of gossypol accumulation in livers of channel catfish fed diets, Table 2

Dietary Level of Gossypol (mg/kg)	Liver Gossypol Content ($\mu\text{g/g}$ dry matter) Total	Liver Gossypol Content ($\mu\text{g/g}$ dry matter) (+) isomer	Liver Gossypol Content ($\mu\text{g/g}$ dry matter) (-) isomer	(+):(-) Ratio
0	0	–	–	–
300	207.3 ^e	92.3 ^e	92.3 ^e	1.226 ^c
600	505.2 ^d	229.3 ^d	229.3 ^d	1.206 ^c
900	709.7 ^{cd}	318.4 ^c	318.4 ^c	1.229 ^{bc}
1,200	901.4 ^b	398.9 ^b	398.9 ^b	1.260 ^{ba}
1,500	1066.1 ^a	472.0 ^a	472.0 ^a	1.259 ^a
Pooled SEM	37.8	16.8	16.8	0.01

Table 2. Isomers of gossypol accumulation in livers of channel catfish fed diets containing various levels of gossypol. Four replicates/treatment. Means with different superscripts significantly different ($P < 0.05$).

The red blood cell counts of fish fed the two highest levels of dietary gossypol were significantly lower than those of fish fed the control diet. However, the values of this parameter did not differ among fish fed the diets containing 300 to 900 mg gossypol per kilogram.

White blood cell counts were unaffected by dietary gossypol levels. Hemoglobin significantly decreased in the fish fed 900 milligram per kilogram or higher gossypol diets. Hematocrit was significantly affected at the 600 milligram per kilogram gossypol level and higher.

Serum total protein concentrations were significantly reduced at 900 mg and higher gossypol, but did not differ for fish fed the lower levels of gossypol. Macrophage chemotaxis ratios were similar for groups fed diets with gossypol, but significantly higher than that of fish fed the control diet.

The serum lysozyme activity of fish fed the diets containing 600 to 1,200 mg gossypol per kilogram was significantly higher than that of fish fed the control and 300-mg gossypol diets. Superoxide anion production by phagocytic cells, as measured by nitroblue tetrazolium reduction assay, was not significantly affected by dietary levels of gossypol.

At 14 days postchallenge with *E. ictaluri*, mortality significantly decreased at dietary gossypol levels of 900 mg and higher. However, antibody titers against *E. ictaluri* 15 days postchallenge were not affected by the dietary treatments. All fish fed the control diet died within seven days after challenge with *E. ictaluri*.

Conclusion

Under experimental conditions, improved macrophage chemotaxis ratio, serum lysozyme activity and survival 14 days postchallenge with *E. ictaluri* were observed in juvenile channel catfish that received dietary gossypol levels of 300 to 900 milligram per kilogram feed. However, since the gossypol adversely affected the growth performance and other physiological conditions of the fish, gossypol appears to be of limited benefit in improving the resistance of catfish to enteric septicemia.

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