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Effects of *Yucca shidigera* Extract on Water Quality and Fish Growth in Recirculating-Water Aquaculture Systems

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Abstract.—In livestock industries such as poultry and swine, an extract of the *Yucca shidigera* plant has shown promise in controlling ammonia buildup in production facilities. In three trials, effects of *Y. shidigera* extract on ammonia levels and fish growth in recirculating-water aquaculture systems were evaluated. When added to in vitro ammonia solutions, extract concentrations of 0.043% and 0.43% caused a significant ($P < 0.05$) reduction in ammonia levels after 24 h. The 0.43% concentration also caused significantly ($P < 0.05$) lower levels of nitrite and higher levels of nitrate. Both concentrations were subsequently found to be toxic to juvenile channel catfish (*Ictalurus punctatus*). In a feeding trial, fish fed diets containing 1.1-g/kg concentrations of *Y. shidigera* extract had decreased ($P < 0.10$) weight gain and increased feed conversion ($P < 0.05$) compared with fish fed control diet, but there were no significant differences ($P > 0.05$) in ammonia, nitrite, and dissolved oxygen levels. These results suggest that *Y. shidigera* could be evaluated as a preconditioning agent for water-recirculating systems and biofilters but should not be used in direct contact with fish.

Water reuse or recirculation aquaculture systems are receiving increased attention because they conserve water, allow greater flexibility in site selection, reduce pollution output, and facilitate regulation of water temperature. Accumulation of nitrogenous wastes (ammonia and nitrite) limits production intensity in water-recirculating aquaculture systems.

In other livestock industries, an extract of the *Yucca shidigera* plant has shown promise in the control of ammonia accumulation. Reduction in ammonia generated from poultry litter has been demonstrated by Berg (1977) and Rowland et al. (1979). Jacques and Bastien (1989) reported that, 24 h after manure collection in gas-sampling bags, ammonia levels averaged 29 mg/L for manure from birds not fed *Y. shidigera* extract and 2 mg/L from birds receiving extract. Suggested modes of action include urease inhibition, increased bacterial use of ammonia (Jacques and Bastien 1989), and direct binding of ammonia (Headon and Dawson 1990).

Improved performance and increased feed efficiency have also been demonstrated or claimed when *Y. shidigera* extract was incorporated into feeds for poultry (Johnston et al. 1981, 1982), swine (Foster 1983; Cromwell et al. 1985; Mader and Brumm 1987), and cattle (Goodall and Matsushima 1980). Johnston et al. (1982) suggested that surfactant properties of components of *Y. shidigera* extract could aid nutrient absorption. However, because of the intimate contact fish have with their culture environment, any compound used in aquaculture systems (and resulting metabolites) must be nontoxic to the fish at the levels required for efficacy.

We conducted tests to evaluate the effects of *Y. shidigera* extract on ammonia concentrations in vitro, its toxicity to fish, and its effects on fish growth and ammonia production when incorporated into feeds.

Methods

Preparation of extracts.—The *Y. shidigera* extract was prepared by mechanically grinding raw *Y. shidigera* plants and collecting water-extractable components. The formulation used in the in vitro ammonia test and the toxicity test was in liquid form and contained 10% *Y. shidigera* extract, *Bacillus subtillis* fermentation extract, and water. The formulation in the feeding test was in powder form and contained 30% *Y. shidegera* extract, dried *B. subtillis* fermentation extract, and calcium silicate as a carrier. These formulations are sold commercially under the trade name De-Oderase (Alltech Biotechnology Center, Nicholasville, Kentucky). In all trials, concentrations and inclusion rates were based on total formulation, not active ingredients.

In vitro ammonia testing.—The in vitro effects of *Y. shidigera* extract on total ammonia levels were determined in twelve 30-L aquaria. Stock ammonia concentrations of 2 mg/L were prepared by mixing ammonium chloride with dechlorinated tap water. Extract was added in amounts necessary to attain aquarium concentrations of 0.0043, 0.043, and 0.43%; no extract (0.000%) was added

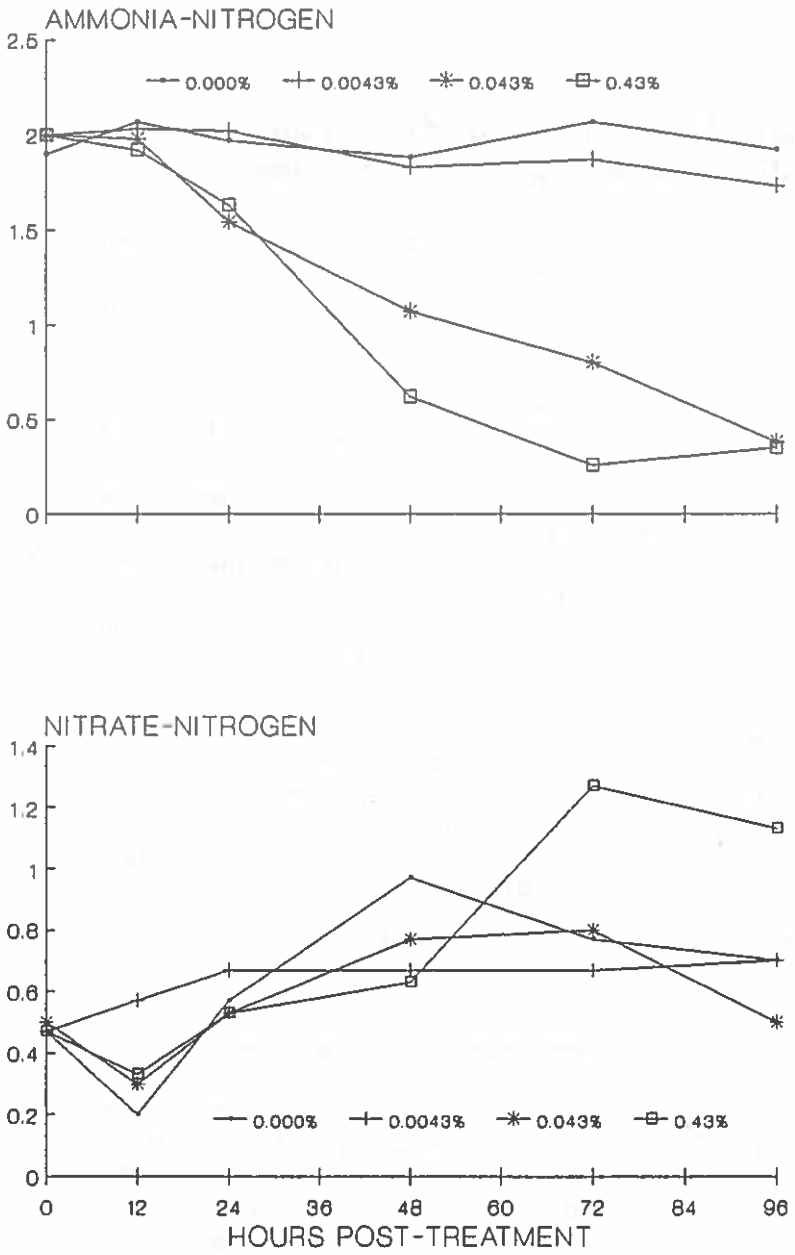

FIGURE 1.—Effects of *Yucca shidigera* extract concentration on concentration (mg/L) of ammonia–nitrogen and nitrate–nitrogen (left panel) and nitrite–nitrogen and dissolved oxygen (right panel) during 96-h in vitro aquarium tests. Starting ammonia concentrations were 2 mg/L. Values are means of three replications.

to control aquaria. There were three replications per treatment. Treatment concentrations were based on direct application rates recommended for poultry litter or livestock lagoons (Jacques 1988). Analyses were conducted on water samples collected at 0, 12, 24, 48, 72, and 96 h. Total ammonia, nitrite, and nitrate were measured with a DREL/5 spectrophotometer (Hach Co., Loveland, Colorado) and pH was measured with an electric pH meter (Omega Engineering, Stamford, Connecticut). Dissolved oxygen (DO) and temperature were measured with a polarographic oxygen meter, YSI model 57 (YSI Industries, Yellow Springs, Ohio).

Toxicity testing.—Ninety juvenile channel catfish (*Ictalurus punctatus*) were randomly stocked into nine 30-L aquaria, 10 fish per aquarium (mean initial weight, 1.9 g; mean total length, 5.9 cm). During the 7-d acclimation period, fish were fed a 38%-protein trout diet (Purina, St. Louis, Missouri) at a rate of 3% of body weight per day. Fish were not fed for 48 h before treatment. The three

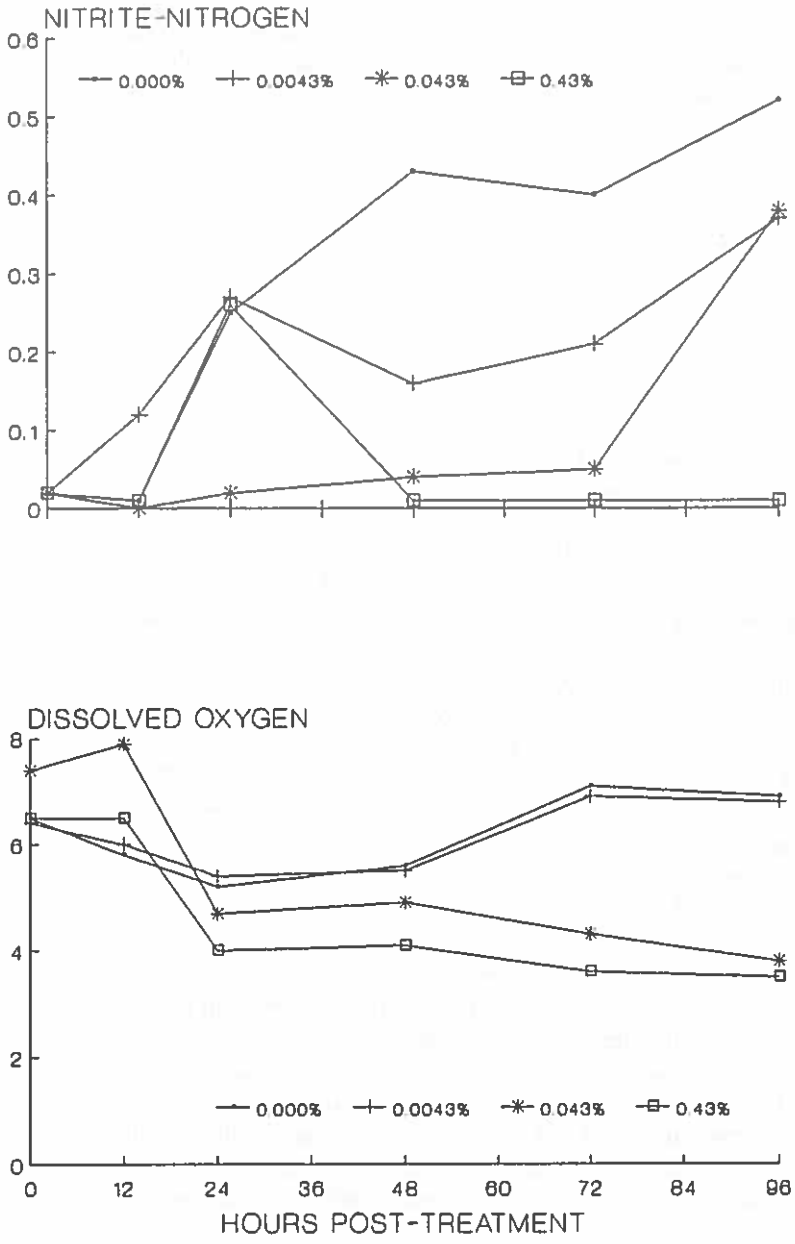

FIGURE 1.—Extended.

treatments were (1) dechlorinated tap water (control), (2) 0.043% *Y. shidigera* extract concentration, and (3) 0.43% *Y. shidigera* extract concentration. Each treatment was replicated in three tanks. All aquaria were well aerated and supplied with constant illumination. Fish were monitored hourly, and dead or moribund fish were removed. At the conclusion of the trial, samples of gill and liver were taken from two fish per aquarium; the tissues were fixed in Bouin's solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Sections were examined with a light microscope.

Diet preparation and feeding trial.—Juvenile channel catfish (mean weight, 12 g) were randomly stocked into twelve 30-L aquaria at a density of 10 fish/aquarium. For 10 weeks fish were fed one of four diets at 3% of body weight per day, split into two equal feedings (0700 and 1500 hours). Fish were weighed weekly and feeding rates were adjusted accordingly. The diets were prepared by grinding a commercial trout diet (38% crude protein; Purina) in a Wiley Mill (250 μm). *Yucca shidigera* extract powder was added at rates of 0.011, 0.110, or 1.100 g/kg diet. Ingredients were thoroughly mixed, and water was added to obtain a

TABLE 1.—Net weight gain, average individual weight, total length, feed conversion, percentage of visceral fat, hepatosomatic index, and survival rate of channel catfish fed diets containing *Yucca shidigera* extract for 10 weeks. Values are means ± SEs for three replications. Means within a row having different letters were significantly different at $P < 0.05$ except for net weight gain which was significant at $P < 0.10$.

Variable	Concentration of *Y. shidigera* extract in feed			
	0.000 g/kg	0.011 g/kg	0.110 g/kg	1.100 g/kg
Net weight gain (%)	157.47 ± 21.91 y	126.72 ± 11.20 y	133.71 ± 16.78 y	104.18 ± 8.72 z
Mean weight/fish (g)	28.44 ± 1.69 z	26.33 ± 1.64 z	26.76 ± 3.15 z	24.92 ± 1.31 z
Total length (cm)	15.10 ± 0.14 z	15.03 ± 0.20 z	15.01 ± 0.50 z	14.74 ± 0.15 z
Feed conversion	2.62 ± 0.31 z	3.05 ± 0.40 z	2.47 ± 0.20 z	3.70 ± 0.24 y
Visceral fat (%)	2.01 ± 0.19 z	1.47 ± 0.34 z	1.85 ± 0.13 z	1.50 ± 0.48 z
Hepatosomatic index	1.73 ± 0.12 z	1.73 ± 0.17 z	1.76 ± 0.28 z	1.92 ± 0.30 z
Survival (%)	100.0 ± 0.0 z	76.7 ± 20.8 z	73.3 ± 28.9 z	100.0 ± 0.0 z

30% moisture level. Diets were passed twice through a mincer with die, formed into 1.6-mm-diameter strands, and dried (25°C) for 16 h in a convection oven. After drying, the diets were broken up and sieved into appropriate pellet sizes.

Ammonia, nitrite, nitrate, temperature, DO, and pH were measured three times per week as described previously. Fish were not fed during the last 24 h before the experiment ended. At the end, all fish were individually weighed and measured. Livers of all fish were removed and weighed. Two fish per aquarium were randomly selected for histopathologic examination, and their liver and gill tissues were extracted and prepared as already described.

Growth performance and feed conversion were measured in terms of final individual fish weight (g), total length (cm), percentage weight gain, percentage survival, and feed conversion. We calculated feed conversion as (total feed fed, g)/(total weight gain, g) and the hepatosomatic index as 100 × (liver weight)/(body weight). Data were subjected to analysis of variance (ANOVA) by the SAS ANOVA procedure (SAS Institute 1988). If significant differences between means were identified, Duncan's multiple-range test was used to separate means. Percentage and ratio data were transformed to arcsine values before analysis (Zar 1984).

Results

In Vitro Ammonia Testing

Aquaria treated with 0.043 and 0.43% *Y. shidigera* extract concentrations had significantly ($P < 0.05$) lower levels of total ammonia after 24 h than the control or 0.0043% treatments (Figure 1). After 72 h, dissolved oxygen was significantly lower ($P < 0.05$) in aquaria treated with 0.043 and 0.43% extracts. After 96 h, aquaria treated with 0.43% extract had significantly ($P < 0.05$) lower nitrite and higher nitrate levels than other treatments.

Toxicity Testing

In aquaria treated with 0.43% *Y. shidigera* extract, all fish died after 2 h. After 4 h, all fish in the 0.043% concentration were dead. No fish died in the control tanks. Histopathologic examination revealed moderate to severe hyperplasia of the gill epithelium in fish exposed to *Y. shidigera* extract; hyperplasia was not present in control fish. Water quality measurements did not differ significantly ($P > 0.05$) between treatments.

Feeding Trial

Fish fed a diet containing 1.1 g *Y. shidigera* extract per kilogram feed had significantly ($P < 0.10$) lower weight gain (104.2%) than fish fed control feed (157.5%; Table 1). There was no significant difference ($P > 0.05$) in total length, visceral fat weight, or hepatosomatic index between treatments. Feed conversion was significantly higher ($P < 0.05$) for fish fed the 1.1 g/kg feed (3.7) than for fish given other feeds (Table 1). There were no significant differences ($P > 0.05$) in survival between treatments. Histopathologic examination did not reveal any differences in gills or livers of fish from different treatments. Inclusion of *Y. shidigera* extract in feeds caused no significant differences ($P > 0.05$) in total ammonia, nitrite, or DO (Table 2). Nitrate concentrations were significantly lower ($P < 0.05$) than control values in all aquaria receiving treated feed, and pH was significantly higher ($P < 0.05$) in aquaria with fish fed diets containing 0.11 and 1.1 g extract/kg.

Discussion

The addition of *Y. shidigera* extract to in vitro ammonia solutions reduced ammonia concentrations. These results agree with those of Headon

TABLE 2.—Summary of water quality analyses for aquaria containing channel catfish fed diets containing *Yucca shidigera* extract. Means (±SE) are based on samples from three replicate aquaria taken daily for temperature and dissolved oxygen and twice weekly for ammonia, pH, nitrite, and nitrate for 10 weeks. Means within a row having different letters were significantly different ($P < 0.05$).

Variable	Concentration of *Y. shidigera* extract in feed			
	0.000 g/kg	0.011 g/kg	0.110 g/kg	1.100 g/kg
Temperature (°C)	26.1 ± 0.1 z	26.2 ± 0.0 z	26.2 ± 0.0 z	26.1 ± 0.1 z
Dissolved oxygen (mg/L)	7.4 ± 0.1 z	7.5 ± 0.1 z	7.5 ± 0.1 z	7.5 ± 0.1 z
pH	7.1 ± 0.0 z	7.2 ± 0.1 zy	7.3 ± 0.1 y	7.3 ± 0.0 y
Total ammonia (as mg N/L)	0.27 ± 0.01 z	0.37 ± 0.01 z	0.36 ± 0.10 z	0.36 ± 0.05 z
Nitrite (as mg N/L)	0.526 ± 0.127 z	0.509 ± 0.178 z	0.389 ± 0.178 z	0.557 ± 0.088 z
Nitrate (as mg N/L)	22.0 ± 1.7 x	16.7 ± 2.5 y	14.3 ± 1.5 z	12.0 ± 2.0 z

and Dawson (1990), who stated that reduction of ammonia could be due to either binding of ammonia with some fraction of the *Y. shidigera* extract or by conversion of ammonia to another compound. The present study supports the theory of conversion. In aquaria treated with 0.43% extract, nitrite concentrations rose as ammonia levels declined; subsequently, nitrite concentrations decreased and nitrate concentrations increased. This indicates the actions of either chemical oxidation or nitrifying bacteria. In aquatic systems, bacteria of the genus *Nitrosomonas* oxidize ammonia to nitrite, which is oxidized to nitrate by bacteria of the genus *Nitrobacter*. Microbial or chemical nitrification is also supported by concurrent declines in oxygen levels, because these are oxygen-consuming reactions.

However, concentrations of *Y. shidigera* extract capable of reducing aqueous ammonia concentrations were toxic to juvenile channel catfish. *Yucca shidigera* extract contains at least three steroid saponins (Kaneda et al. 1987). Basu and Rastogi (1967) stated that saponins tend to alter cell wall permeability. Such a change could affect the osmoregulatory capabilities of the fish because the skin, and especially the gills, are important osmoregulatory organs. Saponins, even in dilute solutions, also cause hemolysis (Basu and Rastogi 1967). Hemolysis could severely impair gill function because blood and water may be separated by only one cell layer in the gill lamellae. Recent work in Europe has shown that the saponin component of the extract can be removed without eliminating ammonia reduction capabilities (D. R. Headon, University College, Galway, Ireland, personal communication); thus, removal of saponin could reduce the toxicity problems and should be evaluated further in aquatic systems.

Previous studies have shown that addition of *Y. shidigera* to formulated turkey diets reduced ammonia accumulation. In our study no reduction in ammonia production or accumulation could be demonstrated when *Y. shidigera* extract was incorporated into prepared channel catfish diets. Reduced growth and increased feed conversion with extract incorporation indicate reduced feed consumption or utilization, which differs from the positive growth responses reported for cattle (Goodall and Matsushima 1980), swine (Foster 1983), and poultry (Johnston et al. 1981).

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