Evaluation of a Bio-Organic Catalyst in Channel Catfish, *Ictalurus punctatus*, Ponds

Julio F. Queiroz
Claude E. Boyd
Amit Gross

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ABSTRACT. A bio-organic catalyst was tested in ponds used to grow channel catfish, *Ictalurus punctatus*, at Auburn, Alabama, for its effect on water quality, soil organic carbon, and channel catfish production. Although there were no significant differences ($P > 0.1$), ponds treated with the bio-organic catalyst tended to have higher concentrations of dissolved oxygen than control ponds during summer months even though all ponds were aerated mechanically. Data on water quality and soil organic carbon suggested that the bio-organic catalyst caused a slight but statistically insignificant inhibition of phytoplankton productivity, which in turn lessened the nighttime oxygen demand. Although fish production did not increase as the result of greater dissolved oxygen availability, fish survival was higher in the treated ponds ($P = 0.1$). At the maximum daily feeding rate of 75 kg/ha, water quality was not severely impaired in any of the ponds. The bio-organic catalyst product might have greater benefits in ponds with higher stocking and feeding rates. Information on the mechanism of action of bio-catalyst additions in pond ecosystems would be useful in determining their potential benefits to pond aquaculture. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworthpressinc.com]*

Julio F. Queiroz, Claude E. Boyd, and Amit Gross, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849.

Address correspondence to Claude E. Boyd at the above address.

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INTRODUCTION

Substances high in enzymatic activity, such as bio-organic catalysts (BOCs), have been developed to accelerate natural microbial process in soil, water, or other media. These BOC products have been used in agriculture, wastewater treatment, clean-up of oil spills, and remediation of sites contaminated with hazardous wastes (Metting 1992). Enzymes are catalysts that accelerate biochemical reactions, and microorganisms excrete extracellular enzymes that are important in the initial stages of decomposition. Extracellular enzymes break large molecules into smaller fragments that can be absorbed by microbial cells (Anderson 1987), and they can cause transformations of various compounds to accelerate microbial processes (Dick and Tabatabai 1992). Enough success has been obtained with some of these products to encourage companies to produce and market them for a variety of applications (Glass 1992). Some products purportedly improve water quality, and these have been recommended for use in pond aquaculture. Manufacturers claim their product can improve water quality through increasing the solubility of hydrophobic material, improving gas diffusion in ponds by increasing dissolved oxygen levels, and accelerating breakdown of organic matter.

The price of channel catfish, *Ictalurus punctatus*, has not increased appreciably over the past decade, and this has lead to greater stocking and feeding rates to increase production per unit area. Water quality problems increase as production rises, and some farmers are interested in the possibilities of using bio-organic catalysts to improve water quality. Because little information is available on the use of these substances in aquaculture, the present study was initiated to evaluate the use in channel catfish ponds of a BOC, which contains derived catalysts, surface modifying synthetic compounds, and other proprietary ingredients.

MATERIALS AND METHODS

Ponds

Nine 400-m^2 levee ponds on the Auburn University Fisheries Research Unit (FRU), Auburn, Alabama, were used in this study. Ponds were square with earthen bottoms that gradually sloped from depths of about 20-40 cm at the vertical edges supported by wooden or concrete walls to 130-150 cm at the drains. Pond volumes ranged from about 450 to 480 m^3. These ponds have been used annually for experiments on water quality and pond fertilization for 25 years.

Soils used for construction of these ponds were typic, Kandiudults (clayey, kaolinitic, and thermic). Such soils are acidic, reddish brown and are of low cation exchange capacity with base saturation less than 35%, and in their native state, these soils have low concentrations of phosphorus and organic matter (McNutt 1981).

Ponds are supplied by water from a reservoir filled by runoff from woodland (Boyd 1990). This water has total alkalinity and total hardness values below 20 mg/L as $CaCO_3$ as well as soluble reactive phosphorus concentrations less than 0.005 mg/L. These ponds are treated most years with 500 to 1,000 kg/ha of agricultural limestone to maintain adequate pH, total alkalinity, and total hardness.

Management

The ponds were stocked at 15,000 channel catfish fingerlings (10.7 g/ha) in April 1996. A 32% crude protein, pelleted feed was fed 7 days per week at 3% of body weight/day. Feeding rates were increased weekly according to an assumed feed conversion (weight of feed applied/weight gained by the fish) of 1.6. Ponds were inspected daily, and any dead fish were removed. Daily feeding rate did not exceed 75 kg/ha. When this rate was reached, it was continued until fish harvest. All ponds had a 0.33-kW, vertical pump aerator (Air-O-Lator Corporation, Kansas City, Missouri[1]) connected to a timer. Aerators were operated from dusk to dawn from the end of June until harvest in October. Water levels in ponds were maintained 10-12 cm below the tops of standing drain pipes to prevent overflow after rains. Water was added from a pipeline when necessary to replace evaporation and seepage losses. Ponds were drained, and fish were harvested and weighed on 15 October 1996.

BOC Treatment

A BOC product, marketed under the trade name AC+, was obtained from Neozymes International, Inc., Aliso Viejo, California. The experiment was designed so that the treatment applied to any given pond was not revealed until after the ponds were harvested. The researchers assigned each pond a letter (A-I) by ballot. The manufacturer bottled 960-mL aliquots of AC+ to provide 2 mg/L in ponds (based on 480 m^3 volume) and equal volumes of a placebo consisting of herbal tea that looked like AC+. Bottles were identified by the application date and letters A to I to correspond to letters assigned to ponds and shipped to Auburn University.

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1. Use of trade or manufacturer's name does not imply endorsement.

This provided three treatments of three replications each, as follows: high AC+ treatment–2 mg/L AC+ weekly (8 mg/L per month); low AC+ treatment–2 mg/L AC+ one week followed by applications of placebo for three weeks (2 mg/L per month); control–placebo at weekly intervals. The manufacturer sent the code for identifying bottle contents to Craig S. Tucker, Delta Research and Extension Center, Stoneville, Mississippi, and he revealed it to the researchers at the end of the study. These treatments were applied between 13 May and 11 October 1996. The placebo and AC+ were splashed over pond surfaces in the vicinity of the aerators, and the aerators were then operated for 1 hour to mix the materials with the pond waters.

Water, Soil, and Fish Analyses

Dissolved oxygen and temperature were measured one or more times per day for management purposes with a polarographic oxygen meter (Yellow Springs Instrument Company, Yellow Springs, Ohio) to verify that fish were not subjected to low dissolved oxygen stress. Water samples were collected between 0630 and 0700 at 2-week intervals with a 90-cm water column sampler (Boyd and Tucker 1992). Samples were taken from three places in each pond and combined to give a composite for analysis. Analyses were conducted for pH (glass electrode), total alkalinity (acidimetry), total hardness (EDTA titration), specific conductance (conductivity meter), chemical oxygen demand (potassium dichromate-sulfuric acid oxidation), biochemical oxygen demand (standard 5-day test), soluble reactive phosphorus (ascorbic acid method), total ammonia nitrogen (phenate method), chlorophyll *a* (membrane filtration, acetone extraction, and spectroscopy), and bacterial abundance (standard plate count). Standard protocol was followed for these analyses (Eaton et al. 1995). Nitrate was determined by the NAS reagent method (van Rijn 1993). Before stocking and one week before harvest, five soil samples (the upper 5 cm layer) were collected from each pond with a 5-cm diameter core sampler. On each sampling, the samples from a given pond were combined to make a composite sample. Soil samples were dried at 60°C and pulverized in a hammer mill. They were analyzed for total carbon by an induction furnace carbon analyzer (Leco Corporation, St. Joseph, Michigan). At harvest time, the following fish production data were collected: number and total weight of fish, percentage survival, average weight of individual fish, and food conversion ratio.

Data Analysis

Means were tested for statistical differences with Duncan's multiple range test. A large degree of variation is typically encountered in water quality and fish production variables in experiments conducted in earthen aquaculture ponds (Boyd et al. 1994). Because of this large variation, the amount of replication necessary to declare significant differences at $P = 0.05$ in pond aquaculture experiments is often prohibitive. Therefore, in this study, means were different if the probability was $P < 0.1$.

RESULTS

Total alkalinity and total hardness concentrations in pond waters were between 25 and 30 mg/L in May, and the concentrations increased to 35 to 40 mg/L in October. Specific conductance of pond waters was within the range 75 to 105 μmhos/cm, and morning pH values were between 7.7 and 8.2. Morning water temperature in ponds increased from 23°C on 16 May to 29°C on 8 August and declined to 18°C by 14 October. No influence of AC+ treatment could be detected on pH, specific conductance, water temperature, alkalinity, and hardness.

Because ponds were aerated nightly, dissolved oxygen concentrations were seldom less than 3 mg/L. From July to September, when water temperature and feeding rates were high, dissolved oxygen concentrations often were 1 or 2 mg/L higher in ponds treated with AC+ than in control ponds (Figure 1), but the differences were not statistically significant ($P > 0.1$). The high AC+ and low AC+ treatments were similar in dissolved oxygen concentration. Concentrations of soluble reactive phosphorus (SRP) were quite similar in all ponds during most of the study, but there were large increases in SRP concentration in the high AC+ treatment on two dates and in the low AC+ treatment on one date (Figure 1). On a few dates, nitrate-nitrogen (NO_3^--N) concentration also tended to be higher in ponds treated with AC+ than in control ponds, but these apparent differences were not statistically significant. During the summer, there was a trend of greater, but statistically insignificant, concentrations of total ammonia nitrogen (TAN) in AC+ ponds than in control ponds (Figure 2). Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) concentrations tended to be lower during the summer in ponds treated with AC+ as compared to controls (Figure 3). Chlorophyll *a* concentration (an index of phytoplankton abundance) tended to be higher during August and September in control ponds than in ponds of the AC+ treatments. From June through August, there was a trend of greater bacterial abundance in control ponds than in AC+ ponds (Figure 4). Nevertheless, none of these apparent differences was statistically significant. Even though trends of difference in water quality between control and AC+ ponds were detectable at certain times, seasonal means for treatments were similar (Table 1).

FIGURE 1. Means and standard errors of dissolved oxygen and soluble reactive phosphorus (SRP), measured biweekly in earthen ponds in the Fisheries Research Unit, Auburn, Alabama, that were stocked with 15,000 channel catfish/ha between May to October 1996. Ponds were treated with no, low, and high concentrations of bio-organic catalysts amendments (Control, Low AC+, and High AC+, respectively).

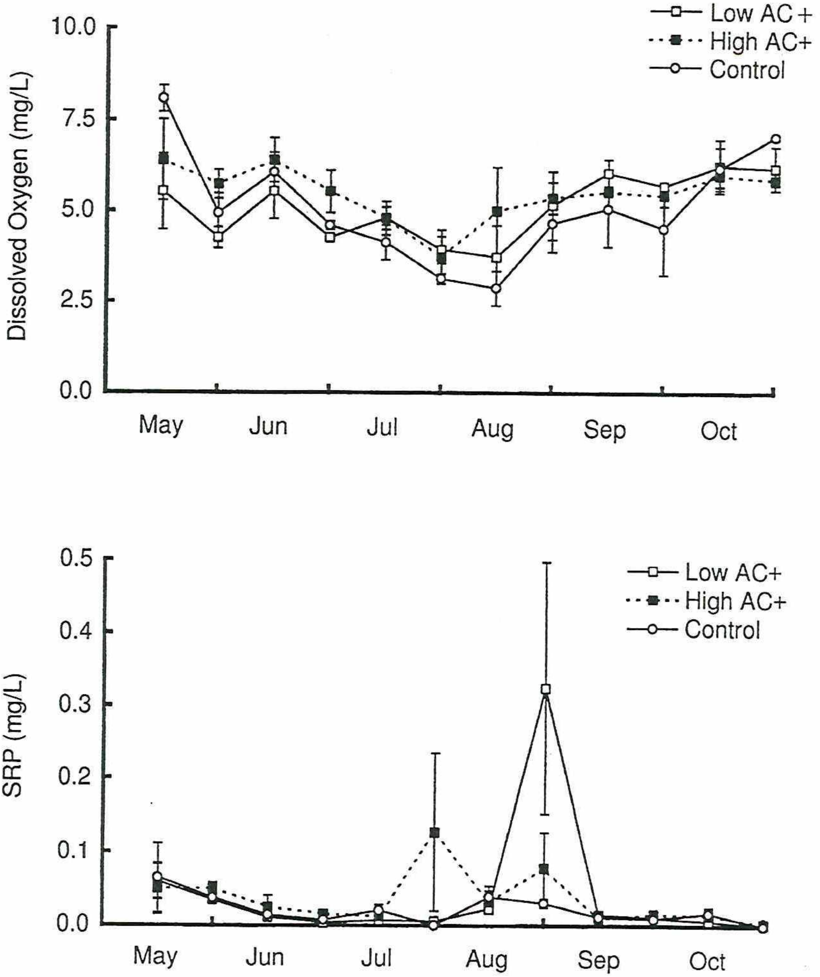


FIGURE 2. Means and standard errors of total ammonia nitrogen (TAN) and nitrate, measured biweekly in earthen ponds in the Fisheries Research Unit, Auburn, Alabama, that were stocked with 15,000 channel catfish/ha between May to October 1996. Ponds were treated with no, low, and high concentrations of bio-organic catalysts amendments (Control, Low AC+, and High AC+, respectively).

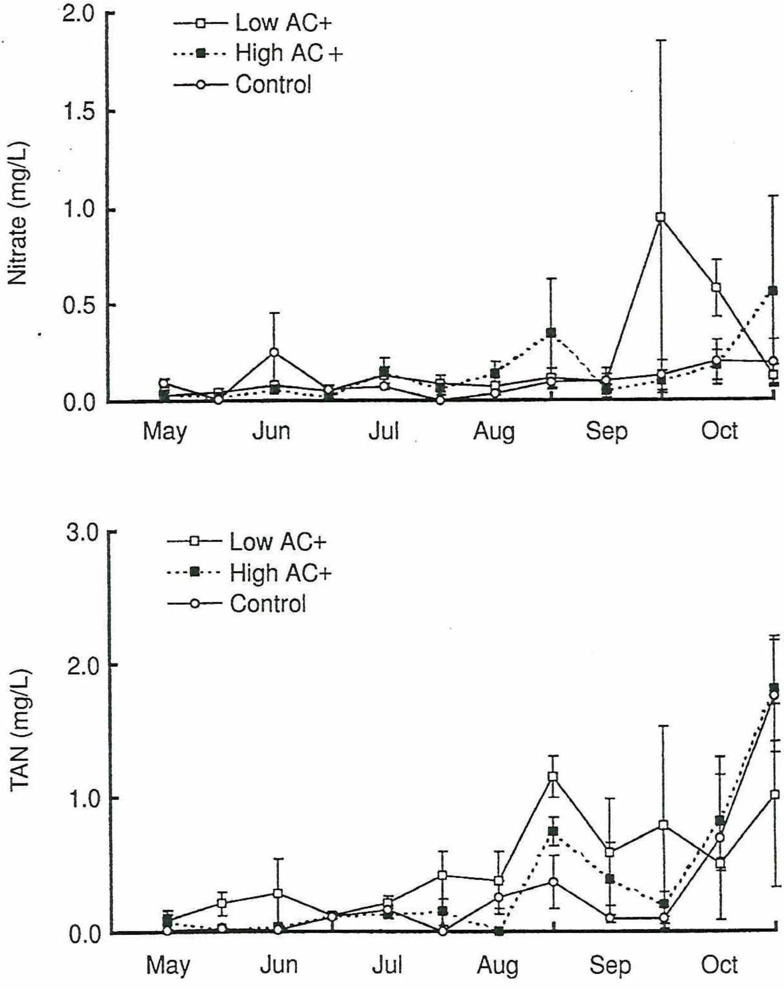


FIGURE 3. Means and standard errors of chemical and biochemical oxygen demand (COD and BOD, respectively) measured biweekly in earthen ponds in the Fisheries Research Unit, Auburn, Alabama, that were stocked with 15,000 channel catfish/ha between May to October 1996. Ponds were treated with no, low, and high concentrations of bio-organic catalysts amendments (Control, Low AC+, and High AC+, respectively).

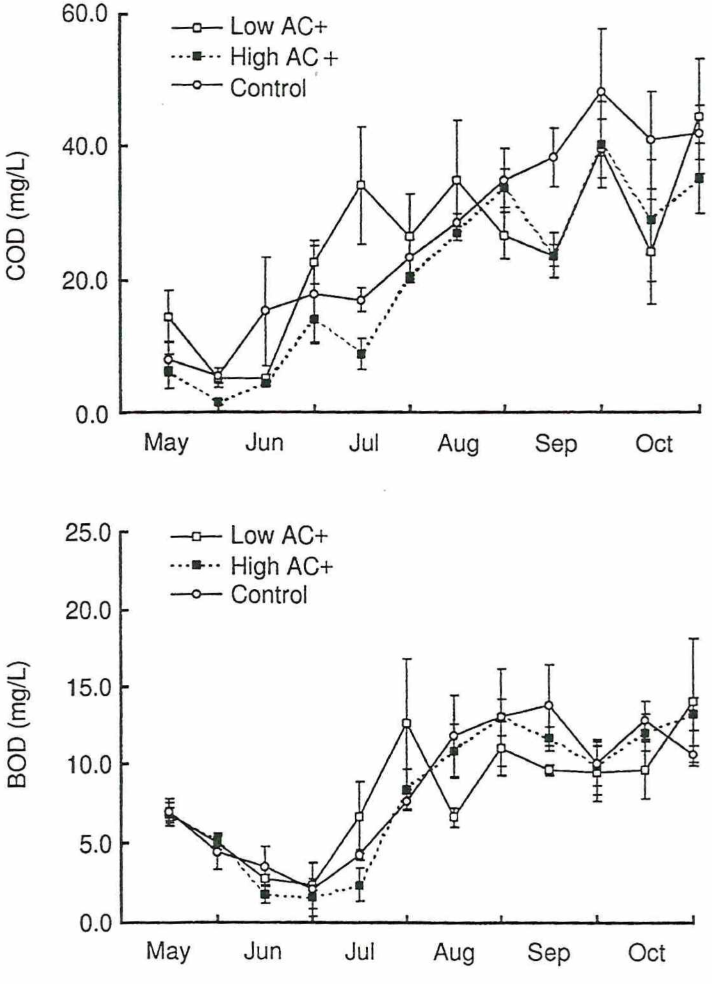
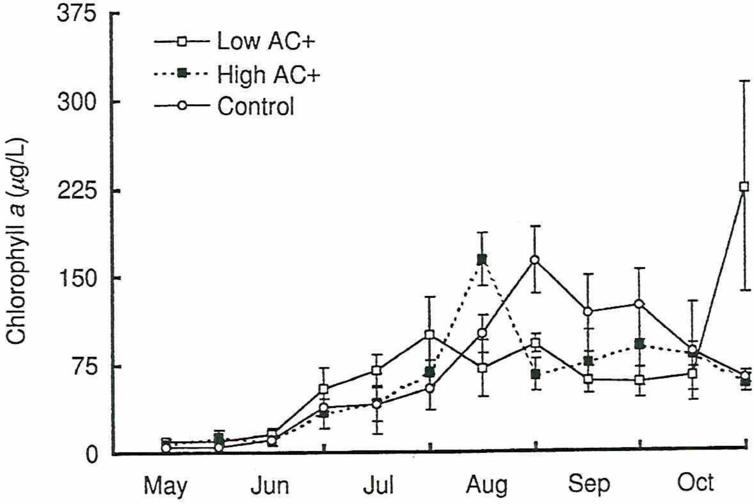


FIGURE 4. Means and standard errors of chlorphyll *a* and bacteria count, measured biweekly in earthen ponds in the Fisheries Research Unit, Auburn, Alabama, that were stocked with 15,000 channel catfish/ha between May to October 1996. Ponds were treated with no, low, and high concentrations of bio-organic catalysts amendments (Control, Low AC+, and High ACT+, respectively).



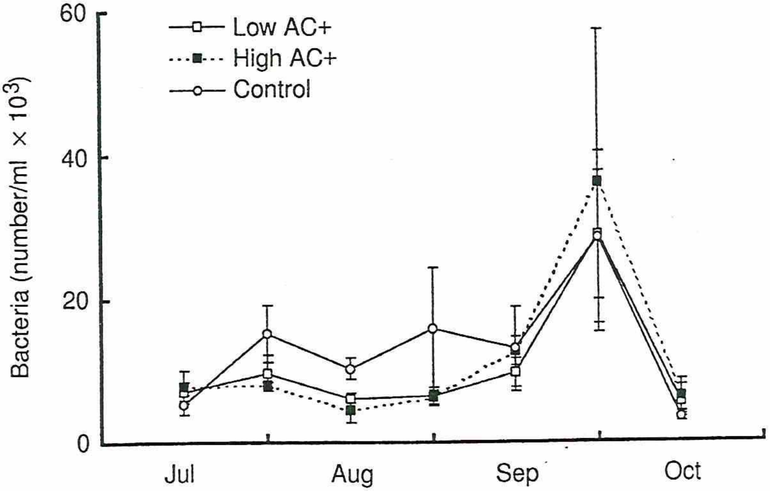


TABLE 1. Seasonal means ± SE of water quality data collected from channel catfish ponds treated with extracellular enzymes at 4-week intervals (low AC+), weekly intervals (high AC+), and controls. There were three replications of each treatment. Means in a row with different letters were significantly different ($P < .01$).

	Treatment		
Variables	Low AC+	High AC+	Control
Dissolved oxygen (mg/L)	5.25 ± 0.44a	5.59 ± 0.41a	5.26 ± 0.59a
Soluble reactive phosphorus (mg/L)	0.039 ± 0.037a	0.038 ± 0.022a	0.020 ± 0.009b
Total ammonia nitrogen (mg/L)	0.450 ± 0.191a	0.350 ± 0.185a	0.280 ± 0.187a
Nitrate nitrogen (mg/L)	0.180 ± 0.15a	0.130 ± 0.093a	0.090 ± 0.046a
Chemical oxygen demand (mg/L)	23.5 ± 5.1a	19.0 ± 4.8a	25.1 ± 5.4a
Biochemical oxygen demand (mg/L)	7.8 ± 1.5a	7.8 ± 1.5a	8.1 ± 1.5a
Chlorophyll *a* (μg/L)	64.5 ± 23a	54.5 ± 16.7a	62.2 ± 19.7a
Bacterial abundance (cells/mL × 10^3)	10.5 ± 3.2a	11.7 ± 5.3a	13.0 ± 4.0a

The only significant difference was for SRP concentration, and the difference resulted from high concentrations of SRP in AC+ ponds on a few dates.

Pond soils on the FRU do not naturally contain free calcium carbonate (McNutt 1981), and only small amounts of residual calcium carbonate could have resulted from agricultural limestone applications. Therefore, the total carbon concentrations in pond soil were considered to result primarily from organic carbon. In May, averages and standard deviations for percentages of soil carbon were as follows: control, 0.81 ± 0.15; low AC+, 1.00 ± 0.30; high AC+, 0.70 ± 0.18. Soil carbon increased in all ponds during the study, and percentages found in October were: control, 1.26 ± 0.47; low AC+, 1.40 ± 0.91; high AC+, 1.21 ± 0.59. These data suggest that AC+ treatment did not influence the rate of increase of soil organic carbon in pond bottoms.

Fish production data are summarized in Table 2. A portion of the fish in all ponds were infected by proliferative gill disease and enteric septicemia of catfish, *Edwardsiela ictaluri*, and survival ranged from 56.1% in controls to 75.7% in the high AC+ ponds. Nevertheless, similar amounts of feed were applied in all ponds, and at harvest fish were similar in size in all ponds. Feed conversion ratio and net fish production did not differ among treatments ($P > 0.1$).

TABLE 2. Mean production data ± SE for ponds treated with extracellular enzymes at 4-week intervals (low AC+), weekly intervals (high AC+), and controls. There were three replications of each treatment. Ponds were stocked at 15,000/ha.

	Treatment		
Variables	Low AC+	High AC+	Control
Feed input (kg/ha)	6,128a	6,298a	6,008a
Survival (%)	68.2 ± 8.6ab	75.7 ± 7.42a	56.1 ± 6.3b
Harvest weight (g)	342 ± 32a	328 ± 36.8a	400 ± 13.8a
Net yield (kg/ha)	3,502 ± 300.8a	3,728 ± 502.8a	3,302 ± 279.2a
Feed conversion ratio	1.75 ± 0.12a	1.77 ± 0.0a	1.82 ± 0.12a

DISCUSSION

Even though ponds were aerated during the night, there was a trend of higher dissolved oxygen concentrations in the ponds treated with AC+ as compared to control ponds (Figure 1). This trend in dissolved oxygen concentration was most obvious in August and September; the trend resulted because of the tendency for higher concentrations of COD and BOD, and more phytoplankton in control ponds than in AC+ ponds caused a greater nighttime demand for oxygen in control ponds during August and September. Nevertheless, when phytoplankton abundance and concentrations of dissolved oxygen, COD, and BOD were averaged over all dates, they did not differ ($P > 0.1$) among treatments (Table 1).

One explanation for the lower oxygen demand at times in AC+ ponds is that additions of BOCs accelerated the decomposition of organic matter to cause lower BOD and COD concentrations. This explanation is consistent with the observation that SRP, TAN, and NO_3^--N concentrations sometimes tended to be greater in AC+ ponds (Figures 1 and 2), because greater decomposition would have caused more nutrient recycling. However, greater nutrient recycling should have stimulated phytoplankton productivity in the AC+ ponds, but this effect was not observed. Also, similar rates of organic carbon accumulation in pond soils of the three treatments is not consistent with enhanced decomposition of organic matter in AC+ ponds.

A more feasible explanation for the apparent trend in higher concentrations of chlorophyll *a*, BOD and COD at times in the control ponds is that AC+ reduced the phytoplankton growth rate. Boyd (1973) and Boyd et al.

(1978) demonstrated that BOD and COD concentrations in pond waters had a strong positive correlation with chlorophyll *a* concentrations (phytoplankton abundance). An increase in chlorophyll *a* concentration should cause an increase in BOD and COD concentrations. Nutrient inputs to all treatments in feed were similar, and the tendency of greater nutrient concentrations in AC+ ponds relative to controls could have resulted from less uptake of nutrients by smaller phytoplankton biomass in AC+ ponds instead of greater rates of nutrient recycling in AC+ ponds. Boyd and Musig (1980) showed that the rate of decline in spikes of SRP made to pond waters increased with increasing phytoplankton abundance. Tucker et al. (1984) demonstrated that TAN concentrations in fish pond waters decreased as phytoplankton abundance increased. Thus, the apparent trend in lower nutrient concentrations and in higher BOD and COD values in control ponds is consistent with the explanation that AC+ reduced the phytoplankton abundance. Further support of the effects of AC+ on phytoplankton growth rates comes from the claim by the manufacturer that AC+ can be used to reduce the abundance of nuisance algal growth in water. Because AC+ is a proprietary product, its composition was not divulged by Neozymes International. Without this information, no speculation can occur on the mechanism by which AC+ might have influenced phytoplankton growth.

Differences in fish survival were observed (Table 2). This result could be related to the actions of the AC+; however, because of the high variation among the ponds, the limited number of replicates, and the disease problems that were observed, further investigation of the effects of AC+ is needed.

The apparent improvement in dissolved oxygen concentration in AC+ ponds did not cause greater fish production. This is not surprising because the stocking rate was conservative and the feeding rate did not exceed 75 kg/ha per day. When mechanical aeration is used, dangerously low dissolved oxygen concentrations, excessive TAN concentrations, and other water quality impairment seldom occur unless feeding rates exceed 100 to 120 kg/ha per day (Boyd 1990; Tucker and Boyd 1995). The likelihood of water quality improvement and enhanced fish production through the use of BOC supplementation would be greater in ponds where stocking and feeding rates are high enough to cause more severe water quality deterioration than was observed in the present study.

This study shows that BOC's supplementation did not seem to improve water quality even in aquaculture ponds stocked and fed at conservative rates. Further studies are needed to show if greater benefit can be achieved in ponds where stocking and feeding rates are higher and water quality conditions are more critical. Information on the mechanisms of action of extracellular enzyme additions in ponds would also be useful in assessing the potential benefits of these products and defining the conditions under which they can be effective.

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