Aquaculture CRSP 21st Annual Technical Report

Use of Clinoptilolite Zeolites for Ammonia-N Transfer and Retention in Integrated Aquaculture Systems and for Improving Pond Water Quality before Discharge

Tenth Work Plan, Appropriate Technologies Research 5 (10ATR5)
Final Report

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Abstract

Five experiments were conducted at the Asian Institute of Technology (AIT, Thailand) during February–July 2002 to assess the potential of crushed (1–2 mm diameter) clinoptilolite zeolite to: 1) transfer ammonia-N from animal manures to fertilize aquaculture ponds, 2) moderate ammonia concentrations in fertilized culture water, and 3) remove ammonia-N from discharged pond water. The clinoptilolite used in this study was found to absorb about 1.91 g N kg^{-1} when soaked in an ammonium chloride solution, but absorbed very little nitrogen when soaked in a solution of urea. When soaked in either fresh swine or chicken manure slurries, the clinoptilolite absorbed about 0.43 g N kg^{-1} after one week—with most of the absorption occurring within the first two days of immersion. Other molecules in the manures likely out-competed ammonium ions for ion exchange sites in the clinoptilolite. Nevertheless, plastic mesh bags containing crushed clinoptilolite soaked in swine manure and replaced weekly were able to provide sufficient N to culture waters to promote algal production and Nile tilapia yields similar to tanks fertilized with urea. Results indicate that clinoptilolite technologies applied to livestock-fish integrated systems could increase on-farm nitrogen utilization efficiencies by capturing and recycling manure-N before it is otherwise lost through ammonia volatilization or nitrate leaching, and to use that nitrogen as a fertilizer to promote algal productivity in culture ponds. Unfortunately, a simple economic analysis does not encourage this approach for small-scale farmers. In addition, the anticipated effect of moderating ammonia levels in culture water by clinoptilolite was not obvious in the experiments. It was also found that clinoptilolite did not significantly reduce nitrogen levels (both total ammonia and total nitrogen) of aquaculture discharge water. These results were likely due to the relatively low ammonia concentrations in the water and the limited contact time with the clinoptilolite. Adequately assessing the potential for clinoptilolite to capture and recycle nitrogen from aquaculture wastewater will require further research.

Introduction

Animal manures have long been used for pond fertilization because they contain the three major algal nutrients: nitrogen (N), phosphorus (P), and carbon (C). A major concern with putting manures into ponds, however, is the biochemical oxygen demand (BOD) from the microbial decomposition of organic matter. Too much manure with too little pond aeration can cause serious depletion of dissolved oxygen (DO) and significant mortalities of culture organisms. The research described below examined the use of clinoptilolite zeolites as a vehicle for transferring manure-N into a pond without including the detrimental BOD in the process.

Natural zeolites are aluminosilicate minerals found in volcanogenic sedimentary rocks worldwide (Mumpton, 1999). Natural zeolites possess several important properties including adsorption, cation-exchange, dehydration-rehydration, and catalysis. Considerable scientific research in the last few decades has identified broad applications for natural zeolites in construction materials, soil improvements for water and nutrient retention, treatment of water and wastewater for removal of heavy metals and nutrients, dietary supplements for farm-raised animals, health care, and other beneficial uses (Mumpton, 1999).

Clinoptilolite zeolites, $(Na_3K_3)6(Al_{12}Si_{30}O_{72}).24H_2O$, are one of about 40 types of naturally existing zeolites. Clinoptilolites possess a cation-exchange capability of about 2.25 meq g^{-1}, and are able to exchange ammonium-N with sodium (Na) and potassium (K) ions (Mumpton, 1999). Theoretically, one gram of clinoptilolite can take in about 2.2 mg ammonium-N. This cation-exchange capability has been utilized effectively for terrestrial agriculture, where clinoptilolites are first saturated with ammonium-N and then incorporated into crop soils. In this way they act as a slow-release fertilizer, with plants able to extract the sequestered ammonia from the clinoptilolite (Barbarick and Pirela, 1984; Lewis et al., 1984; Dwairi, 1998). Not only does clinoptilolite improve nitrogen fertilization efficiencies, it also reduces nitrate leaching by inhibiting the nitrification of ammonium to nitrate (Perrin et al., 1998). Most of the manure-ammonia sequestered in the zeolite is unavailable to nitrifying bacteria because of the small (4–5 angstrom) pore size of the crystal lattice structure (Mumpton, 1999). Furthermore, clinoptilolites are also used for animal waste management. Clinoptilolites are replacing clays in the cat litter industry, and are being used to create an odorless, nitrogen-rich compost from livestock manures.

The use of clinoptilolites in aquaculture has focused on ammonia removal for the aquarium industry and freshwater culture systems (Bower and Turner, 1982; Dryden and Weatherley, 1987). The research below examined an additional use of clinoptilolite for aquaculture analogous to terrestrial applications for agriculture and animal waste management: i.e., as a vehicle for ammonia absorption and subsequent fertilization to stimulate algal productivity.

Specifically, the objectives of the research described in this report were to:

1. Determine the relationship between total ammonia-N (TAN) absorption/saturation by clinoptilolite as a function of exposure time to fresh swine manure and liquified chicken manure,
2. Evaluate the release of TAN from ammonia-enriched clinoptilolite when used as a N fertilizer for stimulating algal production in an outdoor system,
3. Determine the ability of clinoptilolite to moderate TAN concentrations in an outdoor fertilized culture system, and
4. Evaluate the effectiveness of clinoptilolite for removing TAN from discharged pond water.

METHODS AND MATERIALS

This study was conducted at the Asian Institute of Technology (AIT), Pathumthani, Thailand, within the Agriculture, Aquatic Systems and Engineering Program. There were a total of five experiments designed to achieve the above objectives. The crushed clinoptilolite (about 1–2 mm diameter) used in the study originated from Potosí, Mexico (supplied by Geoexplorers International, Inc., Denver, Colorado), and had an exchangeable potassium:sodium ratio of about 8:1. Fresh chicken and swine manures were collected from broiler and pig houses of local farms. Manures were liquified by adding water followed by thorough mixing before each experiment. Making the manures more liquid facilitates the cation-exchange process between K and ammonium ions.

Experiment 1. The first experiment was a preliminary analysis of clinoptilolite's capacity to adsorb ammonia-N and/or urea, and to compare different analytical methods for estimating TAN in clinoptilolite. Fifty grams of clinoptilolite, which had been pre-washed five times with 200 ml deionized water and dried at 105°C for two hours, were placed into each of three 1-l flasks. One flask then received 500 ml of a 2M ammonium chloride solution, the second flask received 500 ml of a 2M urea solution, and the third flask received 250 ml of the 2M ammonium chloride and 2M urea solutions. After immersion for four days, the clinoptilolite was removed from the solutions, washed five times with 200 ml deionized water, and dried overnight at 70°C. Subsamples of about 1 g each were randomly taken from the semi-dried clinoptilolite removed from the three treatment solutions. Clinoptilolite subsamples were analyzed for TAN content directly, or after drying at 105°C for two hours, using either Kjeldahl nitrogen analysis (Brenner and Mulvaney, 1982) or a phenate method (APHA, 1985) following ammonia-N extraction using 50 ml of 2M potassium chloride solution for two days.

Experiment 2. The second experiment used a 2×2 factorial design to test the effects of manure types and agitation on TAN absorption by clinoptilolite. The experiment was conducted in twelve 20-l plastic buckets, with three randomly selected buckets for each treatment. The four treatments were: 1) chicken manure slurry with agitation (CM-A), 2) chicken manure slurry without agitation (CM-NA), 3) swine manure with agitation (SM-A), and 4) swine manure without agitation (SM-NA). Each bucket received 7.5 kg manure and 7.5 l tap water, and was mixed thoroughly using a bamboo stick. Crushed clinoptilolite was placed in small-mesh nylon bags (16 meshes per inch), with about 1 kg clinoptilolite per bag. There was one bag per bucket, suspended in the manure by hanging it from the bucket handle. Bags in the agitation treatments were vertically hand-agitated for 30 seconds before and after each sampling. One clinoptilolite subsample of about 3 g was taken from each bag at 0, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 hours after the bags were first immersed into the buckets, and analyzed for TAN content. Samples of liquified manures taken at the beginning and end of the experiment were also analyzed for TAN.

Experiment 3. The third experiment evaluated the efficiency of TAN release from clinoptilolite enriched with ammonia, and subsequent uptake by algae. The experiment was conducted in eighteen 20-L plastic buckets. Based on the results of Experiment 2, crushed clinoptilolite containing six different levels of ammonia-N was prepared by immersing clinoptilolite in chicken manure slurry for 10 minutes, 0.5, 2, 8, 36, and 72 hours. TAN content in these six batches of clinoptilolite were determined to be 0.07%, 0.12%, 0.16%, 0.22%, 0.32%, and 0.37%, respectively. These were designated as treatments 1 through 6, and allocated randomly to buckets in triplicate. Each bucket was filled with 5 L of water collected from the top 30 cm of a green-water pond and diluted with clear tap water at a 1:2 ratio. To bring the same N level (i.e., 0.045 g per bucket) in all treatments, clinoptilolite was added at 64.3g, 37.5g, 28.1g, 20.5g, 14.1g, and 12.2 g per bucket in treatments 1 through 6, respectively. Triple super phosphate (TSP) was added at a rate of 0.015 g P per bucket in all treatments to provide sufficient P for algal growth.

Water samples were collected daily at 1000 h from all buckets to estimate concentrations of chlorophyll *a* using a hand-held fluorometer (Turner Designs Aquafluor). Dissolved oxygen (DO) concentrations were measured at 1800 h, 0600 h, and 1800 h for two consecutive days using a DO meter (WTW, model Oxi 320) to estimate 12-hour gross (GPP) and net (NPP) primary productivity (Hall and Moll, 1975). Clinoptilolite samples were taken at the beginning and end of the culture period for TAN analysis.

Experiment 4. The fourth experiment was designed to determine the efficiency of ammonia release from different loading rates of ammonia-enriched clinoptilolite in fertilized concrete tanks (2.5m × 2m × 1m) for fish culture, and to assess the ammonia moderation effects of unenriched-clinoptilolite added to fertilized tanks. Clinoptilolite was packed in small-mesh nylon bags at 1.25 kg per bag, immersed in swine manure slurry for one week, and placed in tanks as a fertilizer for the following week at loading rates of 2, 3, and 4 bags per tank (treatments designated as M-Cp-2, M-Cp-3, and M-Cp-4, respectively).

Table 1. Mean (± 1 SE, n = 5) percent total ammonia nitrogen (TAN) measurements of clinoptilolite samples enriched with different solutions using two different analytical methods.

Treatment Combinations			TAN content (%)*
Immersion Solutions	*Drying*	*Analytical Methods*	
NH_4Cl	Dry	Phenate after KCl extraction	0.99 ± 0.05 [d]
NH_4Cl	Semi-dry	Phenate after KCl extraction	1.04 ± 0.04 [d]
NH_4Cl	Dry	Kjeldhal	1.65 ± 0.08 [bc]
NH_4Cl	Semi-dry	Kjeldhal	1.91 ± 0.01 [a]
Urea	Dry	Phenate after KCl extraction	0.03 ± 0.01 [f]
Urea	Semi-dry	Phenate after KCl extraction	0.06 ± 0.00 [f]
Urea	Dry	Kjeldhal	0.08 ± 0.02 [f]
Urea	Semi-dry	Kjeldhal	0.16 ± 0.01 [f]
NH_4Cl + Urea	Dry	Phenate after KCl extraction	0.89 ± 0.04 [e]
NH_4Cl + Urea	Semi-dry	Phenate after KCl extraction	0.93 ± 0.03 [e]
NH_4Cl + Urea	Dry	Kjeldhal	1.60 ± 0.04 [c]
NH_4Cl + Urea	Semi-dry	Kjeldhal	1.76 ± 0.05 [b]

Mean values with different superscript letters in the same column are significantly different.

Table 2. Mean (± 1 SE, n = 3) percent total ammonia nitrogen (TAN) as measured in clinoptilolite samples immersed in chicken and swine manure slurries for different lengths of time with and without agitation.

Time (hours)	TAN (%) Absorbed in Clinoptilolite Immersed in:			
	Chicken Manure		*Swine Manure*	
	Agitation	Non-agitation	Agitation	Non-agitation
0	0.00	0.00	0.00	0.00
0.25	0.10 ± 0.01	0.06 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
0.5	0.12 ± 0.01	0.09 ± 0.02	0.17 ± 0.02	0.15 ± 0.01
1	0.14 ± 0.00	0.09 ± 0.01	0.20 ± 0.00	0.16 ± 0.00
2	0.14 ± 0.01	0.14 ± 0.00	0.21 ± 0.02	0.19 ± 0.01
4	0.20 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
8	0.20 ± 0.00	0.17 ± 0.01	0.25 ± 0.02	0.21 ± 0.01
24	0.24 ± 0.01	0.19 ± 0.01	0.34 ± 0.01	0.32 ± 0.00
48	0.40 ± 0.01	0.33 ± 0.01	0.38 ± 0.02	0.33 ± 0.01
96	0.43 ± 0.01	0.32 ± 0.03	0.38 ± 0.01	0.40 ± 0.02
168*	0.43 ± 0.00[a]	0.37 ± 0.00[b]	0.43 ± 0.03[a]	0.43 ± 0.01[a]

Clinoptilolite bags without ammonia enrichment were placed in tanks fertilized with urea (at a rate of 30 kg N ha^{-1} $week^{-1}$) at the same loading rates as above (treatments designated as NM-Cp-2, NM-Cp-3, and NM-Cp-4, respectively). The seventh treatment served as the control, where tanks were also fertilized with urea at the above rate but did not contain any clinoptilolite bags. All tanks were fertilized with TSP at a rate of 10 kg P ha^{-1} $week^{-1}$. Nile tilapia fingerlings of about 10 g in size were stocked at 3 fish per m^2 in all tanks. All treatments were triplicated and assigned randomly to experimental tanks, and the experiment lasted six weeks.

Clinoptilolite samples were taken for TAN analysis before and after the experiment, and before and after each ammonia enrichment. Water levels were maintained at 1 m using tap water. Water column samples were taken from all tanks weekly at 0600 h for TAN analyses and chlorophyll *a* measurements. Gross and net primary productivities were estimated weekly by integrated changes in diel DO concentrations using a DO meter measured at 30 cm, 50 cm, and 80 cm (Hall and Moll, 1975). All Nile tilapia were weighed individually at the beginning and end of the experiment. Nile tilapia were harvested using a fixed net without discharging water.

Experiment 5. The fifth experiment examined the application of clinoptilolite for reclaiming ammonia-N from culture water discharge. Twelve randomly-selected tanks from Experiment 4 were used for this experiment. Crushed clinoptilolite, packed in small-mesh nylon bags at 250 g per bag, was immersed into tank water for three days. The four treatments were 0 (control), 2, 3, and 4 bags per tank. Treatments were allocated randomly to tanks in triplicate. Column water samples were taken from each tank for the analyses of TAN, nitrate-N, nitrite-N , TKN, and TN before and after the experiment.

Data for all experiments were analyzed statistically using regression analysis and one-way, two-way, and repeated measurement analysis of variance (ANOVA) using SPSS (version 9.01) statistical software package. Differences were considered significantly different at an alpha probability of 0.05. Means are given with ± 1 standard error (s.e.).

RESULTS

Experiment 1. Results indicate that the Kjeldhal method for TAN analysis gave results about 55% greater than the phenate method (Table 1). The differences between the semi-dry and dry clinoptilolite were not significantly different, although the semi-dry gave results about 8% greater than dry within each analytical method. Therefore, subsequent experiments analyzed TAN in semi-dry clinoptilolite using the Kjeldhal method. Under these conditions, clinoptilolite soaked in a NH_4Cl solution had a mean TAN concentration of 1.91%. The ability to absorb urea was significantly less, averaging 0.16%. When ammonia and urea were mixed together, the TAN content in clinoptilolite was about 1.76%.

Experiment 2. TAN absorption by clinoptilolite averaged 0.43% after seven days (168 hours) immersion in swine manure (Table 2). Nearly 80% of the total TAN absorption from swine manure occurred within the first 48 hours of immersion (Table 2, Figure 1). There were no significant differences in TAN absorption between agitated versus non-agitated bags. TAN absorption by clinoptilolite immersed in chicken manure slurry was a little slower than observed with swine manure (Table 2, Figure 1). After 48 hours immersion, however, over 90% of the TAN absorbed after 7 days had already occurred. Unlike swine manure, agitating the clinoptilolite bags in the chicken manure slurries did result in a significantly higher TAN absorption ($P < 0.05$). With agitation, TAN absorption was the same as the swine manure (0.43%). Without agitation, TAN absorption was about 15% less than with agitation after one week. For all four treatments, TAN absorption by clinoptilolite showed a highly significant exponential relationship ($P < 0.001$, r^2 values ranged from 0.912–0.954). Figure 2 illustrates this log-linear relationship for the swine manure non-agitation treatment.

Experiment 3. Results indicate that initial TAN concentrations in the clinoptilolite had no significant effect on the subsequent release of TAN in green water after 7 days (Table 3). Over all treatments, an average of about 86% of the initial 0.045 g TAN per bucket absorbed in the clinoptilolite was released after 7 days immersion. Comparative measurements of GPP, NPP, TAN, and chlorophyll *a* in the water also did not show any significant differences between treatments (Table 4). There was also no significant linear relationship between initial TAN concentrations in the clinoptilolite and either GPP, NPP, and TAN in the water. However, the linear relationship between initial TAN concentrations in the clinoptilolite and chlorophyll *a* concentrations was significantly positive ($P < 0.05$, Figure 3).

Experiment 4. Table 5 provides the fish growth data for the fourth experiment. The percent survival of treatment fish averaged about 97% in the three swine manure treatments (M-Cp), as compared to about 84% in the three clinoptilolite treatments without swine manure (NM-Cp). Fish survival in the control treatment without clinoptilolite bags averaged about 93%. Fish growth in the NM-Cp treatments was not significantly different from the control, averaging about 28–33 g per fish. Fish yields in the M-Cp treatments, however, increased with increasing number of clinoptilolite bags (Figure 4). With four bags, treatment mean weight was 46.7 g per fish. Daily weight gain also increased with as the number of bags increased (Table 5).

Table 6 provides the water quality data collected for Experiment 4. Early morning DO concentrations showed a negative

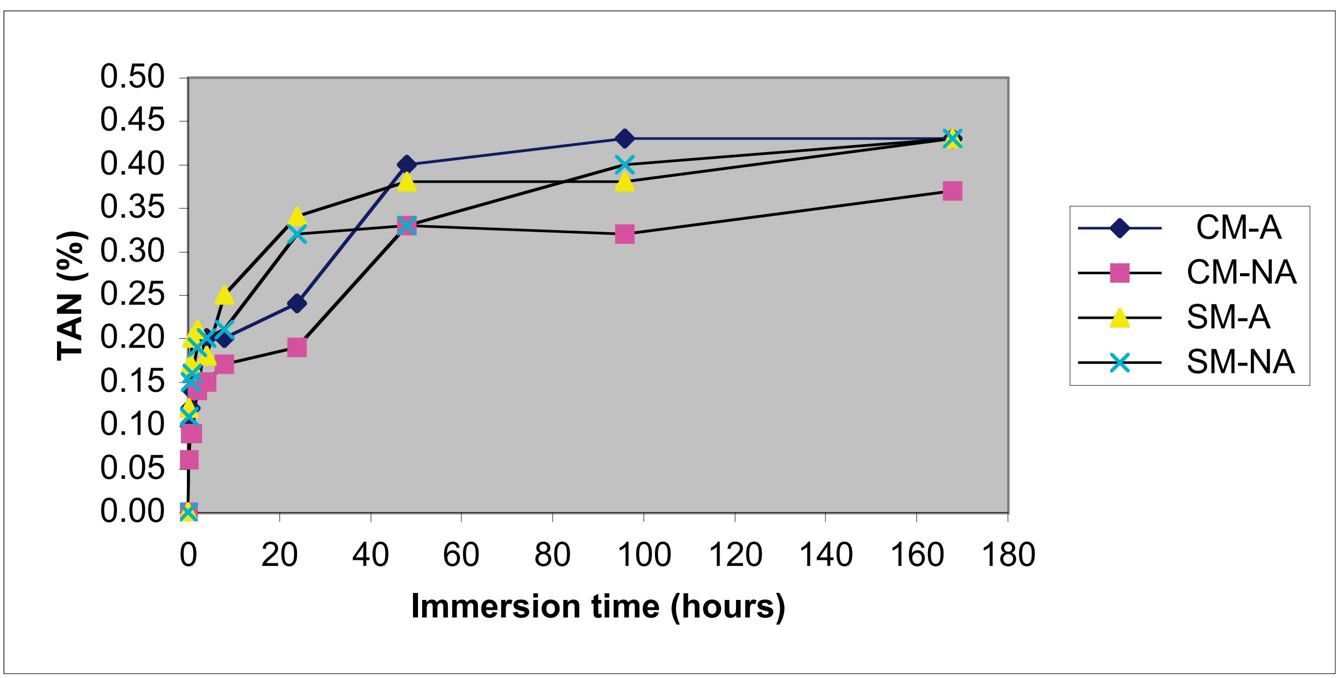

Figure 1. Total ammonia nitrogen (TAN) absorption from liquefied chicken and swine manure by clinoptilolite in Experiment 2. Treatments: CM-A = chicken manure slurry with agitation; CM-NA chicken manure slurry without agitation; SM-A = swine manure with agitation; SM-NA swine manure without agitation.

Table 3. Mean reductions (± 1 SE, n = 3) of percent and actual total ammonia nitrogen (TAN) content in ammonia-enriched clinoptilolite before and after a 7-day immersion in outdoor buckets with green pond water. Treatments were different initial TAN concentrations in the clinoptilolite before immersion in outdoor buckets.

Treatments	DO at 0600 h	DO at 1800 h	GPP	NPP	TAN	CHL-*a*
(% TAN)	*(mg/L)*	*(mg/L)*	*(O_2, mg/L)*	*(O_2, mg/L)*	*(mg/L)*	*(g/L)*
0.07	7.2 ± 0.36	12.7 ± 0.58	11.1 ± 0.77	5.5 ± 0.45	0.02 ± 0.01	77.2 ± 9.8
0.12	7.1 ± 0.37	13.5 ± 0.52	12.4 ± 0.71	6.4 ± 0.46	0.07 ± 0.02	81.3 ± 8.9
0.16	7.0 ± 0.34	13.3 ± 0.54	12.5 ± 0.77	6.4 ± 0.45	0.06 ± 0.01	92.2 ± 13.6
0.22	7.0 ± 0.36	13.0 ± 0.54	11.8 ± 0.74	6.0 ± 0.45	0.03 ± 0.01	81.5 ± 9.5
0.32	7.0 ± 0.36	13.5 ± 0.56	12.9 ± 0.71	6.6 ± 0.43	0.06 ± 0.02	105.5 ± 15.3
0.37	6.6 ± 0.33	12.8 ± 0.61	12.0 ± 0.90	6.1 ± 0.50	0.04 ± 0.01	102.4 ± 14.3

Table 4. Treatment means (± 1 SE, n = 3) of dissolved oxygen (DO), gross primary productivity (GPP), net primary productivity (NPP), total ammonia nitrogen (TAN), and chlorophyll *a* (CHL-*a*) in buckets with green pond water following a 7-day immersion of clinoptilolite with different intitial %TAN concentrations.

Treatments	DO at 0600 h	DO at 1800 h	GPP	NPP	TAN	CHL-*a*
(% TAN)	*(mg/L)*	*(mg/L)*	*(O_2, mg/L)*	*(O_2, mg/L)*	*(mg/L)*	*(g/L)*
0.07	7.2 ± 0.36	12.7 ± 0.58	11.1 ± 0.77	5.5 ± 0.45	0.02 ± 0.01	77.2 ± 9.8
0.12	7.1 ± 0.37	13.5 ± 0.52	12.4 ± 0.71	6.4 ± 0.46	0.07 ± 0.02	81.3 ± 8.9
0.16	7.0 ± 0.34	13.3 ± 0.54	12.5 ± 0.77	6.4 ± 0.45	0.06 ± 0.01	92.2 ± 13.6
0.22	7.0 ± 0.36	13.0 ± 0.54	11.8 ± 0.74	6.0 ± 0.45	0.03 ± 0.01	81.5 ± 9.5
0.32	7.0 ± 0.36	13.5 ± 0.56	12.9 ± 0.71	6.6 ± 0.43	0.06 ± 0.02	105.5 ± 15.3
0.37	6.6 ± 0.33	12.8 ± 0.61	12.0 ± 0.90	6.1 ± 0.50	0.04 ± 0.01	102.4 ± 14.3

Table 5. Mean (± 1 SE, n = 3) growth performance and survival data for Nile tilapia raised in outdoor tanks with either 2, 3, or 4 mesh bags of clinoptilolite soaked in swine manure (M-Cp-2, M-Cp-3, and M-Cp-4, respectively), 2, 3, or 4 bags of clinoptilolite not soaked in swine manure (NM-Cp-2, NM-Cp-3, and NM-Cp-4, respectively), or fertilized without clinoptilolite bags (Control).

Treatment	Initial		Final		Mean Daily Weight Gain (g/d/fish)	Mean Total Weight Gain (g/tank)	Survival (%)
	Total Weight (g/tank)	*Mean Weight (g/fish)*	*Total Weight (g/tank)*	*Mean Weight (g/fish)*			
M-Cp-2	130.6	8.7 ± 0.47	463.4	32.3 ± 4.39	0.62 ± 0.11	332.8	95.6 ± 4.4
M-Cp-3	129.6	8.6 ± 0.43	586.9	40.0 ± 3.73	0.83 ± 0.09	457.3	97.8 ± 2.2
M-Cp-4	147.0	9.8 ± 0.90	677.4	46.7 ± 3.02	0.97 ± 0.37	530.4	96.7 ± 3.3
NM-Cp-2	136.2	9.1 ± 0.33	393.8	32.8 ± 3.54	0.62 ± 0.09	257.6	80.0 ± 7.7
NM-Cp-3	129.3	8.6 ± 0.61	345.9	28.0 ± 3.33	0.51 ± 0.09	216.6	82.2 ± 8.0
NM-Cp-4	130.3	8.7 ± 0.42	430.3	32.3 ± 4.83	0.62 ± 0.12	299.9	88.9 ± 5.9
Control	139.8	9.3 ± 0.71	381.9	27.3 ± 3.07	0.47 ± 0.08	242.1	93.3 ± 3.8

relationship with increasing number of manure bags in the M-Cp treatments (Figure 5). Early morning DO averaged about 6.0 mg l^{-1} in the M-Cp treatments, which was significantly less ($P < 0.001$) than the mean DO of 12.0 for the non-manure treatments. For GPP, NPP, TAN and chlorophyll *a* measurements there was no observed relationship with bag number. Water quality data for the control and NM-Cp treatments were very consistent among all water quality variables. Interestingly, GPP and NPP were a little higher, though not significantly, in the manure treatments as compared to the non-manure treatments. Furthermore, TAN measurements averaged about 0.23 mg l^{-1} in the manure treatments, significantly less ($P < 0.001$) than the

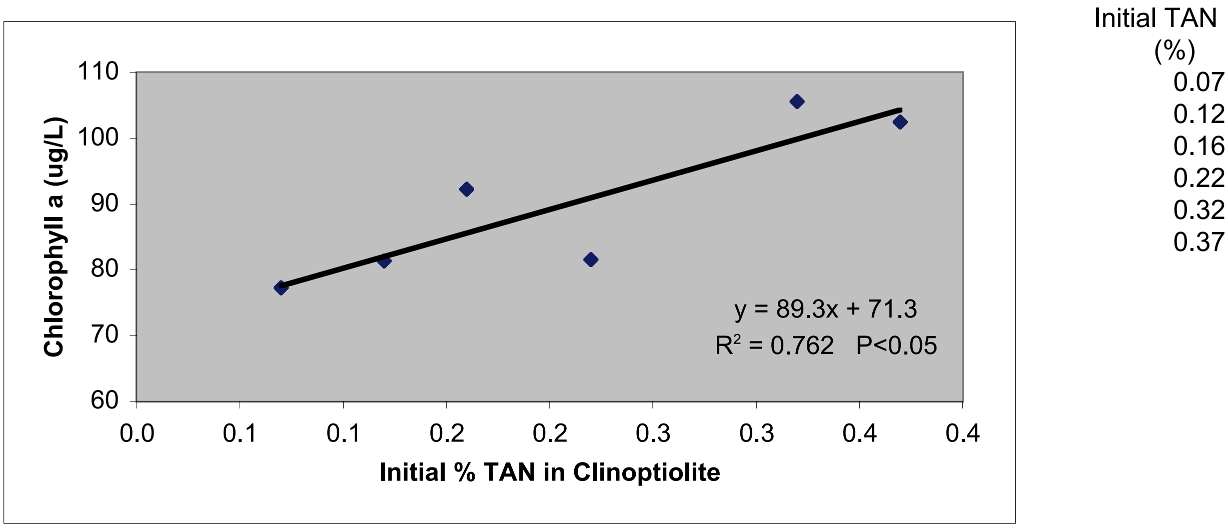

Figure 3. Relationship between initial total ammonia nitrogen (TAN) content in clinoptilolite and resulting chlorophyll *a* concentrations in pond water when the clinoptilolite was used as a nitrogen fertilizer in Experiment 3.

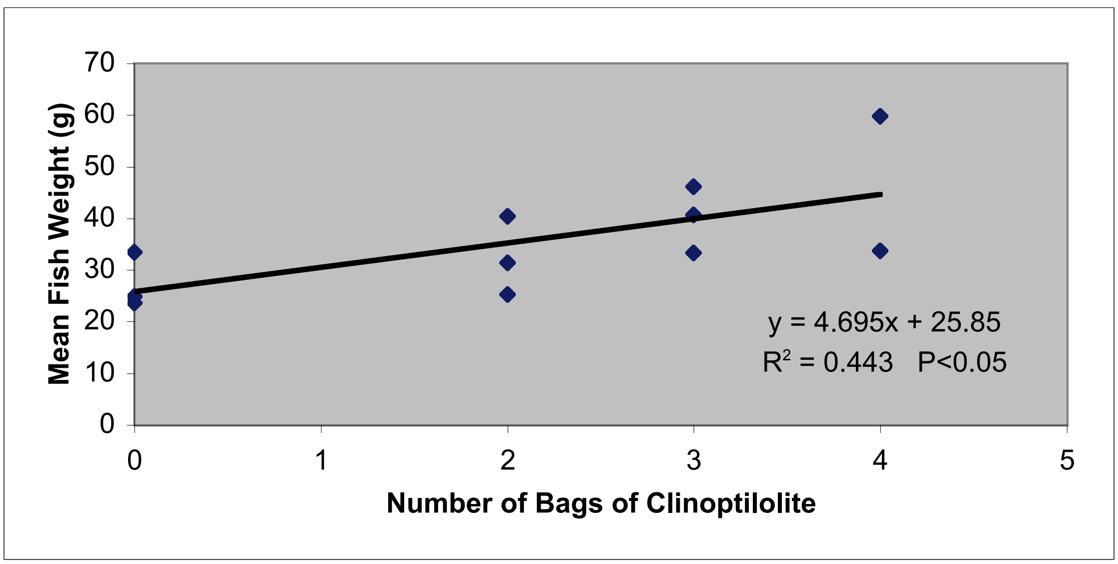

Figure 4. Relationship between the number of bags of clinoptilolite enriched with swine manure versus mean fish weight at harvest from Experiment 4.

1.34 mg l^{-1} average for the non-manure treatments. There was no relationship between TAN concentrations and the number of bags for either the M-Cp or NM-Cp treatments. Water temperatures ranged from 29–33°C during the experiment.

Experiment 5. Tank water quality data from Experiment 5 are given in Table 7. There was no statistically significant, observable reduction in any form of nitrogen in tank water with increasing number of bags of clinoptilolite in the tanks. Consistent with this observation, TAN concentrations in clinoptilolite remained undetectable after three days immersion in tank water.

DISCUSSION

Research described in this report represents the first documented investigation for applying clinoptilolite technologies for integrated aquaculture systems. The two general goals were to investigate whether clinoptilolite zeolite could benefit the farmer by providing a mechanism to transfer manure-N into ponds without adding unnecessary BOD, and to trap/remove ammonia-N from discharge water to be available for future pond fertilizations.

Ammonia absorption tests using ammonium chloride showed that the clinoptilolite used in this study was capable of taking in about 1.91 g N kg^{-1} clinoptilolite. This amount is similar to other reported values (e.g., 2.3 g N kg^{-1}, Perrin et al., 1998). The ability to absorb ammonium-N was significantly better than for urea, which averaged about 0.16 g N kg^{-1}. This makes sense, since the primary mechanism for uptake is through cation exchange, and urea is not an ionically-charged molecule. The clinoptilolite was able to absorb ammonium-N from both swine and chicken manure slurries. Maximum N absorption

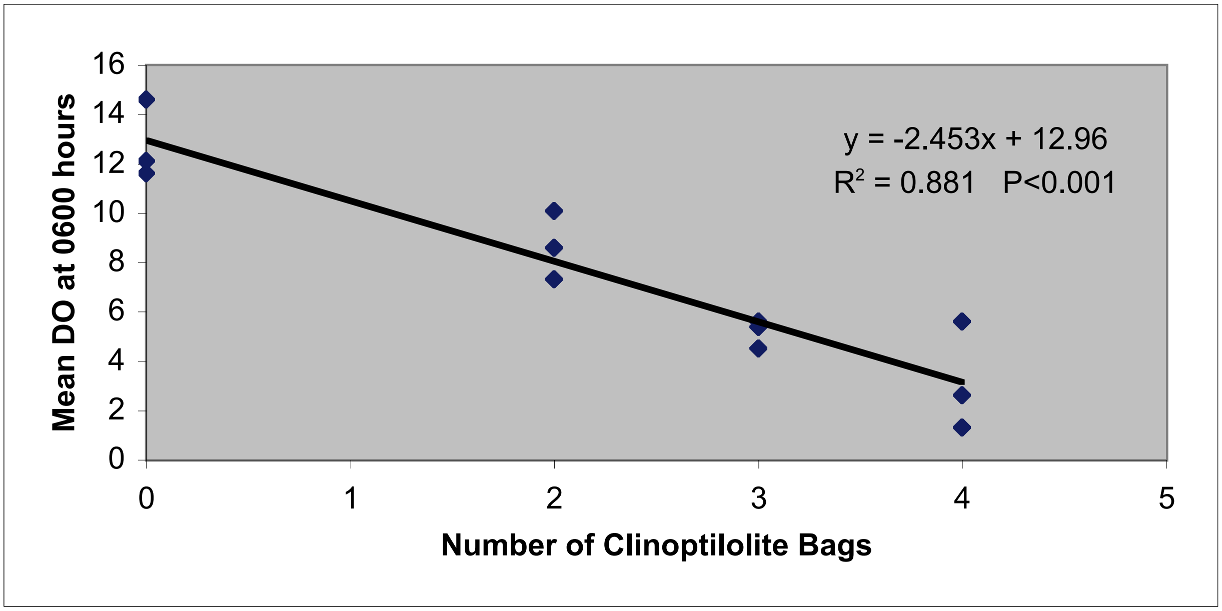

Figure 5. Relationship between the number of bags of clinoptilolite enriched with swine manure versus mean dissolved oxygen (DO) measurements made at 0600 h in Experiment 4.

Table 6. Treatment means (± 1 SE, n = 6) of dissolved oxygen (DO), gross primary productivity (GPP), net primary productivity (NPP), total ammonia nitrogen (TAN), and chlorophyll *a* (CHL-*a*) in outdoor tanks with either 2, 3, or 4 mesh bags of clinoptilolite soaked in swine manure (M-Cp-2, M-Cp-3, and M-Cp-4, respectively), 2, 3, or 4 bags of clinoptilolite not soaked in swine manure (NM-Cp-2, NM-Cp-3, and NM-Cp-4, respectively), or fertilized without clinoptilolite bags (Control).

Treatments	DO (mg/L)		GPP	NPP	TAN	CHL-*a*
	0600 h	*1800 h*	*(O_2, mg/L)*	*(O_2, mg/L)*	*(mg/L)*	*(g/L)*
M-CP-2	8.7 ± 0.5	16.2 ± 0.5	15.0 ± 0.7	7.3 ± 0.49	0.01 ± 0.01	56.4 ± 5.6
M-CP-3	5.2 ± 0.3	12.5 ± 0.4	14.6 ± 0.7	7.4 ± 0.53	0.31 ± 0.08	95.6 ± 8.4
M-CP-4	4.1 ± 1.0	11.1 ± 0.5	14.0 ± 1.2	7.2 ± 0.68	0.37 ± 0.12	68.2 ± 7.3
NM-CP-2	11.9 ± 0.5	18.0 ± 0.4	12.3 ± 1.0	6.5 ± 0.66	1.34 ± 0.20	55.0 ± 7.4
NM-CP-3	11.2 ± 0.8	17.7 ± 0.8	13.2 ± 0.9	6.4 ± 0.78	1.41 ± 0.19	66.8 ± 8.4
NM-CP-4	12.1 ± 0.8	18.2 ± 0.4	12.2 ± 0.8	6.1 ± 0.47	1.19 ± 0.19	59.5 ± 5.1
Control	12.7 ± 0.6	18.9 ± 0.4	12.4 ± 0.9	6.2 ± 0.63	1.40 ± 0.16	64.5 ± 8.8

reached about 0.43 g kg^{-1} clinoptilolite after 7 days immersion, with about 50% absorbed in the first 8 hours and 80–90% absorbed within the first 48 hours (Table 2). This was most likely due to the initial fast exchange of cations with manure-N from near-surface exchange sites, and a subsequent decline in ammonium absorption as it diffused inward towards remaining internal sites. Agitation of the clinoptilolite bags had no effect on ammonium absorption from swine manure, but did enable N uptake from chicken manure slurries to reach the same level as swine manure after 7 days immersion. Nevertheless, maximum manure-N absorption was still only about 20–25% of the maximum amount observed in the ammonium chloride solution (Tables 1, 2). The likely reason for difference was the presence of other materials, particularly dissolved organic matter, which clinoptilolite can also absorb through cation exchange reducing the amount of ammonium-N absorbed from the manures (Mumpton, 1999).

Manure-derived ammonium-N adsorbed by clinoptilolite can be released into the water through diffusion and cation exchange reactions (Perrin et al., 1998). Results from Experiment 3 indicated that initial TAN concentrations in the clinoptilolite had no significant effect on the subsequent release of TAN in green water after 7 days (Table 3), releasing an overall average of about 86% of the initial 0.045 g TAN per bucket previously absorbed in the clinoptilolite. Since the GPP and NPP measurements were similar across all treatments in Experiment 3, it is not clear why there was a significant positive relationship between initial TAN concentrations in the clinoptilolite and chlorophyll *a* concentrations in the water (Figure 3). Nevertheless, this experiment demonstrated the ability of algae to utilize manure-derived nutrients transported to the water via clinoptilolite.

The Nile tilapia growout trial (Experiment 4) also supported the conclusion that clinoptilolite may be a useful vehicle for transferring swine manure-N into culture systems. In fact, results indicated that there might be additional benefits when compared to fertilization with only urea and TSP. Percent survival of treatment fish was significantly greater in tanks with swine manure (M-Cp), averaging about 97% as compared to about 84% in the three clinoptilolite treatments without swine manure (NM-Cp) and 93% in the control treatment without clinoptilolite bags. Although mean fish growth with two manure-soaked bags was similar to that observed in the NM-Cp and control tanks (i.e., 28–33 g $fish^{-1}$), mean fish growth increased to 40.0 g $fish^{-1}$ and 46.7 g $fish^{-1}$ with three and four bags, respectively (Table 5). Tilapia daily weight gain also increased as the number of bags immersed in swine manure increased (Table 5).

The positive significant relationship ($r^2 = 0.443$, $P < 0.05$) between the number of bags soaked in swine manure and fish growth (Figure 4), in light of the similarities of GPP, NPP, and chlorophyll *a* measurements among all treatments, suggests that the swine manure itself was contributing some benefit to the fish. Although GPP and NPP were a little higher in the manure treatments as compared to the non-manure treatments, differences among all treatments were not statistically significant. However, when transported from the manure slurry to the culture tanks, the clinoptilolite bags also had particulate organic matter attached. It is possible that this added organic matter, with associated microbial fauna and flora, served as an additional food source for the fish.

DO measurements made at 0600 support the conclusion that proportionally higher amounts of manure-derived organic matter entered the tanks with increasing numbers of bags. Early morning DO showed a negative relationship ($r^2 = 0.877$, $P < 0.001$) with increasing number of manure bags in the M-Cp treatments (Figure 5). In one of the M-Cp-4 tanks, early morning DOs fell to near zero. Figure 5 suggests that the risk of deoxygenation is too great beyond four bags per tank (or about 1 kg clinoptilolite per m^2 surface area), limiting the fish growth relationship observed in Figure 4.

The last objective for Experiment 4 was to determine the ability of clinoptilolite to moderate TAN concentrations in an outdoor fertilized culture system. Tank water TAN measurements averaged about 0.23 mg l^{-1} in the three manure treatments, suggesting a slow release of ammonia as observed in Experiment 3. However, TAN measurements in the three non-manure clinoptilolite treatments, which represented a dose-response design, averaged 1.34 mg l^{-1}. This was significantly higher ($P < 0.001$) than the average for the manure treatments, and neither the M-Cp nor NM-Cp treatments showed any relationship between TAN concentrations and the number of bags. Furthermore, analyses of clinoptilolite samples following the experiment showed little or no TAN absorption in the NM-Cp bags. Concentrations of TAN in the culture water may have been too low for meaningful uptake by the clinoptilolite.

Therefore, research here does not support the addition of clinoptilolite bags in fertilized systems to moderate ammonia-N concentrations in culture water. This conclusion for culture systems appears contrary to the current use of clinoptilolites for ammonia removal for the aquarium industry (Bower and Turner, 1982; Dryden and Weatherley, 1987). The use of clinoptilolite as a filter medium in recirculating systems (for both aquaria and larger tank systems) provides greater contact over a longer period of time. However, the application examined here was for static systems with only a one week exposure time.

The above analysis may also explain why clinoptilolite was not effective at capturing ammonium-N in the discharge water as examined in Experiment 5. Although reductions in various forms of nitrogen were observed following the three-day exposure of clinoptilolite to tank culture water, none of the reductions was significantly different between treatments or the control (Table 7). There was also no relationship between observed reductions and the number of bags of clinoptilolite immersed in the tanks. And similar to Experiment 4, little or no TAN was measured in clinoptilolite samples following the three-day exposure to culture water. Observed N reductions in tank waters most likely reflected a natural purification process through nitrification, denitrification, volatilization, and/or other biological means. Although clinoptilolites are increasingly being used for wastewater treatment (Ciambelli et al., 1985; Baykal and Guven, 1997), potential applications of clinoptilolite to treat aquaculture water and effluents will require further research under both controlled and field conditions to establish reliable protocols.

Although our research did not support a useful application of clinoptilolite for either moderating TAN concentrations in fertilized ponds or for capturing TAN from pond discharge water for reuse, research did demonstrate that 1 kg bags of crushed clinoptilolite are capable of transferring algal and fish nutrients from fresh swine manure into fish culture systems. This result could have practical applications for improving nutrient utilization efficiency and animal waste management in Asian aquaculture systems.

Small-scale Asian farms are typically nutrition-poor and crop-dominated. Their nutrition-poor status is compounded by the high loss of nutrients from manures, particularly nitrogen, that occurs before collection and reuse from traditional livestock production systems. The nitrogen from backyard poultry waste and urine from ruminants is often almost completely lost through ammonia volatilization (creating noxious odors) and/or leached out as nitrate after ammonium nitrification (Little and Edwards, 1999). On the other hand, large-scale livestock/poultry-fish in integrated systems are constrained by the limited waste digestion capacity of fish ponds. The capacity of the pond's food web to absorb and utilize wastes is limited to about 100 kg dry matter ha^{-1} day^{-1} and about 4 kg N ha^{-1} day^{-1} (Edwards et al., 1996). Direct use of livestock wastes in static-water fish ponds also imposes limitations in terms of both fish species and intensity of fish culture. Trends in urban population growth and demand for animal products in Asia will lead to further industrialization of livestock, particularly pigs and poultry (Simpson, 1979).

The ultimate goal of our research was to adapt existing agricultural technologies using natural clinoptilolite zeolites to provide a more socially acceptable and efficient way to integrate animal manures in pond fertilization, conserve and recycle on-farm resources, and lessen environmental impacts. By capturing ammonia-N in manures before it gets either volatilized or nitrified, and using that nitrogen to promote algal productivity in ponds, the farmer may improve the farm environment by reducing noxious odors and nitrate leaching and recycle

Table 7. Mean reduction (± 1 SE, n = 3) of various forms of nitrogen in tank discharge water with different amounts of clinoptilolite immersed for three days.

Water Quality Parameters		Clinoptilolite Treatments			Control (0 g)
		2 bags (500 g)	*3 bags (750 g)*	*4 bags (1000 g)*	
Ammonia (mg/L)	Before	1.50	0.93	1.42	0.33
	After	0.71	0.72	1.14	0.55
	Reduction	0.79 ± 0.18	0.21 ± 0.34	0.28 ± 0.32	-0.22 ± 0.14
Nitrate (mg/L)	Before	0.91	1.46	0.91	1.37
	After	0.70	0.77	0.85	1.10
	Reduction	0.20 ± 0.32	0.69 ± 0.08	0.06 ± 0.16	0.26 ± 0.35
Nitrite (mg/L)	Before	0.55	0.85	0.48	0.78
	After	0.39	0.46	0.52	0.78
	Reduction	0.16 ± 0.13	0.39 ± 0.13	-0.04 ± 0.09	0.00 ± 0.31
TKN (mg/L)	Before	6.00	6.25	5.99	3.85
	After	5.26	5.20	4.53	3.72
	Reduction	0.74 ± 0.63	1.05 ± 0.53	1.46 ± 0.25	0.13 ± 0.03
TN (mg/L)	Before	7.46	8.56	7.38	6.00
	After	6.36	6.43	5.90	5.60
	Reduction	1.10 ± 1.07	2.13 ± 0.51	1.48 ± 0.44	0.40 ± 0.67

an otherwise lost nutrient for increased farm productivity. Furthermore, clinoptilolite is renewable, since regeneration can be simply accomplished through heating or immersion in a salt solution. And since clinoptilolite is natural, inert, does not degrade, and is even used in animal feeds (Pond and Yen, 1984), it has no associated environmental risks.

However, economics unfortunately limit the small-scale application of clinoptilolite technologies for rural farmers as examined here. Approximate costs in Thailand (US$1 = about 40 Thai baht) are the following: clinoptilolite, Bt 6 per kg; mesh bags, Bt 5 per bag; urea, Bt 6 per kg. Because the clinoptilolite absorbed from manures is only about 0.4 g of releasable N per kg clinoptilolite, this equates to a one-time clinoptilolite cost of about Bt 15,000 for 1 kg N. Since urea provides N at about Bt 13 per kg N, it would take over 1,000 reuses of the clinoptilolite bags to provide an equivalent amount of N. Although results from the growout trial (Experiment 4) suggest a benefit of manure-soaked clinoptilolite beyond just adding N for algal growth, it would still take about 80-100 reuses of the clinoptilolite bags to be economically competitive with urea to achieve comparable yields. Economics certainly would have been more favorable if N absorption by clinoptilolite from manures had equaled the 1.9 g N per kg observed with the ammonium chloride solution, but apparently other compounds in the manure slurries interfered with ammonium absorption.

Although small-scale integrated farmers could still benefit from a one-time donation of clinoptilolite, initial costs and the length of time for equivalent payback make actual purchases of clinoptilolite by these farmers less practical economically. Our research does not indicate a clear, immediate economic benefit beyond traditional integrated systems for small-scale farmers. Clinoptilolite technologies may be better suited for large-scale integrated farming operations, where additional economic benefits may include animal waste management to meet environmental regulations, and the commercial production of high quality terrestrial fertilizers produced by the composting of animal manures mixed with clinoptilolite.

CONCLUSIONS

The five experiments described in this report represent the first comprehensive research designed to evaluate the applicability of existing clinoptilolite technologies for small-scale integrated farming systems. The clinoptilolite used in this study was capable of absorbing about 1.91 g N kg^{-1} when soaked in an ammonium chloride solution, but only about 0.43 g N kg^{-1} when soaked in either swine manure or a chicken manure slurry. Nevertheless, mesh bags containing crushed clinoptilolite soaked in fresh swine manure were able to provide sufficient N to promote algal production and Nile tilapia yields similar to waters fertilized with urea and TSP. Bits of swine manure attached to the bags may have provided an additional benefit to Nile tilapia, as indicated by the positive relationship between the number of bags and fish growth—even though algal production remained similar between treatments. Nevertheless, it would take an estimated 80–100 reuses of the clinoptilolite bags to provide the same benefit as fertilizing with urea, and therefore may not be economically practical for small-scale farmers. Applications of clinoptilolite for moderating ammonia concentrations in ponds and for removing ammonia from pond discharge water were less promising, likely due to relatively low ammonia concentrations in the water and limited contact time with the clinoptilolite.

ANTICIPATED BENEFITS

Applying clinoptilolite technologies for livestock-fish integrated systems has the potential to improve farm sustainability by increasing on-farm nutrient utilization efficiencies while reducing undesirable farm outputs. By capturing manure-N before it gets either volatilized or nitrified, and using that nitrogen to promote algal productivity in ponds, the farmer not only improves the farm environment by reducing noxious odors and nitrate leaching, but recycles an otherwise lost nutrient for increased farm productivity. The farmer can fertilize ponds with manure-N while reducing BOD loading and the associated risk of anoxic pond water. Depending on the scale of farm operation, there could also be an economic savings over time with reduced need for purchasing additional fertilizers through the retention and recycling of nutrients on farm.

ACKNOWLEDGMENTS

The authors wish to acknowledge the Asian Institute of Technology, Thailand, for providing the field research and laboratory facilities. Mrs. P. Naditrom, Mr. Manoj Y., and Mr. Supat P. are greatly appreciated for their field and laboratory assistance.

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Cite as: [Author(s), 2004. Title.] In: R. Harris, I. Courter, and H. Egna (Editors), Twenty-First Annual Technical Report. Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]