

MINISTRY OF EDUCATION AND TRAINING
CAN THO UNIVERSITY
School year: 2010-2012

TICK NOUANTHAVONG

**EFFECT OF BIODIGESTER EFFLUENT AND
LEAVES FROM TARO (COLOCACIA -
ESCULENTA) AND DUCKWEED (LEMNA MINOR)
ON GROWTH OF TILAPIA (OREOCHROMIS
NILOTICUS) IN OUTDOOR SYSTEM**

**MASTER OF SCIENCE THESIS IN AGRICULTURAL SCIENCES ANIMAL
HUSBANDRY**

Code: 60 – 62 - 40

Scientific supervisors:

1- Associate Professor Dr Nguyen Thanh Phuong; CanTho University.

2- Professor. Dr. T R Preston

AN APPROVAL OF THE SCIENTIFIC EVALUATION COMMITTEE

The thesis with the title: “*Effect of biodigester effluent, duckweed and leaves from Taro (Colocacia esculenta) on growth of Tilapia (Oreochromis niloticus) in outdoor system* ” by **Tick Nouanthavong** was approved by the Scientific evaluation Committee at the Can Tho University

Committee member

Secretary

Opponent number 1

Opponent number 2

Can Tho City, March, 2012

Chair person

CURRICULUM VITAE

BIO-DATA

Full name: Tick Nouanthavong Gender: Female
Date of birth: 15 July, 1980 Place of birth (*ward/commune, district, city/province, country*)
Phonsasanh village, Pak district, Xiengkhouang Province,
Father's birthplace: Khammoun Province Nationality: Lao
Position (before participating MSc program): Technical Assistant of aquaculture (fish)
Workplace (current or before participating MSc program): Living Aquatic Research Resource
Center
Residence address (house number, district, ward/district, city/province): Sivilay Village, Sxythani
district, Vientiane Capital, Lao P.D.R
Mailing address: P.O.Box: 9108
Tel: 856(20)22203310 Fax: 856(21)214855
E-mail: ticknv_15@hotmail.com- ticknounthavong@yahoo.com.

II. EDUCATIONAL BACKGROUND

1. Undergraduate education

Type of training (*Permanent or impermanent*): *Permanent*
Institution awarding degree: Faculty of Agriculture, National University of Laos
Major: Animal Science
Year earned: 2003-2004
Country: Laos
Institution awarding degree: Faculty of Agriculture, National University of Laos
Country: Laos
Name of the thesis: Study on inventory of local production in Champhone district Savannaket
Province
Name of supervisor: Dr Silinthone Saklokham

2. Master of Sciences

Type of training (*Permanent or un-permanent*):
Institution awarding degree: Faculty of Agriculture and Apply & Biology, Cantho University
Major: Animal Production
Year earned: 2010-2012
Country: Vietnam
Name of the thesis Effect of biodigester effluent, duckweed and leaves from Taro (*Colocacia
esculenta*) on growth of Tilapia (*Oreochromis niloticus*) in outdoor ponds
Name of supervisor: 1. Associate professor Dr. Nguyen Thanh Phuong
2. Professor Dr. Thomas Reg Preston

3. Foreign languages

1. English	Level of proficiency (IELTS): Good (Overall score: 4.5)
2. Mother tongue' name	Level of proficiency: Excellent

4. BSc Degree awarding:

- Major: Animal Science

- Degree number: 0103/NOUL2005, date/month/year 21March, 2005 at Vientiane Country Lao P.D.R

Cantho City, *March, 2012*

Signature

Tick Nouanthavong

COMMITMENT

I assure that this thesis is a scientific work which was implemented by myself. All the figures and results presented in the thesis are true and not published in any previous theses. .

Author

Tick Nouanthavong

ACKNOWLEDGEMENT

The experiments presented in this thesis were carried out at An Giang University, Vietnam and the Living Aquatic Resource Research Center (LARReC) belonging to National Agriculture and Forestry Research Institute (NAFRI), in Vientiane Capital Lao PDR. The research was funded by Swedish International Development Agency (Sida)

I would like to express my best gratitude to:

The Swedish International Development Agency (Sida) and the Norwegian Programme for Development, Research and Higher Education (NUFU) for the financial support for this research (Sida) and for my participation in the MSc program (NUFU).

Dr. Thomas Reg Preston, my teacher and adviser, for valuable guidance and support during the study.

I would also like to extend sincere thanks to Dr. Ngo Van Man, Co-ordinator of the SAREC-MEKARN project, the staff of Sida-SAREC regional office in NLU, and the administration and teaching staff of An Giang University for providing resources and advice for conducting the first part of my research.

The research forms part of the requirement by the author for the MSc degree from Cantho University, Vietnam.

Effect of biodigester effluent and leaves from Taro (*Colocacia esculenta*) and duckweed (*Lemna minor*) on growth of Tilapia (*Oreochromis niloticus*) in outdoor system

By

Tick Nouanthavong

Living Aquatic Resource Research Center (LARReC), Lao PDR
ticknv_15@hotmail.com

Table of contents

Abstract	9
Paper 1	9
Paper 2	9
Conclusion	9
Abbreviations	10
Effect of biodigester effluent and leaves from Taro (<i>Colocacia esculenta</i>) and duckweed (<i>Lemna minor</i>) on growth of Tilapia (<i>Oreochromis niloticus</i>) in outdoor system	11
Introduction	11
Hypotheses	12
Paper 1	12
Paper 2	12
Literature review	13
Factors influencing raising of fish in natural pond systems	13
Introduction	13
Intensive with artificial feed	13
The natural food system	14
Phytoplankton and zooplankton	14
Sources of fertilizer	16
Lime	16
Livestock manure	17
Biodigester effluent	17
Supplementation with feed resources produced on the farm	17
Appropriate fish species	18
References	19
Comparison of natural pond (outdoor) system with an intensive (indoor) system for raising Cachama (<i>Colossoma macropomu</i>) and Tilapia (<i>Oreochromis niloticus</i>)	23
Abstract	23
Introduction	23
Hypothesis	24
Materials and methods	24
Location and climate	24
Experimental design	24

System:	24
Species of fish:	25
Procedure.....	25
Measurements.....	26
Chemical analyses	26
Statistical analyses.....	26
Results and discussion.....	26
Water quality	26
Growth of Tilapia and Cachama	26
Natural system.....	28
Intensive system	29
Feed costs of Intensive and Natural systems.....	29
Conclusions	30
Acknowledgements	30
References	30
Effect of biodigester effluent, duckweed and leaves from Taro (<i>Colocacia esculenta</i>) on growth of Tilapia (<i>Oreochromis niloticus</i>) in outdoor system	32
Abstract	32
Introduction	32
Hypothesis.....	33
Objective	33
Materials and Methods	33
Location and climate	33
Treatments and experimental design.....	33
Ponds, fish and management.....	34
Data collection.....	36
Fish weight and length	36
Water quality	36
Statistical analyses.....	36
Results and discussion.....	37
Acknowledgements	41
References	41

Abstract

The objective of the thesis was to study systems for raising of Tilapia in outdoor system using natural resources.

Paper 1

The objective of this experiment was to compare two systems of fish culture: intensive in indoor tanks and natural in outdoor ponds; and two species of fish: Tilapia (*Oreochromis niloticus*) and Cachama (*Colossoma macropomu*). The treatments were compared in a 2*2 factorial arrangement with 3 replications. The fish in the intensive system were raised in plastic tanks of 0.5 m³ capacity (50 fish per tank) and given commercial fish feed (35% crude protein) at 3-5% of live weight. The ponds in the natural system (2*2*1.5m) were lined with polyethylene film and fertilized with biodigester effluent (240 mg N/m²/day); supplementation was with fresh duckweed (*Lemna minor*) and Taro leaves (*Colocacia esculenta*).

Over a 60 day period, both Tilapia and Cachama grew faster in weight and length, and in weight/length ratio, in the intensive system than in the natural system. In both systems the Tilapia grew faster than the Cachama. Less supplement DM was required per unit live weight gain for the Tilapia in the natural than in the intensive system.

It was concluded that despite the slower growth rates of the fish in the outdoor system, the economic analysis would be better than in the intensive system.

Key words: *biodigester effluent, duckweed, fish feed, phytoplankton, supplementation, Taro*

Paper 2

The objective of the experiment was to measure the growth performance of Tilapia in outdoor ponds using biodigester effluent as fertilizer and Taro leaves and Duckweed as protein supplements. Leaves from Taro (*Colocacia esculenta*) and Duckweed (*Lemna spp*) were compared as supplements for Tilapia (*Oreochromis niloticus*) grown in open ponds fertilized with biodigester effluent or not fertilized. The design was a 3*2 factorial arrangement with 3 replications.

Fresh duckweed (*Lemna minor*) was grown in adjacent ponds fertilized with biodigester effluent. Taro leaves (*Colocacia esculenta*) were harvested from natural stands in the Centre. Both supplements were given ad libitum. The Tilapia had an average starting weight of 2.52 g and a length 5.3 cm. The density was 5 fish/ m². The 18 ponds were each 3*2 m and 1 m depth. The biodigester effluent was taken from a tubular polyethylene plug-flow biodigester charged with pig manure. The quantity applied was 520 mg N/pond/day.

Growth rates were four-fold higher for Tilapia supplemented with duckweed compared with Taro leaves or no supplement; and were 22% higher in ponds fertilized with biodigester effluent compared with no fertilizer. DM feed conversion was much superior on the duckweed supplement, ranging from 0.76 to 1.08, the latter being the adjusted value after allowing for the growth rate supported by the natural food chain in the ponds. There were no benefits from feeding Taro leaves. Survival was high on all treatments (98-99%). Values for pH, ammonia and nitrous dioxide were higher in ponds fertilized with biodigester effluent than in unfertilized ponds.

It was concluded that raising Tilapia in outdoor ponds fertilized with biodigester effluent and supplemented with fresh duckweed was an appropriate way to improve the economy of fish production for small scale farmers with beneficial effects on the environment.

Key words: *ammonia, feed conversion, nitrous oxide, pH, survival, temperature,*

Conclusion

It was concluded that raising Tilapia in outdoor ponds fertilized with biodigester effluent and supplemented with fresh duckweed was an appropriate way to improve the economy of fish production for small scale farmers with beneficial effects on the environment.

Abbreviations

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Ca(OH ₂)	Hydrated lime
CaCO ₃	Unslaked lime or quicklime
CaO	Slaked lime
CC	Cachama (<i>Colossoma macropomu</i>)
°C	Temperatura
DM	Dry matter
DO	Dissolved Oxygen
FCR	Feed conversion ratio
GLM	General Liner Model
LW	Live weight
Mekarn	Mekong basin animal research network
N	Nitrogen
NH ₃	Ammonia
NO ₂	Nitrite
ON	Tilapia (<i>Oreochromis niloticus</i>)
pH	Power of/potential Hydrogen
Prob/P	Probability
RCD	Randomized complete design
SEM	Standard error of the mean
SE	Asian
IS	Indoor system
OS	Outdoor system

Effect of biodigester effluent and leaves from Taro (*Colocasia esculenta*) and duckweed (*Lemna minor*) on growth of Tilapia (*Oreochromis niloticus*) in outdoor system

Introduction

In this initial section we introduce the rationale for the study of fish culture by the intensive system and in natural outdoor ponds. The sustainability and importance of these systems are then considered in a broader context, to satisfy needs and if they fit into the resource base or the environment and if they are socially and economically viable.

One of the reasons why tilapia is such a popular fish among fish farmers worldwide is that it is sturdy, adaptable and will eat a wide range of feeds. Pond culture is the most commonly used method of raising tilapia. One of the big advantages with a pond culture is that it closely resembles the life of wild tilapia and makes it possible for the fish to feed on naturally occurring food. However, this can result in a situation where fry and fingerlings compete for food with the adults, resulting in a lower growth rate for the fish in the pond. One way of solving the problem is to cultivate male fish only in the pond. Ponds also tend to become a part of the natural landscape, for good and for bad. When animals such as birds and snails are attracted to the pond, they can bring parasites that cause trouble for tilapia. <http://www.aquaticcommunity.com/tilapia/pondculture.php>.

Growing out strategies for tilapia range from the simple to the very complex. Simple strategies are characterized by little control over water quality and food supply and by low fish yields. When greater controls over water quality and fish nutrition are imposed, the production cost and fish yield per unit area increases. Across this spectrum, there is a progression from low to high management intensity. Increasing stocking densities places increasing demands on the production system. As pond production becomes more intensive and feed rates increase, supplemental aeration and some water exchange are required to maintain good water quality.

All tilapia production systems must provide a suitable environment to promote the growth of the aquatic crop. Critical environmental parameters include the concentrations of dissolved oxygen, un-ionized ammonia nitrogen, nitrite nitrogen, and carbon dioxide in the water. Other important parameters include nitrate concentration, pH, and alkalinity levels within the system. To produce tilapia in a cost effective manner, production systems must be capable of maintaining proper levels of these water quality variables during periods of rapid fish growth.

Since the aim of the natural pond system is to increase fish production by using natural food organisms within the pond it is relevant to first describe the basic aquatic food chain or ecosystem and the underlying primary nutrient cycles operating within a pond ecosystem. Fertilizers are added to the pond to improve the level of nutrients present in the water.

In the natural pond system the strategy is to fertilize the pond with fresh animal manures, or effluents from biodigesters to increase plankton or other natural food for fish in the pond. Thus close integration with livestock necessitates that the livestock living quarters are located adjacent to or above the fish pond (Bui Xuan Men et al 2010). The advantages of integrating livestock production directly with aquaculture production are many, and include: the nutritional value of the manure and feed remnants is preserved because losses of nitrogen and energy due to natural

wastage, fermentation, evaporation and non-reversible coagulation are eliminated. The manure producing animals are often located near the fish ponds to save money and effort on storage and transportation. Fresh manure can then easily be added to the pond on a regular basis.

The most important of the above advantages is the decrease in waste products. Improved soil structure, through the use of pond bottom silt in agriculture as fertilizer, means that water is better retained and less erosion takes place. These long-term advantages outweigh any others which lead only to an increase in fish production. The soil must be suitable for making a fish pond, and suitable fish species must be available.

Hypotheses

Paper 1

A natural pond system for raising Cachama and Tilapia fish will be more economical than raising them indoors in plastic tanks with purchased fish feed

Paper 2

Tilapia will grow well in natural pond systems when biodigester effluent is used as fertilizer to stimulate growth of plankton. Growth rates of Tilapia will be increased when they are also fed fresh leaves of Taro and duckweed

Literature review

Factors influencing raising of fish in natural pond systems

Introduction

Tilapia is poised to make a substantial contribution to aquaculture. It is hardier and more prolific than other species. Also, tilapia produces a fine-tasting white, flakey meat. Initial consumer taste panels have indicated that tilapia is a delicious fish. The main impediment to an increase in tilapia consumption is the lack of familiarity of tilapia to the consumers.

The two main systems of fish culture are: “intensive” in which fish in an indoor system or outdoor system are supplied with concentrate balanced feed usually in the form of pellets; and “natural” in which fish in natural or excavated ponds depend for their feed on the products of photosynthesis, in the form of phytoplankton and zooplankton and on higher plants that grow on the water surface.

Intensive with artificial feed

Tilapia grows well at high densities in the confinement of tanks when good water quality is maintained. This is accomplished by aeration and frequent or continuous water exchange to renew dissolved oxygen (DO) supplies and remove wastes. Culture systems that discard water after use are called flow-through systems while those that filter and recycle water are referred to as recirculating systems.

Intensive tank culture offers several advantages over pond culture. High fish density in tanks disrupts breeding behavior and allows male and female tilapia to be grown together to marketable size. In ponds, mixed sex populations breed so much that parents and offspring compete for food and become stunted. Tanks allow the fish culturist to easily manage stocks and to exert a relatively high degree of environmental control over parameters (water temperature, DO, pH, waste) that can be adjusted for maximum production. With tanks, feeding and harvesting operations require much less time and labor compared to ponds. Small tank volumes make it practical and economical to treat diseases with therapeutic chemicals dissolved in the culture water. Intensive tank culture can produce very high yields on small parcels of land.

Tank culture also has some disadvantages. Since tilapia has limited access to natural foods in tanks, they must be fed a complete diet containing protein, vitamins and minerals. The cost of pumping water and aeration increases production costs. The filtration technology of recirculating systems can be fairly complex and expensive and requires constant and close attention. Any tank culture system that relies on continuous aeration or water pumping is at risk of mechanical or electrical failure and major fish mortality. Backup systems are essential. Confinement of fish in tanks at high densities creates stressful conditions and increases the risk of disease outbreaks. Discharges from flow-through systems may pollute receiving waters with nutrients and organic matter.

There are several advantages of pelleted feeds when properly made. They can provide the correct balance of nutrients needed, the cultured species usually grows very quickly, and they are easily stored for relatively long periods. For disadvantages they are usually expensive and unsuitable for some species. They may pollute the water, lead to allergic reactions if not properly made, and may lead to pathogen virulence.

An intensive fish culture unit, with its inputs and outputs, can be regarded as a system which supplies fish with feed and water and produces fish and waste water. Among these inputs and outputs, stocking and harvesting are specific events, while feed and water and waste water are continuously supplied to or removed from the system. Such systems are efficient in producing fish when the characteristics of the water and feed, as well as other inputs, are well suited to the biological requirements of the fish. In planning and operating such systems, biological requirements need the greatest attention, although, at the planning stage, economic evaluation of feed sources and that of the final product could be of equal importance (Scarves 1984).

A major problem with intensive systems is pollution. The concentration of $\text{NH}_3\text{-N}$ and other nitrogenous compounds is higher in intensive systems due to the decomposition of organic matter (mainly from unused feed) and the excretion of feces by the large biomass of fish (Shaker et al 2002; Mousa 2004). The other issue is the relative economic value of fish produced with artificial feed which is less than when the fish are produced with natural food. These differences may be because the zooplankton diets are better digested than artificial feed (Wilcox et al 2006).

The natural food system

Phytoplankton and zooplankton

Phytoplanktons are photosynthesizing microscopic organisms that inhabit the upper sunlit layer of almost all bodies of fresh water. They are agents for "primary production," the creation of organic compounds from carbon dioxide dissolved in the water, a process that sustains the aquatic food web. Phytoplanktons obtain energy through the process of photosynthesis and must therefore live in the well-lit surface layer of an ocean, sea, lake, or other body of water (<http://en.wikipedia.org/wiki/Phytoplankton>). They are at the base of the natural food chain. Many small fish and whales eat them. Most of the phytoplankton in inland waters obtains all of their energy from light and their chemical requirements from dissolved inorganic solutes. Even in photolithographic phytoplankton, cell phosphorus and combined nitrogen can be obtained from dissolved organic phosphate and organic nitrogen. Phytoplankton use carbon dioxide for growth, which means that the more phytoplankton there are, the more carbon will be sucked out of the air.

Zooplanktons are the consumers of the planktonic world. As such, they feed on other plankton to obtain the energy and nutrients they need to survive. Zooplankton includes the larvae of fish and crustaceans. (<http://animals.about.com/od/p/g/plankton.htm>.) Cladocerans and Rotifera are the dominant species and their relative proportions affect the grazing intensity and thus the rate of primary production of phytoplankton

The biology of a fish pond

Fish are not the only organisms living in the water of a pond. Food for the fish also grows naturally in a pond. The naturally occurring food sources include very small plants (algae or phytoplankton) and very small animals (zooplankton) (figure). Both these sorts are too small to see with the naked eye. If a large amount of algae is present, the water will have a green colour.



Figure: Naturally occurring fish food seen through a magnifying glass (Edwards & Kaewpaitoon, 1984.) A Zoo plankton ; b:Algae

Water plants are larger plants, which can be seen with the naked eye, and grow in the fish pond all the year round. Some grow on the bottom of the pond, some in the water and others float on the surface of the pond. Some fish species eat water plants. The water in a pond must be of good quality so that the fish will be healthy and grow well. In order to grow, fish need oxygen. This is produced mainly by the algae floating in the water, which makes the water green in colour. Climate is important as it determines the temperature of the water in the pond. The higher the water temperature the faster the algae and zooplankton grow. However, most tropical algae, Zooplankton and fish species grow fastest at a water temperature between 25 and 30°C.

Water quality

The two most important factors which determine the quality of the water are the temperature of the water and the amount of oxygen dissolved in the water. The plants in the pond (especially algae) produce oxygen with the help of sunlight, some of this oxygen they use themselves. The more sunlight the pond receives the higher the oxygen production. When it is dark no oxygen is produced by plants as there is no sunlight. As oxygen continues to be used by all living organisms in the pond water, however the amount of oxygen in the water decreases during the night. In the early morning the amount of oxygen in the water is at its lowest level, as fish, algae and zooplankton have been using oxygen all night, and no oxygen production has taken place. The oxygen content of the water is usually highest at the end of the afternoon, as oxygen is produced throughout daylight hours. Climate also influences the oxygen content of the water. The amount of oxygen in the water depends on the temperature of the water. Less oxygen can dissolve in warm water than in cold water. However, fish need more oxygen in warm water as they are more active. The optimal temperature varies depending on the fish species but the average is between 25 and 30°C. Algae produce less oxygen in cloudy weather, as less sunlight falls on the water. Windy conditions lead to a rise in oxygen content as more air mixes with the water.

Management of fertilizer application

Good fertilizer practice is important to maintain water quality and to maintain a good amount of naturally occurring fish food available in the water. The amount of fertilizer added to the water depends on the number of fish in the pond. If too little fertilizer is put in less natural food will grow and less fish will be produced. Putting in too much fertilizer or fertilizing irregularly can lead to oxygen shortage and fish will die. A well managed and fertilized pond can sustain 3 kg fish per 100 m² per day.

The organisms in the pond water which break down plant and animal waste need oxygen. The process of breaking down the waste products releases many nutrients, which are used by the algae to grow. The algae in their turn produce oxygen.

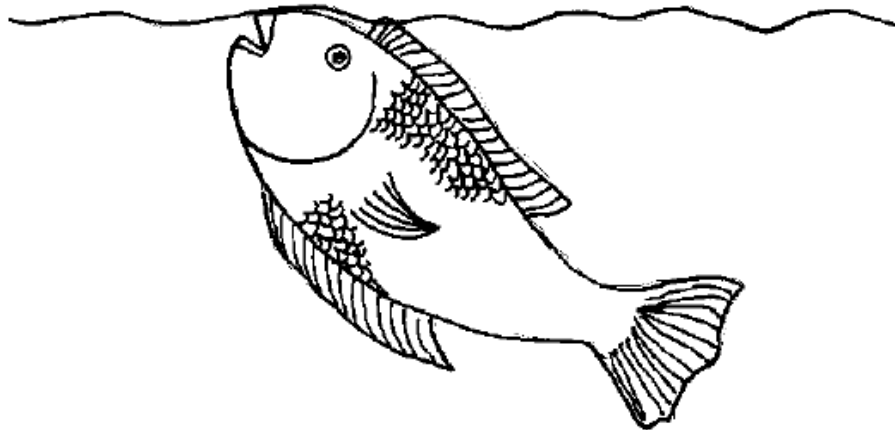


Figure : When a pond receives too much fertilizer you will often see fish gasping for oxygen at the surface in the early morning

However, if there are too many algae in a pond (water colour is dark green) the algae use too much oxygen during the night so the fish and zooplankton will die due to a lack of oxygen in the early morning. If too much fertilizer is spread, too much oxygen may be used by the waste processors to break down the fertilizer. This will also lead to oxygen shortage for the fish, and they may die. To sum up, there is an optimum amount of fertilizer which a pond requires whereby the algae produce enough oxygen and whereby there are no fish gasping at the surface for oxygen at sunrise (figure).

Fertilizing the bottom of the pond

Fertilizer can be applied to the bottom of the pond before filling the pond with water. Tiny plant and animal organisms in the soil break down the fertilizer. When the pond is filled, the nutrients available from the fertilizer are taken up by the water. These nutrients form food for the algae and zooplankton, which in turn are eaten by the fish.

After the fish have been harvested, and before the pond is refilled, the bottom will not need fertilizing if the sludge layer is not removed. This sludge is made up of organic material from fish manure and uneaten fish food which has sunk to the bottom of the pond.

Sources of fertilizer

Lime

Lime is applied in fishponds primarily as a soil conditioner. Liming corrects soil acidity, promotes the release of soil nutrients, precipitates suspended materials which hamper light penetration and reduces incidence of fish diseases. Agricultural lime (CaCO_3) is the most common form that is used in fishponds. Unslaked lime or quicklime (CaO) and slaked lime ($\text{Ca}[\text{OH}]_2$) or hydrated lime may also be applied.

Livestock manure

The natural food system is enhanced by adding fertilizers such as urea and triple superphosphate or organic manures such as chicken manure, buffalo manure, goat manure, crop residues and kitchen wastes.

Pig, chicken and duck manures increase fish production more than cow and sheep manure. Animals fed high quality feeds (grains) produce manure that is better as a fertilizer than those fed diets high in crude fiber. Fresh manure is better than dry manure. Finely-divided manure provides more surface area for the growth of microorganisms and produce better results than large clumps of manure. Manure should be distributed evenly over the pond surface area. Large accumulations of manure on the pond bottom produce low oxygen conditions in the sediment that reduce microbial activity and sometimes result in the sudden release of toxic chemicals into the water column. If early mornings dissolved oxygen (DO) is less than 2 ppm, manuring should be reduced or stopped until the DO increases.

Suggested application rates for earthen ponds having an area of 0.03 ha each are cattle manure 90 kg pond⁻¹ (3 tonnes ha⁻¹) and poultry manure 45 kg pond⁻¹ (1.5 tonnes ha⁻¹) (Jena and Das 2006; Sahu et al 2007)

Biodigester effluent

Pich Sophin and Preston (2001) compared manure from pigs before and after processing in a biodigester, with chemical fertilizer (80% urea and 20% diammonium phosphate) biodigester effluent, using five fish species (Tilapia, Silver carp, Bighead carp, Silver barb and Mrigal) stocked at a density of 2 fish/m². The net fish yield was 55% greater in ponds fertilized with biodigester effluent rather than with fresh manure. The improvement with effluent compared with chemical fertilizer was 27%.

Supplementation with feed resources produced on the farm

Duckweeds refers to a group of small floating aquatic plants that are found in natural ponds, lakes and flooded rice field. They can be grown to recycle nutrients from waste water and provide a good source of protein for many species of live stock (Leng No date; Cheng and Stomp 2009). Duckweeds, being tiny surface floating plants, are easy to harvest and have a high protein content of up to 39.3% in dry basis (Bui Xuan Men et al 1996). Duckweeds hold an immense potential for both nutrient recovery from village ponds and utilization as animal feed due to their fast growth rate, efficient nutrient extracting capability, easy harvesting, high nutritive value and good digestibility (Leng et al 1995). They have been used as the basis for fish production in Bangladesh (Skillicorn et al 1993).

Water spinach is a common plant species grown widely in tropical SE Asian countries. In Cambodia, besides growing in inland waterways, this aquatic plant is commonly cultivated all year round in lagoon and lakes. Water spinach is a good source of protein and can be used as feed for all kinds of animal and for humans. The foliage contains protein in the range of 24 to 27% in the DM (Nguyen Nhuy Xuan Dung 1996). The mineral contents in the leaves are high. The leaves contain moderate concentrations of Na, Ca, Mg and P and Zn (Umar et al 2007). It has been used as a supplement for fish in experiments in Cambodia (San Thy et al 2008; Sorphea et al 2010).

Wild taro (*Colocasia esculenta*) originates from India and Southeastern Asia. It is a perennial herb 1.5 m tall, with thick stems, very small corms, and with leaf blades around 60 in length and 50 cm in width. Wild taro is very easy to grow, develops fastest in wet land and is highly resistant to pests and diseases. The wild taro leaf has a high nutritional value, with 22.5-26.3% crude protein

in the dry matter (Malavanh Chittavong et al 2008; Chhay Ty et al 2007), There are no published reports on using the leaves as feed for fish. However, observations in Colombia (Preston T R, personal communication) indicate that it is readily eaten by Cachama and Tilapia

Yields can be increased by giving the fish extra food. In ponds which are well fertilized the fish will usually receive more than enough protein. However, they may not obtain sufficient energy, which can limit production. By feeding the fish grain which is rich in energy you can supplement this deficiency. The by-products from grain production, such as wheat and rice bran, or broken rice, make excellent food supplements for fish ponds which are fertilized using animal dung. In Cambodia the leaves from the ipil-ipil tree, Sesbania tree and kapok tree, together with tender leaves from water hyacinth and morning glory plants are cooked with rice husks and used as fish food. Termites are also a good source of protein-rich food.

Appropriate fish species.

The way in which the pond is fertilized also determines the fish species that can be raised. Alternatively, the fish species present will determine how the pond is fertilized. Snakehead and catfish can obtain oxygen from the air as well as from the water and are therefore less sensitive to changes in the oxygen content of the water. Tilapia cannot obtain oxygen from the air, but they are less sensitive to oxygen shortages in the water than other fish species. The amount of fertilizer that can be used will depend on how sensitive the fish are to oxygen shortages.

journeytoforever.org/farm_library/AD21.pdf.

A wide range of fish species has been cultivated in open ponds, including common carp (*Cyprinus carpio*), Indian major carps (*Catla catla*, *Cirrhina mrigala* and *Labeo rohita*), Chinese silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), crucian carp (*Carassius auratus*), Nile carp (*Osteochilus hasseltii*), tilapia (*Oreochromis spp.*), milkfish (*Chanos chanos*), catfish (*Pangasius spp.*), kissing gourami (*Helostoma temminckii*), giant gourami (*Osphronemus goramy*), silver barb (*Puntius gonionotus*) and freshwater prawn (*Macrobrachium lanchesterii*). The choice of any one species often reflects local culture rather than if the fish are optimally-suited to such environments. For example, Chinese carps and Indian major carps are the major species in excreta-fed systems in China and India, respectively. In some countries, a polyculture of several fish species is used. Tilapia are generally cultured to a lesser extent than carps in excreta-fed systems although, technically, they are more suitable for this environment because they are better able to tolerate adverse environmental conditions than carp species. Milkfish have been found to have poorer growth and survival statistics compared with Indian major carps and Chinese carps in ponds fed with stabilization pond effluent in India. (<http://www.fao.org/docrep/T0551E/t0551e09.htm>.)

Common carp inhabits the surface layer of water and feeds upon plankton. It attains sexual maturity during the second year. It is a surface dweller feeding mainly upon zooplankton during its early stages and gradually becomes predominantly a phytoplankton feeder. Its relatively longer branchiostyles provide a fine filter capable of retaining planktonic organisms. It readily accepts supplementary feed like oil cakes and rice bran mixture in pond culture systems. It does not breed in pond condition. Growth mainly depends upon the bottom biota, stocking density and the rate of supplementary feed. In composite fish culture ponds it grows to about 1 kg within one year.

Tilapias are frequently cultured with other species to take advantage of many natural foods available in ponds and to produce a secondary crop, or to control tilapia recruitment.

Nile tilapia (*Oreochromis niloticus*) were introduced to China in the late '70s. The rate of development was extremely rapid (Figure 1) approaching 1 million tonnes annually by 2003. In

the main, intensive methods are used with stocking rates of 3 -3.7 fish/m² and high protein supplementary feed (28-35% crude protein) at 3 to 6% of live weight. Net yields in a 200-240 day cycle were reported to be 15 to 20 tonnes/ha (Lai Qiuming and Yi No date).

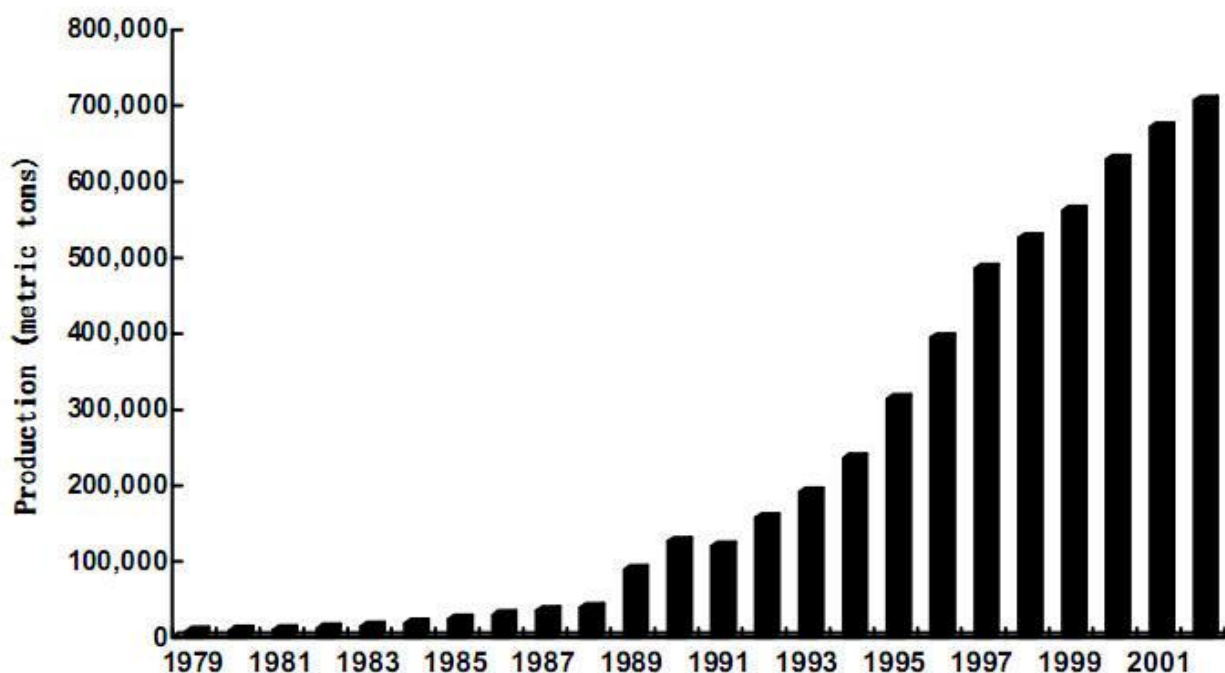


Figure 1. Annual production of tilapias in China (from Lai Qiuming and Yang Yi [No date])

References

- Bui Xuan Men, Ogle and Preston T R 1996.** Use of restricted broken rice in duckweed based diets for fattening Common and Muscovy ducks. *Livestock Research for Rural Development* .Volume 8, Number 3, September 1996. <http://www.lrrd.org/lrrd8/3/mcn832.htm> .
- Bui Xuan Men, Ogle B and Preston T R 2010** Evaluation of an integrated fish, ducks and rice system to reduce pollution and improve resource utilization. <http://www.mekarn.org/workshops/pakse/html/bxmen.htm>
- Buntha P, Borin K, Preston T R and Ogle B 2008.** Digestibility and nitrogen balance studies in pigs fed diets with ensiled taro (*Colocasia esculenta*)leaves as replacement for fish meal. *Livestock Research for Rural Development* . Volume 20.supplement. Retrieved June 6, 2010, from . <http://www.lrrd.org/lrrd20/supplement/bunt2.htm>
- Chang J J and Stomp A M 2009** . Growing duckweed to recover nutrients from waste waters and for production of fuel ethanol and animal feed. www.ctcan-journal.com
- Edwards P and Kaewpaitoon K 1984** Fish culture for small-scale farmers. Environmental Sanitation Center, Asian Institute of Technology (AIT), Bangkok, Thailand. 44p.
- FAO 1993:** Tropical feeds by B. Gohl. Computerized version 4.Edited by A Speedy, Rome, Italy.

- Khov kuong, Willtam Leschhen and Little D 2007.** Food, incomes and urban waste water treatment in Phnom Penh. Cambodia . Aquaculture News 33/ January 2007.
<http://www.aqua.stri.ac.uk/AquaNews/downloads/issuc33/33p810.pdf>.
- Le Ha Chau 1998.** Biodigester effluent versus manure, from pigs or cattle, as fertilizer for duckweed (*Lemna* spp.) Institute of Agricultural Sciences, Ho Chi Minh City, Vietnam. Livestock Research for Rural Development Volume 10, Number 3, 1998. <http://www.lrrd.org/lrrd10/3/chau2.htm>.
- Leng R A, Stambolie J H and Bell R 1995** Duckweed - a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development Volume 7, Number 1. <http://www.lrrd.org/lrrd7/1/3.htm>
- Nguyen Nhuy Xuan Dung 1996.** Identification and evaluation of indigenous plant for livestock and humans in the Mekong Delta region in Vietnam .MSc thesis ,Swedish University of Agriculture Sciences. Uppsala
- Moussa S M S 2004.** Impact of inorganic pollutants on aquatic environment and fish performance in Lake Borollus. Ph. D. Thesis, Inst. of Environment Stud. and Res., Biol. & Phys. Dep., Ain hams Univ., Egypt.
- Jana J K and Dan P C 2006.** In handbook of fisheries and aquaculture (eds S A Verma A T Kumar and S Pradhan), Indian Council of Agricultural Research New Delhi Indian pp 265-285.
- Morris I, Smith A E and Glover H E 1981.** Products of photosynthesis in phytoplankton off the Orinoco River and in the Caribbean Sea. Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575
<http://www.jstor.org/pss/2835873>.
- Kaensombath Lampheuy, San Thy and Preston T R 2004.** Manure or biodigester effluent as fertilizer for duckweed Livestock Research for Rural Development Voloume16 /3/2004.
<http://www.lrrd.org/lrrd16/3/lam16017.htm>.
- PD/A CRSP 1996.** Social, economic and institutional impacts of aquacultural research on tilapia. The PD/A CRSP in Rwanda, Hoduras. The Philippines and Thailand. Research and development series No. 40. International Center for Aquaculture and Aquatic Environments, Alabama Agriculture Experiment Station, Auburn University, USA
- San Thy and Preston T R 2003.** Effluent from biodigesters with different retention times for primary production and feed of Tilapia (*Oreochromis niloticus*). Livestock Research for Rural Development 15 (9).
<http://www.lrrd.org/lrrd15/9/sant159.htm>
- San Thy, Khieu Borin, Try Vanvuth, Pheng Buntha and Preston T R 2008:** Effect of water spinach and duckweed on fish growth performance in poly-culture ponds. Livestock Research for Rural Development. Volume 20, Article #16. <http://www.lrrd.org/lrrd20/1/sant20016.htm>
- Sorphea S, Lundh T, Preston T R and Borin K 2010** Effect of stocking densities and feed supplements on the growth performance of tilapia (*Oreochromis* spp.) raised in ponds and in the paddy field. Livestock Research for Rural Development. Volume 22, Article #227. <http://www.lrrd.org/lrrd22/12/sorp22227.htm>
- Sahu P K, Jean J K, Das P C, Mondal S and Das R 2007.** Production performance of Labeo calbasu(Ham) in polyculture with three India major carps Calla calla (Ham), Labeo rohila (Ham) and Cirrhinius mrigala (Ham) with provision of fertilizers feed and periphytic substrate as varied inputs Aquaculture
- Skillicorn P, Spira W and Journey W 1993** "Duckweed Agriculture. The New Aquatic Farming System for Developing countries" The World Bank. Washington DC, USA.
- Szarvas and Hungary 1984** Chapter 17 Artificial Feeding in Intensive Fish Culture, I. Csengeri Fish Culture Research Institute <http://www.fao.org/docrep/X5744E/x5744e0i.htm>
- Shaker I M A , Ibrahim N A , Dawa M A A and Zakar A H 2002.** Effect of stocking density on water quality and mullet growth in earthen ponds at Sahl El-Teena-Senai- Egypt. The 6th Vet. Med. Zagazig. Conference, 7-9 Sep. 2002, Hurghada, Egypt.

Umar K J, Hanssan L G, Dangoggo S M and Ladan M J 2007. Nutritional composition of water spinach (*Ipomoea aquatica* forsk) leaves .J Applied Science 7.
<http://sc ialert.net/abstract/?doi=jas.2007.803.809>

Wilcox J A, Tracy P and Marcus H N 2006. Improving Live Feeds: Effect of a Mixed Diet of Copepod Naupli (*Acartia tonsa*) and Rotifers on the Survival and Growth of First-Feeding Larvae of the Southern Flounder, *Paralichthys lethostigma*. Journal of the World Aquaculture Society, Vol. 37, No. 1

Comparison of natural pond (outdoor) system with an intensive (indoor) system for raising Cachama (*Colossoma macropomu*) and Tilapia (*Oreochromis niloticus*)

Tick Nouanthavong and T R Preston*

Living Aquatic Resource Research Center (LARReC), Lao PDR

ticknv_15@hotmail.com

* TOSOLY, AA #48, Socorro, Santander, Colombia

Abstract

Two systems of fish culture: intensive in indoor tanks and natural in outdoor ponds; and two species of fish: Tilapia (*Oreochromis niloticus*) and Cachama (*Colossoma macropomu*) were compared in a 2*2 factorial arrangement with 3 replications. The fish in the intensive system were raised in plastic tanks of 0.5 m³ capacity (50 fish per tank) and given commercial fish feed (35% crude protein) at 3-5% of live weight. The ponds in the natural system (2*2*1.5 m) were lined with polyethylene film and fertilized with biogas effluent (240 mg N/m²/day); supplementation was with fresh duckweed (*Lemna minor*) and Taro leaves (*Colocacia esculenta*).

Over a 60 day period, both Tilapia and Cachama grew faster in weight and length, and in weight/length ratio, in the intensive system than in the natural system. In both systems the Tilapia grew faster than the Cachama. Less supplement DM was required per unit live weight gain for the Tilapia in the natural than in the intensive system, with the implication that despite the slower growth rates, the economic analysis would favour the former.

Key words: Biogas effluent, duckweed, fish feed, phytoplankton, supplementation, Taroleaves

Introduction

There is a need to study alternative systems of fish production that do not depend on purchased feeds and which make better use of available resources in farming systems that recycle organic wastes. The objectives are to reduce the present dependency on imported concentrate feeds and to encourage farmers to adopt farming methods that are more economically viable. In an integrated farming system nothing is wasted, the byproduct of one system becomes the input for another. By contrast, in the intensive system, the fish are given supplementary feeds the residues from which can be a source of pollution.

In the natural pond system the strategy is to fertilize the water to encourage the growth of phytoplankton which becomes the main source of feed. Supplements may also be given but usually these are in the form of water plants such as duckweed and water spinach, which can be grown locally. By contrast, in the intensive system the feed is provided in a concentrated form with a high protein content made from ingredients that are purchased from outside the farm.

The importance of the natural system has been realized of late and the scientific basis is being investigated to evolve appropriate technologies to get optimum productivity of the land, labour, waste and water. Better integration of the systems to involve crop and livestock production with recycling of wastes for aquaculture can help small-holder farmers to diversify their farm production, increase cash income, improve quality and quantity of food produced and exploitation

of unutilized resources particularly labour and waste. More emphasis is needed on the integration of fish farming with agriculture and irrigation, livestock farming, sewage utilization and water pollution control not only to increase the productivity of land and water and improve the economic conditions of poor farmers but also to maintain health and hygiene of the rural poor and city dwellers alike.

The two main components of the natural system are: (i) the means to fertilize the growth of phytoplankton; and (ii) the choice of water plants that can be a direct feed source to the fish. Early research used animal manures as replacement for chemical fertilizers (Cruz and Shehadeh 1980). Later research evaluated the processing of the manure in biodigesters and the use of the biodigester effluents as fertilizer in the pond (Edwards et al 1988; Pich Sophin and Preston 2001; San Thy and Preston 2003). Biodigester technology has developed considerably in the last decade and the use of low-cost tubular polyethylene has enabled the process to be within the reach of poor farmers (Bui Xuan An et al 1997; Doung Nguyen Khang and Le Minh Tuan 2002). The recycling of waste gives additional value to both human and animal wastes through gas production, production of good quality fertilizer and the control of pathogens.

Duckweed has been used successfully as feed for mixed fish species in outdoor ponds fertilized with biodigester effluent (San Thy et al 2008; Sen Sorphea et al 2010). The leaves of Taro (*Colocasia esculenta*) have been shown to have high nutritive value for pigs (Chhay Ty et al 2010; Du Thanh Hang and Preston 2010; Manivanh Noupone and Preston 2011) and ducks (Giang et al 2010). Recent observations indicate that the fresh whole leaves of Taro are readily consumed by Cachama (*Colossoma macropomum*) and Tilapia (Rodriguez Lylian, personal communication). Duckweed and Taro grow naturally in most villages in SE Asia.

Hypothesis

A natural pond system for raising Cachama and Tilapia fish will be more economical than raising them indoors in plastic tanks with purchased fish feed.

Objective

- The objective of the experiment was to compare two systems: Indoor system (IS) versus Outdoor system (OS) with two species of fish: Tilapia and Cachama.

Materials and methods

Location and climate

The experiment was conducted from 15 August to 15 November 2010 at An Giang University, An Giang Province, Long Xeing district of Vietnam. In An Giang Province in the Mekong Delta, the rainy season is from June to November. Average temperature is around 25°C, with a maximum of about 40°C in April, while the coldest month is January, when the temperature is around 22°C, with a maximum of about 35°C.

Experimental design

Four treatments were compared in a 2*2 factorial arrangement with 3 replications.

System:

- IS: Intensive (indoor system) using fish with commercial feed in the tanks
- OS: Natural (outdoor system) with supplementation of duckweed (*Lemna minor*) (Photo 1) and leaves of Taro (*Colocacia esculenta*) (Photo 2)



Photo 1. Duckweed (*Lemna minor*)



Photo 2. Taro leaves (*Colocacia esculenta*)

Species of fish:

- ON: Tilapia (*Oreochromis niloticus*) (Photo 3)
- CC: Cachama (*Colossoma macropomu*) (Photo 4)



Photo 3. Tiapia (*Oreochromis spp*)



Photo 4. Cachama (*Colossoma macropomum*)

The treatments were applied to 8 plastic tanks in the Aquatic Resources building (Photo 5) and to 8 ponds situated outdoors (Photo 6).



Photo: 5. Intensive system



Photo: 6. Natural pond system

Procedure

The ponds in the NP system were 2*2m in area and 1.5 m deep. They were lined with polyethylene sheet to prevent filtration. Biodigester effluent was added to the ponds at weekly intervals equivalent to 240 mg N/m²/day (about 1 liter biodigester effluent/m²/day). The effluent was taken from a “plug-flow” tubular polyethylene biodigester (0.5 m³ liquid volume with 20 days

of retention time) charged daily with pig manure (5 kg of fresh manure and 20 litres of water) collected from a nearby farm. The N content of the effluent was 600 mg/ litre with 535 mg/litre as NH₄-N. Duckweed was obtained from a pond managed to optimize duckweed production by application of biodigester effluent. The duckweed was added to the outdoor system in amounts that resulted in the plants covering half the surface of the ponds; Taro leaves were given at the rate of 350 g /week per pond. Stocking rate was 20 fish/ pond (5 fish / m²) for both species.

In the IS system, the fish were cultured in plastic (PVC) tanks of capacity 0.5 m³. The stocking density was 50 fish / m³. Pelleted commercial fish feed was given at 3-5% of LW (DM basis). The water in the tanks was exchanged every day at 7: 00.

Measurements

The weights and length of the fish were measured before releasing them into the ponds/tanks and at the end of the trial. The pH and ammonia-N were determined weekly in the morning (7: 00). Temperature of the water was measured every day at 7: 00, 12: 00 and .17:00 .

Chemical analyses

Feeds were analyzed for N following the method of AOAC (1990) and for DM using a micro-wave oven (Undersander et al 1993). Ammonia-N in the water was estimated by colour generation with a test kit.

Statistical analyses

The data were subjected to analysis of variance using the General Linear Model (GLM) of the ANOVA option in the Minitab software (MTB 2000). Sources of variation were: species, system, and species *system interaction and error.

Results and discussion

Water quality

Water temperature was lower in the intensive (indoor) tanks than in the natural (outdoor) ponds (Table 1). There were no differences in the average pH but ammonia-N was much higher (100 times) in the water in the intensive tanks than in the natural ponds. It is assumed the origin of the ammonia in the intensive system was from the decomposition of the an-ingested feed and the fecal material.

Table 1. Mean values for pH, temperature and ammonia-N in the water in the ponds/tanks of the natural and intensive systems

	Indoor	Outdoor
pH	7.25	7.25
Temperature, °C	26.5	30.0
Ammonia-N, mg/litre	2.5	0.03

Growth of Tilapia and Cachama

Both species of fish grew faster in weight and length, and weight/length ratio, in the indoor system than in the outdoor system (Tables 2 and 3 and Figures 1-3). In both systems the Tilapia had better growth performance than the Cachama.

Table 2. Mean values for initial and final weights and length, and daily increases in weight and length and weight:length ratio for effect of species and system

	Cachama	Tilapia	Prob.	NP	IS	Prob.	SEM
Weight, g							
Initial	13.2	22.9		18.1	18.1		
Final	26.6	70.1	0.001	33.3	63.5	0.001	2.48
Length, cm							
Initial	9	11.5		10.3	10.3		
Final	12.3	15.2	0.001	13.1	14.4	0.003	0.33
Daily increase							
Weight, g	0.224	0.787	0.001	0.25	0.76	0.001	0.041
Length, cm	0.055	0.061	0.26	5.93	10.1	0.003	0.0039
W/L, g/cm	4.10	11.9	0.001	9.11	14.7	0.001	0.21

Table 3. Mean values for daily increases in weight and length and weight/length ratio for effect of species within production system

	Outdoor		Indoor		SEM	Prob.
	Cachama	Tilapia	Cachama	Tilapia		
Daily increase						
Weight, g	0.149	0.358	0.299	1.22	0.0584	0.001
Length, cm	0.054	0.0391	0.055	0.0832	0.0055	0.001
W/L, g/cm	2.75	9.11	5.46	14.7	0.296	0.001

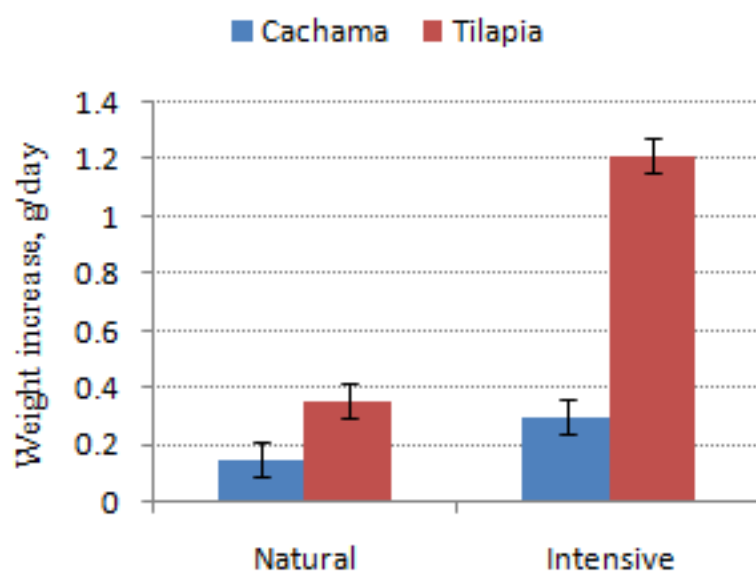


Figure 1. Growth in weight of Cachama and Tilapia in natural and intensive systems

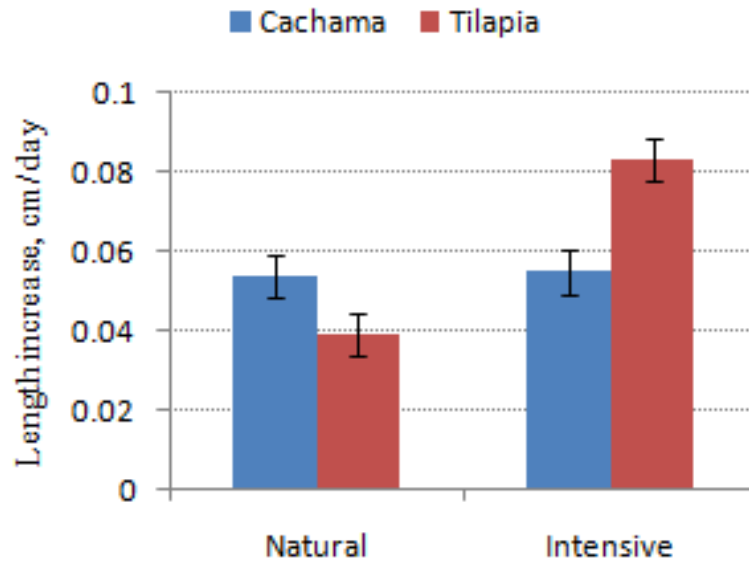


Figure 2. Growth in length of Cachama and Tilapia in natural and intensive systems

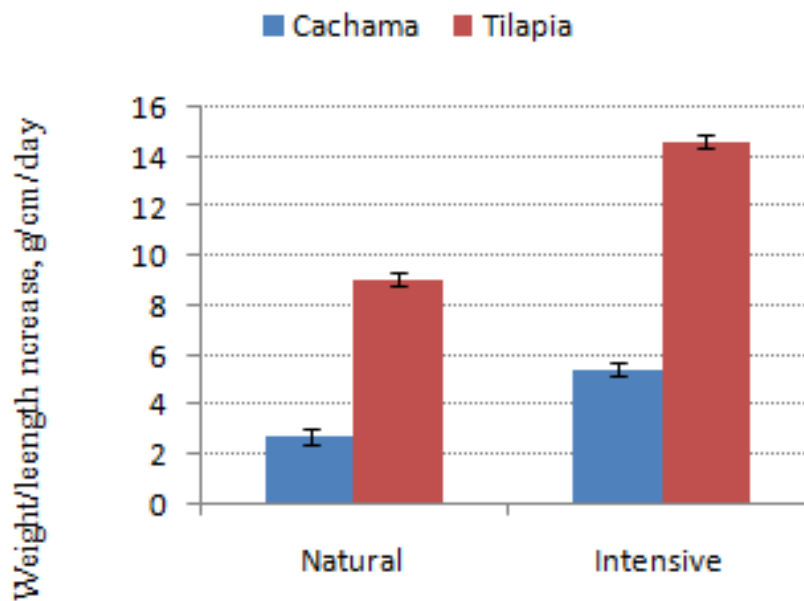


Figure 3. Growth in weight/length ratio of Cachama and Tilapia in natural and intensive systems

Natural system

Pich Sophin and Preston (2001) compared 5 species of fish in ponds fertilized with biodigester effluent (117 mg N / m² / day), pig manure (as used in the biodigester) or chemical fertilizer. Daily growth rates of Tilapia were 0.499 g and 0.0405cm resulting in a weight/length increase of 12.3. These results were superior to those in the present experiment, especially for the weight/length ratio. The application of effluent N was lower (117 mg N / m²/day) in the experiment of Pich Sophin and Preston (2001) than in our study (240 mg N / m²/day), and the fish density was less (2 fish / m²); also mixed cultures were used by Pich Sophin and Preston (2001). In terms of net fish yield (kg / ha/day) the results were similar (17 kg in our study compared with 15 kg for Pich

Sophin and Preston 2001). Growth rates in the natural system in our experiment were better than those reported by Edwards et al (1988) in earthen ponds fertilized with biodigester effluent and with pelleted feed supplement (range in weight gain with 5 Tilapia / m² was 0.15 to 0.23 g / day).

Results from two experiments with Tilapia grown in a natural system, compared with the present study (Table 4), indicate comparable findings to those in our study.

Table 4. Comparison of three studies with Tilapia in natural system using biodigester effluent

	Effluent	Effluent + water spinach and duckweed	Effluent + duckweed + Taro leaves
Daily increase			
Weight, g	0.27	0.7	0.36
Length, cm	0.042	0.044	0.039
W/L ratio	6.0-10.0	6.86	9.10
Fish, m ²	2	5	5
	San Thy and Preston 2003	Sen Sorphea et al 2010	This study

Intensive system

Nile tilapias (*Oreochromis niloticus*) were introduced to China in the late '70s. The rate of development was extremely rapid approaching 1 million tonnes annually by 2003. In the main, indoor system methods are used with stocking rates of 3 - 3.7 fish / m² and high protein supplementary feed (28-35% crude protein) at 3 to 6% of live weight. Net yields in a 200-240 day cycle were reported to be 15 to 20 tonnes / ha (Lai Qiuming and Yi 2004). In our study with 5 fish / m² given pelleted feed at 3-5% of live weight the calculated net fish yield would have been 12.2 tonnes / ha assuming the growth rate of the Tilapia (1.22 g / day) would be maintained over a production cycle of 200 days.

Feed costs of Intensive and Natural systems

Estimates of feeds offered and DM feed conversion ratios for the Tilapia in the indoor system and outdoor systems (Table 5) show major differences with much higher intakes of DM in the indoor than in the outdoor system. Although growth rates were lower in the outdoor system (0.358 g /day) than in the indoor system (1.22 g /day), supplement intakes were much lower with the result that the estimated DM feed conversion in the outdoor system was only one half of that in the indoor system. The cost of the fish feed was USD 0.35 / kg and the value of the net fish yield about USD 1.00/ kg thus with a feed conversion of 1.58 the income over feed would be about 0.50 USD per 1 kg of fish. In the outdoor system, it is assumed the Taro and duckweed are grown on the farm and have no direct cost, as family labour would be used to grow and harvest them. In this case the estimated return would be about twice that in the indoor system.

Table 5. Intake of supplements and feed conversion ratio of Tilapia grown in intensive or natural system

	Total, kg#	Per pond, kg#	Per pond, g DM/d	LWG, g/pond /d	FCR, g DM/g LWG
Indoor					
Fish feed	18	6	96.4	61	1.58
Outdoor					
Taro leaves	2.5	0.833	2.78		
Duckweed	8.25	2.75	3.21		
Total			5.99	7.16	0.836

In 60 days

Conclusions

- Both Tilapia and Cachama grew faster in weight and length, and in weight/length ratio, in the intensive system than in the natural system.
- In both systems the Tilapia grew faster than the Cachama.
- Less supplement DM was required per unit live weight gain for the Tilapia in the natural than in the intensive system, with the implication that despite the slower growth rates, the economic analysis would favour the former.

Acknowledgements

The authors would like to thank the Swedish International Development Agency (Sida) and the Norwegian Programme for Development, Research and Higher Education (NUFU) for the financial support for this research. The research forms part of the requirement by the senior author for the MSc degree from Cantho University. The administration and teaching staff of An Giang University are gratefully acknowledged for providing resources and advice for conducting the research.

References

AOAC 1990 Official Methods of Analysis. Association of Official Analytical Chemists. 15th edition (K Helrick, editor). Arlington pp 1230

Bui Xuan An, Preston T R and Dolberg F 1997 The introduction of low-cost polyethylene tube biodigesters on small scale farms in Vietnam. *Livestock Research for Rural Development*. 9 (2) #16
<http://www.lrrd.org/lrrd9/2/an92.htm>

Chhay T, Borin K and Preston T R 2010 Effect of Taro (*Colocasia esculenta*) leaf + stem silage and mulberry leaf silage on digestibility and N retention of growing pigs fed a basal diet of rice bran. *Livestock Research for Rural Development*. Volume 22, Article #109. <http://www.lrrd.org/lrrd22/6/chha22109.htm>

Cruz E M and Shehadeh Z H 1980 Preliminary results of integrated pig-fish and duck-fish production tests. **In:** *Integrated Agriculture-aquaculture farming systems*, pp. 225–238, Pullin R S V and Shehadeh Z H (editors). Proceedings of the ICLARM-SEARCA Conference, Manila, Philippines, 6–9 August, 1979. 258 pp.

Duong Nguyen Khang and Le Minh Tuan 2002 Transferring the low cost plastic film biodigester technology to farmers. *Proceedings Biodigester Workshop March 2002* <http://www.mekarn.org/procbiod/khang2a.htm>

Hang D T and Preston T R 2010 Effect of processing Taro leaves on oxalate concentrations and using the ensiled leaves as a protein source in pig diets in central Vietnam. Livestock Research for Rural Development. Volume 22, Article #68. <http://www.lrrd.org/lrrd22/4/hang22068.htm>

Edwards P, Polprasert C, Raput V S and Pacharaprakiti C 1988 Integrated Biogas Technology in the Tropics 2. Use of Slurry for Fish Culture. Waste Management & Research Volume 6, Issue 1, 1988, Pages 51-61

Lai Qiuming and Yi 2004 Tilapia culture in mainland China. <http://aquanic.org/species/tilapia/documents/018.pdf>

Manivanh Nouphone and Preston T R 2011 Taro (*Colocacia esculenta*) silage and rice bran as the basal diet for growing pigs; effects on intake, digestibility and N retention. Livestock Research for Rural Development. Volume 23, Article #55. Retrieved , from <http://www.lrrd.org/lrrd23/3/noup23055.htm>

MTB 2000 Minitab Release 13.31 for windows, . Minitab Inc., State College Pennsylvania, USA.

Giang N T, Preston T R and Ogle B 2010 Effect on the performance of common ducks of supplementing rice polishings with taro (*Colocacia esculenta*) foliage. Livestock Research for Rural Development. Volume 22, Article #194. <http://www.lrrd.org/lrrd22/10/gian22194.htm>

Pich Sophin and Preston T R 2001 Effect of processing pig manure in a biodigester as fertilizer input for ponds growing fish in polyculture. Livestock Research for Rural Development. 13 (6) <http://www.lrrd.org/lrrd13/6/pich136.htm>

San Thy and Preston T R 2003 Effluent from biodigesters with different retention times for primary production and feed of Tilapia (*Oreochromis niloticus*). Livestock Research for Rural Development 15 (9). <http://www.lrrd.org/lrrd15/9/sant159.htm>

San Thy, Khieu Borin, Try Vanvuth, Pheng Buntha and Preston T R 2008 Effect of water spinach and duckweed on fish growth performance in poly-culture ponds. Livestock Research for Rural Development. Volume 20, Article #16. <http://www.lrrd.org/lrrd20/1/sant20016.htm>

Sorphea S, Lundh T, Preston T R and Borin K 2010 Effect of stocking densities and feed supplements on the growth performance of tilapia (*Oreochromis spp.*) raised in ponds and in the paddy field. Livestock Research for Rural Development. Volume 22, Article #227. <http://www.lrrd.org/lrrd22/12/sorp22227.htm>

Undersander D, Mertens D R and Theix N 1993 Forage analysis procedures. National Forage Testing Association. Omaha pp 154

Effect of biodigester effluent, duckweed and leaves from Taro (*Colocasia esculenta*) on growth of Tilapia (*Oreochromis niloticus*) in outdoor system

Tick Nouanthavong and T R Preston*

National Agriculture and Forestry Research Institute (NAFRI),
Vientiane, Lao PDR
ticknv_15@hotmail.com

* Finca Ecológica TOSOLY, AA 48 Socorro, Colombia

Abstract

Leaves from Taro (*Colocasia esculenta*) and Duckweed (*Lemna minor*) were compared as supplements for Tilapia (*Oreochromis niloticus*) grown in open ponds fertilized with biodigester effluent or not fertilized. The design was a 3*2 factorial arrangement with 3 replications. Fresh duckweed (*Lemna minor*) was grown in adjacent ponds fertilized with biodigester effluent. Taro leaves (*Colocasia esculenta*) were harvested from natural stands in the Centre. Both supplements were given ad libitum. The Tilapia had an average starting weight of 2.52 g and length 5.3 cm. The density was 5 fish/ m². The 18 ponds were each 3*2m and 1 m depth. The biodigester effluent was taken from a tubular polyethylene plug-flow biodigester charged with pig manure. The quantity applied was 520 mg N/pond/day.

Growth rates were four-fold higher for Tilapia supplemented with duckweed compared with Taro leaves or no supplement; and were 22% higher in ponds fertilized with biodigester effluent compared with no fertilizer. There were no benefits from feeding Taro leaves. Survival was high on all treatments (98-99%). Values for pH, ammonia and nitrous dioxide were higher in ponds fertilized with biodigester effluent than in unfertilized ponds.

Key words: ammonia, feed conversion, nitrous oxide, pH, survival, temperature,

Introduction

In an earlier paper (Nouanthavong Tick and Preston 2011) we stressed "the need to study alternative systems of fish production that do not depend on purchased feeds and which make better use of available resources in farming systems that recycle organic wastes". The conclusion from a preliminary 60 day trial with Tilapia and Cachama was that, although both species grew faster in tanks with artificial feed, than in open ponds fertilized with biodigester effluent and supplementary duckweed, less supplement DM was required per unit live weight gain for the Tilapia in the natural than in the intensive system, with the implication that despite the slower growth rates, the economic analysis would favour the former. The present study aimed to provide further information on the response of Tilapia to supplements of duckweed and taro leaves, in open ponds with and without fertilization from biodigester effluent.

Duckweed (*Lemna minor*) has been used successfully as a complete feed for fish (Skillicorn et al 1993; Leng et al 1995) and as the sole source of protein for pigs (Rodríguez and Preston 1996a). It grows well in earthen ponds and in ponds lined with concrete or polyethylene film (Rodríguez and Preston 1996b). It responds with linear increases in biomass yield and crude protein content when fertilized with biodigester effluent (Rodríguez and Preston 1996b; Dang Thi My Tu et al 2011). The leaves of several members of the Araceae plant family (eg: *Colocasia esculenta*, *Xanthosoma sagittifolium*) are also rich in crude protein which is highly digestible and with good biological value in pigs (Rodríguez et al 2009; Nouphone and Preston 2011). However, apart from our

earlier report (Nouanthavong Tick and Preston 2011) there appears to be no information on its potential value as a supplement for fish.

Hypothesis

- Tilapia will grow well in natural pond systems when biodigester effluent is used as fertilizer to stimulate growth of plankton
- Growth rates of Tilapia will be increased when they are also fed fresh leaves of Taro and duckweed

Objective

To measure the growth performance of Tilapia (Photo 1) using biodigester effluent as fertilizer and Taro leaves (Photo 2) and Duckweed (Photo 3) as protein supplements.



Photo 1: Tilapia (*Oreochromis niloticus*)



Photo 2: Taro leaves (*Colocasia esculenta*)



Photo 3: Duckweed (*Lemna minor*)

Materials and Methods

Location and climate

The experiment was carried out at the Nangtang hatchery belonging to the Living Aquatic Resource Research Center. The site is 15 km from Vientiane Capital in Sikottabong District, Vientiane, and Lao PDR. The experiment was started in January 2011 and finished in July 2011. The climate in Lao PDR is tropical with an average daily temperature of 31 °C and an average annual precipitation of 1500 mm, about 75% of which occurs in the monsoon season (May to October) (Kottel at 2001).

Treatments and experimental design

Six treatments in a Completely Randomized Design (CRD) were arranged as a 3*2 factorial with 3 replications (Table 1). The factors were:

Feed supplement

- NS: No supplement
- TL: Taro leaves
- DW: Duckweed

Fertilizer

- E: With biodigester effluent
- NE: No fertilizer

Table 1. Experimental layout

1	2	3	4	5	6
DWNE	NSE	NSE	NSNE	NSNE	DWE
7	8	9	10	11	12
NSE	TLE	TLNE	DWE	TLE	DWNE
13	14	15	16	17	18
TLNE	DWNE	NSNE	TLNE	TLE	DWE

Ponds, fish and management

The 18 ponds were 2m x 3m in area and 1 m deep, lined with plastic film to avoid water leakage through the sandy soil (Photo 4). Ten days before stocking with fish, quick-lime (CaO) was applied to the bottoms of all ponds at the rate of 100 g/ m². This was done to eliminate parasites and pathogenic organisms and to increase the pH (Pich Sophin and Preston 2001). Two ponds were managed solely to produce the duckweed used in the experiment (Photo 5).



Photo 4. Arrangement of the ponds



Photo 5. Two ponds dedicated to the production of duckweed

The Tilapia fingerlings had an average initial weight of 2.52 g and a length of 5.3 cm. They were put first in a nursery pond to adapt them to the local conditions. During this time they had free access to duckweed and taro leaves. After 2 weeks they were allocated to the treatment ponds at the rate of 30 fish per pond, giving a density of 5 fish / m².

Biodigester

A plug-flow tubular polyethylene biodigester was installed to supply the effluent for the duckweed ponds and for the fish ponds. It had a length of 6 m and a diameter of 1m. The liquid volume was 2 m³. It was charged with fresh pig manure from the piggery adjacent to the project site. The charging rate was 200 litres daily of a mixture containing 4% of DM derived from the pig manure (about 20 kg pig manure [25% DM] and 180 litres water).



Photo 6: The tubular polyethylene biodigester



Photo 7: The inlet to the biodigester



Photo 8: Collecting the effluent

The duckweed pond

Duckweed was first collected from a waste water surface (Photo 9) and washed thoroughly with fresh water prior to introduction to the two ponds dedicated to duckweed production. These were fertilized with biodigester effluent at the rate of 20 litres daily (assumed N concentration was 500 mgN/litre of effluent).

(<http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGA/AGAP/FRG/recycle/default.html>)

Taro leaves

Taro leaves were harvested from the edges of the ponds in the Nangtang hatchery where it grows naturally.

Feed and feeding

The Taro leaves were harvested once per week and fed ad libitum as the whole leaf, which floated on the water surface (Photo 10). Duckweed was harvested daily from the two dedicated duckweed ponds. And also fed ad libitum (Photo 10). The actual quantities offered of Taro leaves and duckweed was based on the observed rates of consumption. The biodigester effluent was added at the rate equivalent to 150 kg N/ha/year (about 2 litres/ pond/day).



Photo 9: Collecting duckweed from a waste water surface



Photo 10. Feeding Taro leaves



Photo 11. Feeding the duckweed

Data collection

Fish weight and length

The average weight of the fish was recorded before releasing them into the ponds and at the end of 150 days of experiments. A random sample of fish ($n =$ about 10) was weighed and measured every 30 days. The growth in weight was calculated from the linear regression of weight on time, disregarding the weight from 0 to 30 days, as this was considered to be an adaptation phase (see Figure 2).

Water quality

pH, ammonia-N and NO_2 in pond water were determined at 7:00 am two times per month using colorimetric test kits (Photos 12-14). Temperature was measured every day, three times at 7:00, 12:00 and 17.00 h.



Photo 12: The colorimetric kit to measure pH

Photo 13: The colorimetric kit to measure nitrite

Photo 14: The colorimetric kit to measure ammonia-N

Samples of effluent were taken weekly before application to the fish pond for determination of pH, DM, OM, N and Ammonia-N. (San Thy et al 2003). Samples of taro leaves and duckweed were taken weekly and analysed for DM (Undersander et al 1993) and N (AOAC 1990).

Chemical analyses

Feeds were analyzed for N following the method of AOAC (1990) and DM using the micro-wave oven (Undersander et al 1993). pH, NO_2 (Nitrite) and ammonia-N in the pond water were estimated by colorimetric test kits.

Statistical analyses

The data were subjected to analysis of variance by using the General Linear Model (GLM) option of the ANOVA program in the MINITAB software (Release 13.3, 2000). The sources of variation in the model were supplements, effluent, interaction effluent*supplement and error.

Results and discussion

The protein content of the duckweed was higher than for the leaves of Taro (Table 2).

Table2. Composition of the supplements

	Duckweed	Taro leaves
Dry matter, %	8.2	16.
Crude protein, % in DM	29.2	22.5

The weight gain of the Tilapia was four-fold higher when they were supplemented with duckweed compared with Taro leaves or no supplement (Table 3; Figure 1). The growth rate of the Tilapia (0.96 g /day) in ponds supplemented with duckweed and fertilized with effluent (Table 4) was similar to that (0.95 g/day) reported by Yen Nhi and Preston (2011) in an identical system in Vietnam with the same stocking rate of 5 fish / m². With a lower stocking rate of 3 fish/ m², the growth reported by these authors was 1.32 g /day. There was no advantage in giving Taro leaves as compared with no supplement.

The Tilapia gained weight 22% faster when the ponds were fertilized with biodigester effluent. On effluent only, the gain of 0.18 g/day was lower than the 0.27 g/day reported by San Thy et al (2008) for Tilapia stocked at a lower density of 2 fish / m². These authors reported that growth rates were doubled to 0.43 g/day when the effluent was from biodigesters managed with a longer hydraulic retention time (30 compared with 20 days). The moderate response to fertilization with biodigester effluent may have been the consequence of the level of N that was applied. According to Knud-Hansen et al (1991) and Lin et al (1997) the optimum input of nitrogen for fish culture especially Tilapia is 4 kg N/ha/day or 400 mg N/m² per day. This level (equivalent to 1460 kg N/ha/year) is almost ten times greater than the amount applied in our experiment (150 kg N/ha/year).

The DM intake was three times greater for duckweed than for the Taro leaves, which explains to a major degree the three-fold greater growth rate for the Tilapia fed duckweed compared with the Taro leaves. DM feed conversion appeared to be similar on duckweed as on Taro leaves, but this comparison was confounded by the contribution from the effluent. When the growth rates were corrected for the effect of the effluent (weight gain on [duckweed / Taro leaves] - weight gain of Tilapia in [effluent-fertilized ponds]), the DM feed conversion changed dramatically, increasing to 18.4 for the Taro leaves treatment compared with 1.08 for Tilapia supplemented with duckweed. The feed conversion ratios on the duckweed with or without correction for effects of the natural feed supply (0.76 to 1.08) were much better than was reported in Brazil (Tavares et al 2008) for Tilapia managed in open ponds and supplemented with 39% crude protein (DM basis) dried duckweed (DM FCR 3.0) or a commercial fish feed of 40% crude protein (DM FCR 1.6). The weight gains in our experiment for fresh duckweed (0.95 g/day) were also higher than those recorded by Tavares et al (2008), which were 0.36 g/day on fish feed compared with 0.22 g/day on dried duckweed.

Table 3. Mean values (main effects) for change in live weight, DM intake (DMI) and DM feed conversion for Tilapia in open ponds, fertilized with biodigester effluent or not fertilized, and supplemented with duckweed or Taro leaves or not supplemented

	No effluent	Effluent	SEM	P	DW	Taro	NS	SEM	P
Initial, g	2.52	2.52			2.52	2.52	2.52		
Final, g	55.2	67.7	3.68	0.034	119 ^a	37.0 ^b	28.5 ^b	4.50	<0.001
Gain, g/d	0.425	0.518	0.029	0.047	0.927 ^a	0.255 ^b	0.232 ^b	0.036	<0.001
DMI	0.473	0.466	0.016	0.76	0.742	0.197		0.016	<0.001
FCR	0.922	0.746	0.10	0.26	0.818	0.869			0.73
FCR#					1.06	5.63			

ab Means without common letter are different at P<0.05

After correcting for weight gain by unsupplemented fish

Table 4. Mean values for change in live weight, DM intake (DMI) and DM feed conversion for Tilapia in open ponds, fertilized with biodigester effluent or not fertilized, and supplemented with duckweed or Taro leaves or not supplemented (individual treatments)

	With effluent			No effluent			SEM	P
	Duckweed	Taro	No sup.	Duckweed	Taro	No sup.		
Initial, g	2.52	2.52	2.52	2.52	2.52	2.52		
Final, g	124 ^a	44.0 ^b	34.7 ^b	113 ^a	30.0 ^b	22.2 ^b	6.36	<0.001
Gain, g/d	0.964 ^a	0.300 ^b	0.289 ^b	0.89 ^a	0.21 ^b	0.175 ^b	0.0512	<0.001
DMI, g/d	0.730	0.202		0.730	0.202			
FCR	0.759	0.733		0.857	0.988			
FCR#	1.08	18.4						

ab Means without common letter are different at P<0.05

After subtracting the weight gain supported by the natural feed

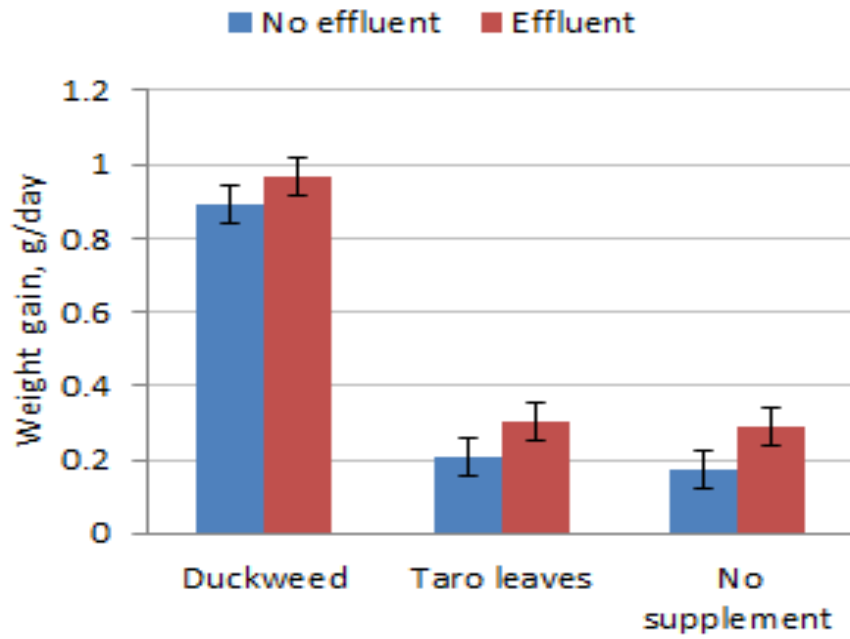


Figure 1. Effect of biogas effluent and supplementation with duckweed or Taro leaves on weight gain of Tilapia raised in open ponds

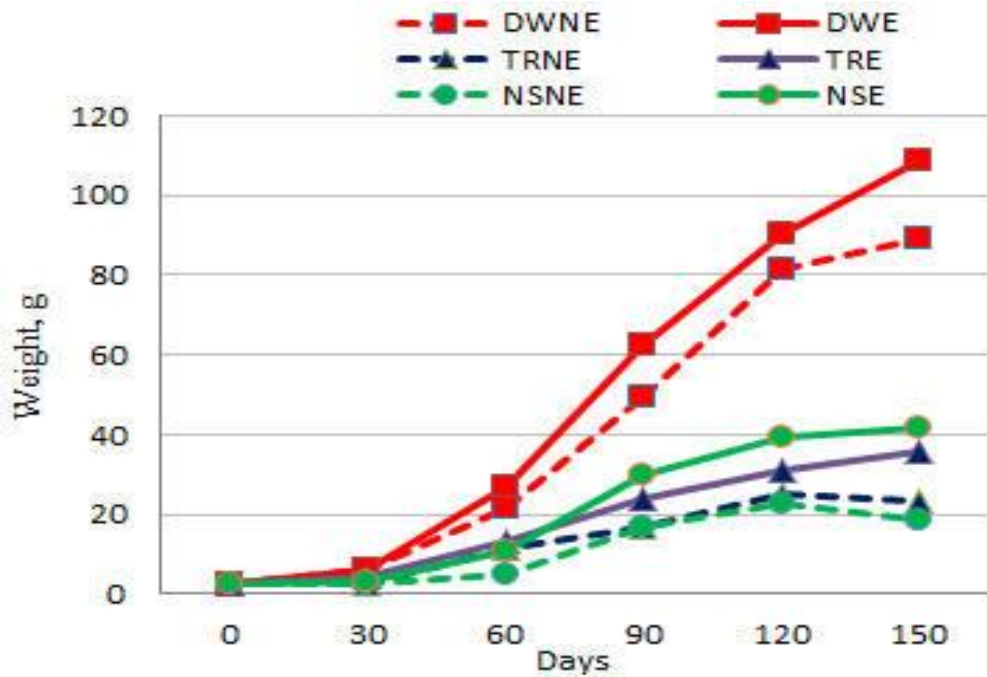


Figure 2. Growth curves of Tilapia raised in open ponds fertilized with biogas effluent (E) or no fertilizer (NE), and supplemented with duckweed (DW), Taro leaves (TR) or not supplemented (NS)

Water quality

There were differences in water temperature among supplementation treatments but not between ponds fertilized or not with effluent (Table 6). However, the differences were small.

Table 6. Mean values of water temperature in the ponds stocked with Tilapia fed supplements of duckweed or Taro leaves, and with addition of biodigester effluent or none

	Duckweed	Taro leaves	NS	SEM	P	No effluent	Effluent	SEM	P
Morning	26.70	27.30	26.70	0.12	<0.001	27.00	26.80	0.096	0.10
After noon	27.90	28.50	28.50	0.11	<0.001	28.20	28.40	0.095	0.27
Evening	28.00	28.70	28.10	0.11	<0.001	28.30	28.20	0.093	0.67

The values for pH, and especially ammonia (Figure 3), were higher in ponds fertilized with biodigester effluent than in ponds not fertilized (Table 7) and tended (P=0.10) to be higher also for nitrous oxide. However, all the values were within the range considered suitable for fish culture (Boyd 1990).

Table 7. Mean values of pH, ammonia and nitrous oxide in the ponds stocked with Tilapia fed supplements of duckweed or Taro leaves, and with addition of biodigester effluent or none.

	Duckweed	Taro leaves	NS	SEM	P	No effluent	Effluent	SEM	P
pH	6.45	6.15	6.28	0.104	0.130	5.98	6.60	0.085	<0.001
NH3	0.02	0.02	0.02	0.020	0.168	0.04	0.19	0.014	<0.001
NO2	0.04	0.01	0.03	0.016	0.270	0.01	0.04	0.013	0.1

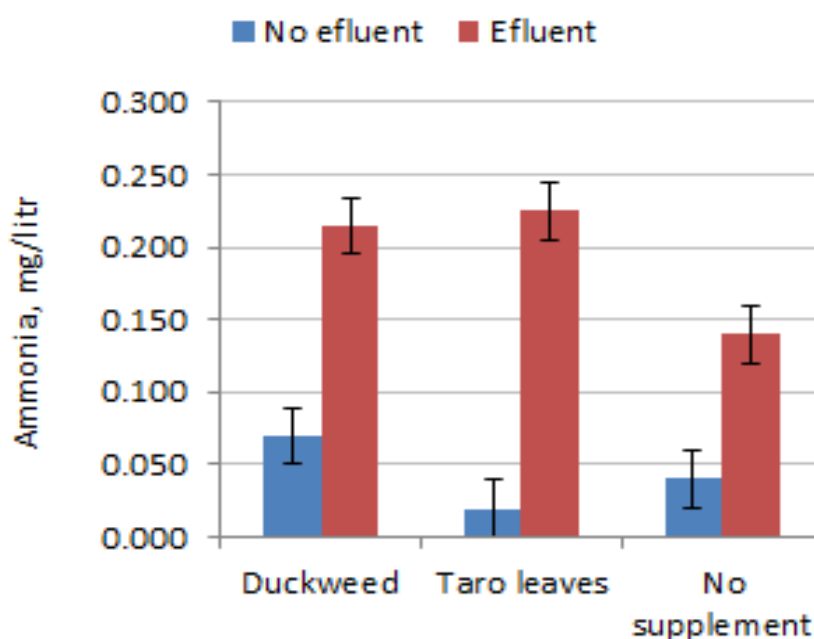


Figure 3. Effect of fertilization with biodigester effluent on ammonia concentration in the ponds

Conclusion

- The weight gain of Tilapia was four-fold higher (0.96 g/day) (when they were supplemented with duckweed compared with Taro leaves or no supplement; and was 22% higher in ponds fertilized with biodigester effluent compared with no fertilizer).
- There were no benefits from feeding Taro leaves.
- DM feed conversion was much superior on the duckweed supplement, ranging from 0.76 to 1.08, the latter being the adjusted value after allowing for the growth rate supported by the natural food chain in the ponds.
- Survival was high on all treatments (98-99%).
- Values for pH, ammonia and nitrous dioxide were higher in ponds fertilized with biodigester effluent than in unfertilized ponds.
- Tilapia fed duckweed in ponds fertilized with biodigester effluent had similar growth rates and better feed conversion than reported in several experiments where they were fed high-protein commercial feed
- Raising Tilapia in outdoor ponds fertilized with biodigester effluent and supplemented with fresh duckweed is an appropriate way to improve the economy of fish production for small scale farmers with beneficial effects on the environment.

Acknowledgements

The authors would like to thank the Swedish International Development Agency (Sida) and the Norwegian Programme for Development, Research and Higher Education (NUFU) for the financial support for this research. The research forms part of the requirement by the senior author for the MSc degree from Cantho University. The Living Aquatic Resource Research Center (LARReC) belonging to National Agriculture and forestry Research Institute (NAFRI) was the supporting location for the experiment.

References

- AOAC 1990** Official Methods of Analysis Association of Official Analytical Chemists 15th Edition (K Helrick editor) Arlington pp 1230
- Boyd C E 1990** Water quality in ponds for aquaculture. Alabama agriculture experiment station, Auburn University. Birmingham Publishing Co. Birmingham, Alabama. First printing 5M, December 1990.
- Dang Thi My Tu, Nguyen Thi Kim Dong and Preston T R 2011** Effect on duckweed composition of different levels of biodigester effluent in the growth medium and of transferring nutrient-rich duckweed to nutrient-free water. Workshop on Reducing Greenhouse Gas Emissions from Livestock and Soils (Editors: Reg Preston and Sisomphone Southavong). National Institute of Animal Science, Hanoi, 14-15 November 2011.
<http://www.mekarn.org/workshops/GHG-CC/mytu.htm>
- Fasakin E, Balogun A M and Fasuru B E 1999** Use of duckweed, *Spirodela polyrrhiza* L. Schleiden, as a protein feedstuff in practical diets for tilapia, *Oreochromis niloticus* L. *Aquacult. Res.* **30**:313–318 (1999).
- Gaigher I G, Porath D and Granoth G 1984** Evaluation of duckweed (*Lemna gibba*) as feed for tilapia (*Oreochromis niloticus* X *O. aureus*) in a recirculating unit. *Aquaculture* 41: 235-244.
- Knud-Hansen C F, McNabb C D and Batterson T R 1991** Application of limnology for efficient nutrient utilization in tropical pond aquaculture. *Proceedings of the International Association of Theoretical and Applied Limnology* 24:2541-2543.
- Kottelat M 2001.** Fishes of Laos. WHT Publications, Colombo

Leng R A, Stambolie J H and Bell R 1995 Duckweed - a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development. (7) 1: <http://www.lrrd.org/lrrd/7/1/3.htm>

Leng R A 1995 Duckweed; a tiny aquatic plant with enormous potential for agriculture and environment. FAO Animal Production and Health Paper 143

Lin C K, Teichert-Coddington D R, Green B W and Veverica K L 1997 Fertilization regimes. In: H.S. Egna and K.L. Boyd (Editors), Dynamic of pond aquaculture. CRCP Press, Boca Raton/ New York.

Manivanh N and Preston T R 2011 Taro (*Colocacia esculenta*) silage and rice bran as the basal diet for growing pigs; effects on intake, digestibility and N retention. Livestock Research for Rural Development. Volume 23, Article #55. <http://www.lrrd.org/lrrd23/3/noup23055.htm>

Minitab 2000 Minitab Release 13.31 for windows, Windows* 95/98/2000/xp. Minitab Inc., State College Pennsylvania, USA.

Nouanthavong Tick and Preston T R 2011 Comparison of natural pond system with an intensive (indoor) system for raising Cachama (*Colossoma macropomu*) and Tilapia (*Oreochromis niloticus*) . <http://www.lrrd.org/lrrd23/4/tick23100.htm>.

Pich Sophin and Preston 2001 Effect of processing pig manure in a biodigester as fertilizer input for ponds growing fish in polyculture. Livestock Research for Rural Development. (10) 6: <http://www.lrrd.org/lrrd13/6/Pich136.htm>

Rodríguez Lylian and Preston T R 1996a Comparative parameters of digestion and N metabolism in Mong Cai and Mong Cai*Large White cross piglets having free access to sugar cane juice and duck weed. Livestock Research for Rural Development. (8) 1: <http://www.lrrd.org/lrrd8/1/lylian.htm>

Rodríguez Lylian and Preston T R 1996b Use of effluent from low-cost plastic biodigesters as fertilizer for duck weed ponds. Livestock Research for Rural Development (8) 2: <http://www.lrrd.org/lrrd8/2/lylian2.htm>

Rodríguez Lylian, Peniche Irina, Preston T R and Peters K 2009 Nutritive value for pigs of New Cocoyam (*Xanthosoma sagittifolium*); digestibility and nitrogen balance with different proportions of fresh leaves and soybean meal in a basal diet of sugar cane juice. Livestock Research for Rural Development. Volume 21, Article #16. <http://www.lrrd.org/lrrd21/1/rodr21016.htm>

San Thy and Preston T R 2003 Effluent from biodigesters with different retention times for primary production and feed of Tilapia (*Oreochromis niloticus*). Livestock Research for Rural Development 15 (9). <http://www.lrrd.org/lrrd15/9/sant159.htm>

San Thy, Khieu Borin, Try Vanvuth, Pheng Buntha and Preston T R 2008 Effect of water spinach and duckweed on fish growth performance in poly-culture ponds. Livestock Research for Rural Development. Volume 20, Article #16. <http://www.lrrd.org/lrrd20/1/sant20016.htm>

Skillicorn P, Spira W and Journey W 1993 "Duckweed Agriculture. The New Aquatic Farming System for Developing countries" The World Bank. Washington DC, USA.

Tavares Flávia de A, Rodrigues João Bosco R, Machado Fracalossi D, Esquivel J and Roubach R 2008 Dried duckweed and commercial feed promote adequate growth performance of tilapia fingerlings. Biotemas 21 (3): 91-97

Undersander D, Mertens D R and Theix N 1993 Forage analysis procedures. National Forage Testing Association. Omaha pp 154.

Yen Nhi N H and Preston T R 2011 The growth and economics of integrated culture of Tilapia (*Oreochromis niloticus*) and Common carp (*Ciprinus carpio*) in an indoor intensive system with earthworms as feed and in natural ponds fertilized with biodigester effluent and supplemented with duckweed. Livestock Research for Rural Development. Volume 23, Article #161. <http://www.lrrd.org/lrrd23/7/nhi23161.htm>

Received 1 January 2012; Accepted 21 January 2012; Published 1 February 2012