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Studies into the Utilization of Pig Manure for Aquaculture in Central Vietnam

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Appendix

This thesis is based on the following papers, which are referred to in the text by their Roman numerals I, II.

I. Tram, N. D. Q., Ngoan, L. D., and Ogle, B., 2004. Effect of processing pig manure through a biodigester as fertilizer for fish ponds on water quality and growth performance of three fish species.
II. Tram, N. D. Q., Ngoan, L. D., and Ogle, B., 2004. Culturing earthworms on pig manure and the effect of replacing trash fish by earthworms on the growth performance of Hybrid Catfish (*Clarias macrocephalus x Clarias gariepinus*).

1. Introduction

Aquaculture is the cultivation of aquatic animals and plants. Its primary aim is to produce aquatic food organisms for human consumption, but includes other purposes such as the cultivation of ornamental and aquarium fishes (Egna, 1997). According to FAO (1995) the total capture fisheries of the world peaked at about 90 million metric tons in 1989 and so aquaculture will become increasingly important in the future. Aquaculture is usually sustainable because it normally makes use of locally available resources. Integration of aquaculture with other forms of agriculture diversifies farm productivity. This, in turn, provides opportunities for intensified production with more efficient allocation of land, water, labor, equipment and other limited inputs than enterprises which are independently operated. Stored pond water may serve as a catalyst for rural development because a variety of activities may be simultaneously undertaken. For example fish culture integrated with garden irrigation, livestock watering, and various domestic uses are all possible. (www.ag.auburn.edu/icaae/intraqua.htm).

Fresh water fish culture has in many ways with a diversification of cultured species (Grass Carp, Common Carp, Silver Carp, Bighead Carp, etc) and applied-aquaculture production systems (VAC system, fresh water fish culture in reservoirs, rice-fish culture system, running system, etc). Demand for aquaculture products for food and export is increasing and aquaculturists are trying to increase production in various ways. One technical application for the appropriate use of available resources to increase cultured fish production is fertilization. There are many kinds of potential fertilizers for fish pond fertilization, including inorganic fertilizer and organic fertilizer (manure, green fertilizer, sewage) in which manures from livestock are used more often. Farmers usually apply manure directly to the fish ponds, which is not appropriate as it causes water pollution, fish disease and eutrophication because of the high organic matter content. Livestock excreta are frequently a source of environmental pollution in intensive, specialized animal agriculture. In contrast, the efficient recycling of manure in integrated farming systems can lead to increased profits and reduced environmental damage. To solve the problem of pollution from animal excreta, there are several techniques that can be applied at present. The plastic biodigester is one of the most efficient technologies for small-scale animal farms because of its low cost, fast payback, simplicity and positive effect on pollution (Bui Xuan An, *et al*., 1997) The main products from the biodigester are biogas and effluent, which is a potential fertilizer because the anaerobic digestion process results in conversion of organic nitrogen from manure to ionized ammonia (NH_4^+), which can be used directly by plant roots. Thus it has been found in Vietnam that effluent was a better fertilizer compared with raw manure for application to cassava and duckweed (Le Ha Chau, 1998), although there are few reports of trials to compare the two sources of plant nutrients. It is also important to note that biodigester effluent, as well as behaving as organic fertilizer, also contains the organic materials from the digestion of bacteria that fish can use as food to grow. Increased productivity in polyculture fish ponds when biodigester effluent, rather than manure, was used as fertilizer was reported by Han Yuqin and Ding Jieyi (1984), and yields of fish were increased by 26% when the effluent was applied compared with the original manure.

However, organic wastes from livestock, plant and vegetable industries are becoming increasingly difficult and expensive to dispose of using conventional technology. Waste management using earthworms is an increasingly attractive option, with earthworms being commercially produced on a large scale using organic waste. To achieve this organic wastes from cattle and pigs may require solid separation from slurry, and poultry waste requires composting, washing or ageing to remove inorganic salts and ammonia. However, horse manure, paper waste, paper pulp solids, brewery waste and spent mushroom compost require no further modification. Urban waste including food scraps and grass clippings are suitable for earthworms, but are best fed after mulching and mixing to produce a uniform feed stock (Edwards and Bohlen, 1996). The worms themselves can also be fed to chickens as a high quality protein supplement (Rodríguez *et* *al.,* 1995). Earthworms are also important in improving the quality of soil by recycling decaying material, and are also a source of protein for other animals. The cultivation of earthworms using local resources such as livestock manure would seem to be a valuable intervention for the improvement of living standards of smallholders in the countryside. This research is aimed at finding the most suitable way of utilizing manure to develop fresh water fish culture and increase the incomes of rural people in Central Vietnam.

2. Objectives

- To evaluate the effect of fresh pig manure and biodigester effluent on physico-chemical and biological parameters of pond water and on the growth of three fish species (Tilapia, Silver Carp and Hybrid Catfish) in a polyculture system, thus providing data on the integration of fresh water aquaculture and biogas utilization.
- To evaluate the nutritive value of earthworms in replacing trash fish as a protein source for growing Catfish.

3. General discussion

3.1. Biological characteristics and feeding behaviors of three fish species

3.1.1. Silver Carp (Hypophthalmichthys molitrix)

Silver Carp live in the upper layer and are active jumpers. They grow in river tributaries, and spawn in the main river. Silver Carp feed mainly on plankton with their gill rakers. In the larval stage, they filter zooplankton but switch to phytoplankton when the gill rakers are properly developed (Sifa li *et al.,* 1994).

3.1.2. Hybrid Catfish (Clarias macrocephalus x C. gariepinus)

Hybrid Catfish are characterized by omnivorous feeding, fast growth, simple artificial propagation and high disease resistance. Catfish can be grown in monoculture or in polyculture systems. They can also be reared in small tanks in highly intensified culture systems. The Catfish has an accessory respiration organ, so it can survive when dissolved oxygen is low, or even out of water for several days. This species characteristic enables it to survive in highly stressed environments, which other species cannot tolerate (Sifa li *et al*., 1994).

3.1.3. Tilapia (Oreochromis niloticus)

Tilapia originated in Africa and were introduced to many areas in the world. *Oreochromis niloticus* (Nile Tilapia), *Oreochromis mossambicus* (Black Tilapia) and *Oreochromis aureus* (Blue Tilapia) are the most common suitable cultivated species. Tilapia are more tolerant to poor water quality than most commonly cultured fish. This is an important reason for the successful culture of these fish in many different kinds of system. Tilapia are an excellent culture species, partly because they grow well on a variety of natural food organisms, including plankton, green leaves, benthic organisms, aquatic invertebrates, larval fish, detritus, and decomposing organic matter (Schroeder, 1978).

Table 1. Feeding habits of some fish species

Feeding habit	Species
Algae and plankton	Silver Carp *(Hypophthalmichthys molitrix)* Milkfish (*Chanos chanos*) *Sarotherodon galileus* *Sarotherodon niloticus* Grey mullet (*Mugil cephalus*)
Zooplankton	Bighead Carp (*Aristichthys nobilis*)
Macrophytes	Grass Carp (*Ctenopharyngodon idella*)
Benthos	Common Carp (*Cyprinus carpio*) Black Carp (*Mylopharyngodon piceus*) Mud Carp (*Cirrhina molitorrela*)
Detritus	*Sarotherodon aureus* Common Carp *(Cyprinus carpio)* Milk fish *(Chanos chanos)* *Sarotherodon niloticus*

3.2. Aquaculture systems

In general, there are two forms of fish culture: monoculture and polyculture. Each system has its own important characteristics and the decision as to which system to use will depend on the purpose of the operation. Mostly, fish are raised in polyculture systems for extensive and semi-intensive culture, while monoculture is the choice for intensive systems.

3.2.1. Monoculture

Monoculture, which is practiced in many countries, is the stocking of a single species in a pond. Within a monoculture system, there are several stocking practices that affect the fish production of a pond. Monoculture is generally not as popular in extensive and semi- intensive cultivation systems as it is in intensive culture. In intensive culture the fish are confined in a small volume, such as in cages and concrete ponds, and derive all their sustenance from high protein, processed feeds. In such cases, a specially developed feed is provided for a specific species of fish. Where fish are raised primarily on natural food sources, monoculture results in ineffective utilization of several levels of the pond's natural food chain.

Nevertheless, some of the highest yields observed in integrated systems have been achieved in monoculture. High yields of the Nile Tilapia, Oreochromis niloticus, for instance, in waste-fed ponds, suggest such a system is practical.

3.2.2. Polyculture

A fish pond, especially a fresh water pond, usually produces a variety of food organisms in different layers of the water. Therefore, stocking species (or different sized classes of a given species) that have complementary feeding habits, or that feed in different zones, will efficiently utilize space and available food in the pond and increase total fish production. Moreover to maximize fish production with available food organisms in ponds, polyculture, with a variety of fish in different feeding niches, has been commonly practiced (Ling, 1967; Olah, 1980). Tang (1970) described multispecies polyculture as a harmonious system where the available fish foods and stocked fish species are balanced.

Chinese fish culturists usually polyculture the following fish: (1) Grass Carp (*Ctonopharyngodon idellus*) which roams in all strata of the water and feeds mainly on higher aquatic plants; (2) Silver Carp (*Hypothalmichthys molitrix*), a midwater dweller that prefers phytoplankton as food; (3) Bighead Carp (*Aristichthys nobilis*), also a midwater dweller, which consumes zooplankton; (4) Black Carp (*Mylopharyngodon piceus*), a bottom-dwelling carnivore that feeds on molluscs; and (5) Mud Carp (*Cirrhinus motilorella*), a bottom-dwelling omnivore that feeds on benthic animals and detritus. Grass Carp is the major species usually stocked when plants are abundant in the pond. In plankton-rich ponds, Silver Carp and bighead Carp are the major species usually stocked. In deeper ponds, the productivity of the bottom water is substantially reduced and the stocking rate of the bottom dwellers is usually low.

Based on these theories and practices the fish used in our study were raised culture in a polyculture with three fish species (Hybrid Catfish, Silver Carp and Tilapia) in order to improve the efficiency of utilization of natural foods (Paper I)

3.3. Animal manures and effluent for fish production

The use of animal manures for fish culture is an extension of traditional land-crop cultivation, which uses available on-farm resources within reach of many small-scale farms in Asia (Zhu *et al*., 1990; Edwards, 1993). It is well recognized that pond fertilization with animal manures stimulates production of bacteria, phytoplankton, zooplankton, and benthos. The obvious advantages of using animal manures as a nutrient source for fish culture are that they are (1) relatively inexpensive, (2) readily available on-farm, and (3) suitable for a variety of fish in polyculture. This procedure mitigates the problem of solid waste disposal. However, there are also a number of negative aspects of using animal manures. There are aesthetic objections and sanitary concerns related to the fish products from manured ponds also it is time-consuming to collect and apply bulk materials to ponds on a routine basis. These procedure results in unpredictable nutrient quality and high biochemical oxygen demand, which may cause oxygen depletion of pond water when applied at high rates. It is unsuitable for intensive, high-yield culture systems.

3.3.1. Manure composition

A large variety of animal manures have been used to fertilize fish ponds. In general, the moisture and nutrient contents of manure vary considerably, depending on factors such as the diet, purity and treatment of manure, and duration and conditions of storage. In our study, manure from pigs fed a mixture of concentrate and maize as fed (content 20% CP) had a mean content of N of 3.1% in DM (Paper I). The quality of manures depends on their composition, as they are often mixed with decomposed animal manure, plant residues and lime. In many instances animal manure may contain a significant amount of spilled animal feed, which contributes directly to the fish's diet when applied to the pond (Kwei Lin, 1997). The typical composition of the manures of various livestock species is shown in Table 2.

Table 2. Average composition of manure from different livestock species

	Composition, % of DM			
	Moisture	N	P	K
Dairy cows	79	0.5	0.1	0.5
Fattening cattle	78	0.7	0.2	0.5
Sheep	64	1.1	0.3	1.1
Pigs	74	0.5	0.2	0.4
Hens	76	1.1	0.4	0.4

Source: Schroeder, 1980
DM = dry matter

3.3.2. The mode of action of manure recycling to fish

Feeding animal wastes to fish is an old practice. The mechanism of manure fish recycling via fish is illustrated in Figure 1.

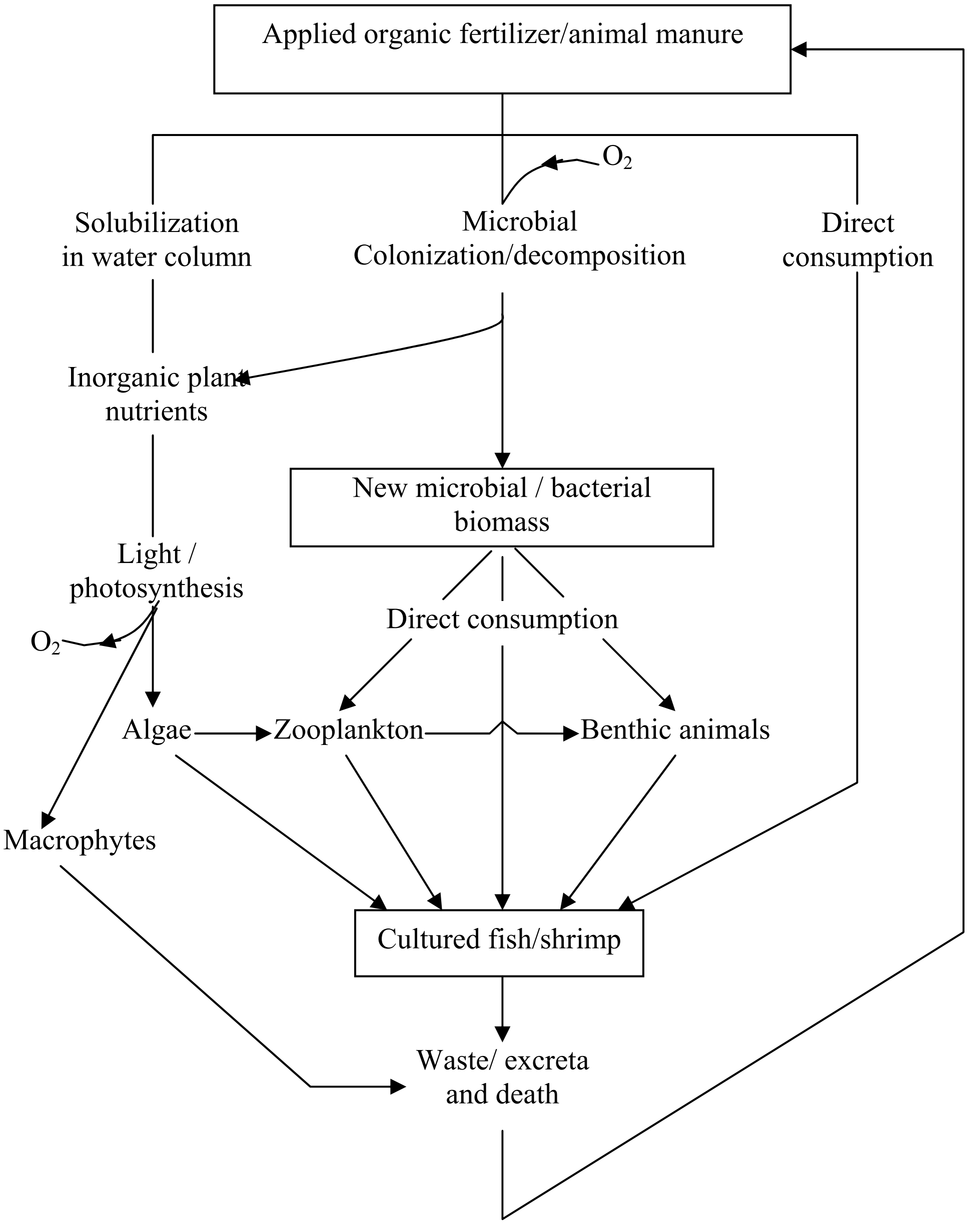

Figure 1. Fate of applied organic fertilizer in aquatic systems (Adapted from Edwards, 1982)

The fish is the final result of a complex biological cycle. The chain or cycle of natural fish production includes the following links: (a) mineral nutrients- plant production; (b) microbial production (c) intermediate animal consumption and production leading to the final product which is the fish; and (d) reduction. The origin of this cycle lies in the mineral nutrients of water which come from soluble substances, carried to the water by exogenous detritus (animal waste) and also by rainfall. By means of photosynthesis, the green vegetation transforms these inorganic substances into organic matter which forms vegetable tissue (higher and lower plants). Living or dead, the plants are consumed by numerous small animals. These then serve as a food for larger water animals which in turn are eaten by fish, either directly or after death and decomposition.

The last stage is the reduction, which is brought about by bacteria. Bacteria, by a mineralization mechanism permit the return in solution of all dead components of organic matter-vegetable and animal-and their re-integration into the biological cycle.

The production of a fish pond depends, in the final analysis, on the production of vegetation, which in turn is dependent on the nutrients found in the pond. The vegetable growth of the fish pond can be increased by introducing fertilizers and animal manures.

3.3.3.Manure fertilization through composting and fermentation

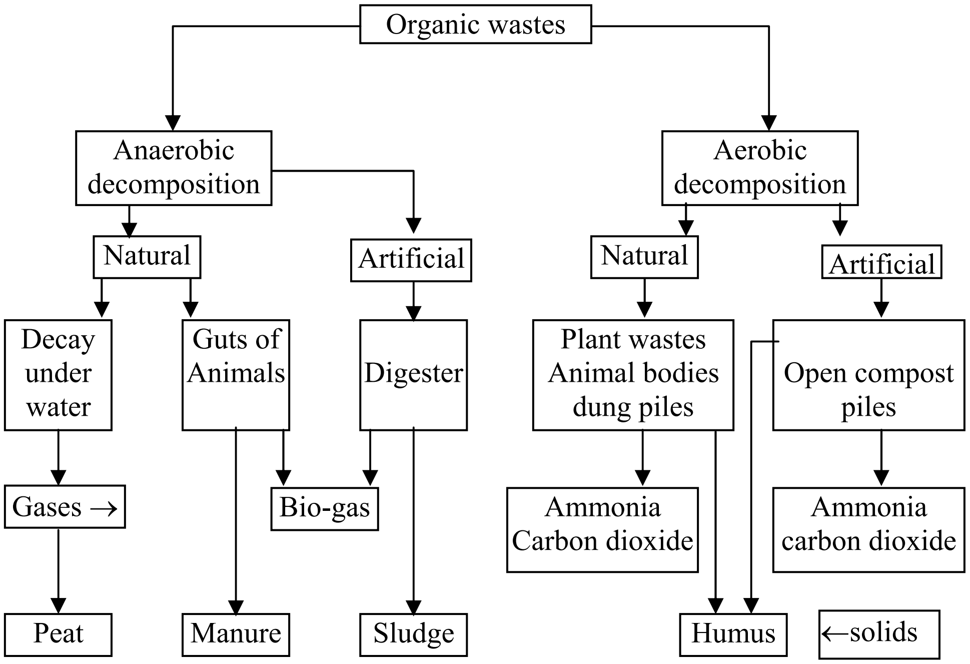

Figure 2. End products of organic decay (Fry, 1976)

In many parts of the world organic manures and wastes are first biologically stabilized by aerobic composting or by anaerobic fermentation prior to application as pond fertilizers. Both these stabilization processes rely on the controlled microbial decomposition of an organic waste substrate; the former (composting) in the presence of atmospheric oxygen, and the latter (fermentation) in the absence of atmospheric oxygen (Figure 2). The rationale behind the use of these stabilization techniques is to speed up the natural decomposition process and so reduce the time lag between fertilizer application and increased natural productivity. Apart from yielding useable by-products such as heat energy (composting) and biogas (mixture of methane and carbon dioxide; anaerobic fermentation), these stabilization techniques permit the use of agricultural wastes which in their natural or undecomposed state would have a low fertilizer value (such as coffee pulp, sugar cane waste, rice straw, palm oil waste, etc), facilitate the destruction of potentially hazardous pathogens and parasites which may be present in the raw waste material (ie. human faecal wastes), reduce the bulk weight of the original waste material, and also reduce the oxygen demand of the stabilized waste when applied to a water body.

3.4. Biodigesters

Biodigester technology for converting manure into methane for fuel is neither new nor uncommon. In many parts of Asia, Central America and Europe, biodigester use is widespread. Biodigesters are installed in these areas in response to organic waste (manure) disposal problems and/or high-energy costs (Halter, www.csale.usask.ca/PDF Documents/biodigesterDevelop.pdf)

3.4.1. The process inside the biodigester

The process of bio-digestion is the collection of organic material into a containment body, which is isolated from the external environment. Within this body a microenvironment optimal for methanogenic bacteria (methanogens[1]) is provided. These methanogens digest organic material (in this case manure) and emit CH_4 gas and NPK slurry[2] (NPK concentrate[3] and grey water[4]). This process greatly reduces the emission of volatiles[5] normally associated with manure and converts the solids into a more uniform sterilized product. Carbon that normally would be released in the form of gaseous hydrocarbons, CO_2 and CH_4, is contained and burnt as fuel to heat the barn and biodigester. If the gaseous hydrocarbons are burnt in a microturbine [6], then electricity to power the barn and biodigester facilities can also be generated. The residual heat from the microturbine is available to maintain a consistent climate within both barn and biodigester (see Figure 3).

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[1] Methanogens are anaerobic bacteria, which digest organic material and release methane as a byproduct.

[2] NPK slurry refers to the liquid material that has passed through the biodigester. It is a mixture which, when separated, constitutes NPK concentrate and grey water.

[3] refer to a product that can be used as input for the production of a commercial product like compost.

[4] Grey water is the water that is removed from the NPK slurry. This water might be purified to potable standards,
however, this has yet to be verified by independent study.

[5] For this report volatiles are gaseous substances like NH_3, CH_4, CO_2, or N_2O which are emitted from manure.

[6] Microtubine: For this report shall refer to a small, portable gas-fired electricity generating plant of 75kW capacity.

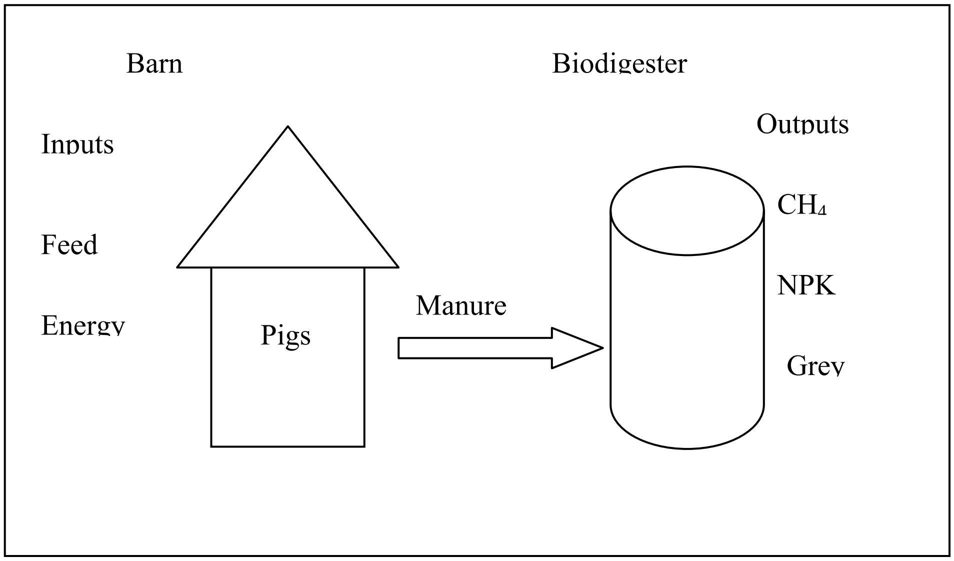

Figure 3. Biodigester Schematic

3.4.2. Animals waste and effluent from biodigester as fertilizer in aquaculture

Organic waste utilization in aquaculture can either be extensive, with wastes occurring naturally or being added, with little or no further production management (Bardach *et al.,* 1972). An example is a duck farm where the wastes can produce up to 1.3 tons fish/ha in 100 days.

In China, when animal wastes are applied there is often additional extraneous feeding (Ding Jieyi and Han Yujin 1984). In these situations, the 100-day fish yield reaches 3 to 4 tons/ha. Moscoso and Nava (1990) reported that in a Tilapia pond fertilized with effluent, the fish production was 2290 kg/ha in 154 days. The fish yield in study by San Thy and Preston (2003b) was 1015 kg/ha in 120 days. In a polyculture fish farm in Israel, yields were reasonably high (4150 kg/ha/yr). It should be noted that in polyculture systems, nutrients are reused directly as they pass through the digestive tracts of the various component species (Rawitscher and Mayer, 1977). The use of manure and domestic sewage, however, represents a saving for the fish farmer only when these materials are locally available. Overall productivity of biomass was increased by 67 % with no additional feeding or fertilizer by providing pig wastes to a polyculture pond. It was also found (Paper I) that in a polyculture with three fish species (Hybrid Catfish, Silver Carp and Tilapia) applied raw pig manure or effluent from a biodigester gave a total net fish yield of 2.1 and 1.7 tons/ha, respectively.

Hogan (1933) and Meehean (1934) showed an increase in the production of fish due to fertilization, and Judy and Schloemer (1938) found that both plankton abundance and fish growth rate increased after the application of fertilizers. Howell (1942) concluded that fertilization led to increase in plankton abundance and fish production. Smith and Swingle (1943) also studied the effects of both inorganic and organic fertilizers on plankton life and found an increase in plankton as well as fish production. Hepher (1962) conducted experiments on the fertilization of fish ponds in Israel for ten years and found positive effects on the growth of fish. Similarly,

Biodigesters have been suggested as a way to alleviate pollution concerns as well as offering new revenue opportunities. These opportunities are realized via the fertilizer resource. One product of bio-digestion is the conversion of raw manure slurry into enriched NPK fertilizer. Relative to raw manure, the enriched NPK concentrate has more available nutrients, is relatively odourless, is free of disease, germs, weeds and seeds and is less prone to cause water contamination (Hazeltine and Bull, 1999 cited by Halter, www.csale.usask.ca/PDF Documents

/biodigesterDevelop.pdf). Hence, the digested product is characterized by greater consistency and minimizes offensive odours. In effect, a biodigester is an environment that transforms carbon and hydrogen in the raw slurry into gaseous CH_4 for combustion. The remaining liquid mixture is sterilized and rendered into a consistent NPK concentrate via bacterial activity. The liquid NPK fertilizer can then be de-watered to become NPK concentrate. The latter process adds significant value since water is heavy and expensive to transport. When water is removed only the nutrient-rich solids remain and can be transported longer distances at less cost. The residual "grey" water can possibly be used to flush manure from the barns into the biodigester. These factors combine to alleviate the problem of excessive fertilization close to the hog barns and opens opportunities for hog manure in the higher value residential compost market. Injection is a method of manure application that uses an apparatus similar to an air seeder to place manure below the surface.

3.5. Water quality in ponds

The various chemicals dissolved in the water, as well as the temperature and other physical attributes of water, all combine to form what is called water quality. For aquaculture systems, changes in water characteristics that improve the production of an aquatic crop would be considered improvements in water quality, while those changes reducing production would be considered as degradation of water quality (Diana *et al.,* 1997). Water quality is one of the most important factors affecting successful pond fish culture. If water quality is excellent, then survival, growth, and reproduction can achieve high values; otherwise fish production will be reduced or impossible. Some of the more commonly cultured warm water pond fish species are relatively tolerant of poor water quality. They can exist and grow over a wide range of salinity and temperature, and can tolerate low oxygen concentrations for brief periods (Table 3) (Fast, 1983). These species were undoubtedly selected by early aquaculturists for their hardiness.

Table 3. Temperature and dissolved oxygen values for selected warm water pond fishes [a]

	Temperature, ^{o}C			Min, Oxygen, mg/l	References
	Min	Optimum	Max		
Clarias Catfish	20	29-32	37	5(fry), 0.0 (adult)	Doudoroff and Shumway, 1970
Tilapia	10-15	25-33	35-42	0.2-2.0	Balarin and Hatton, 1979
Silver Carp	15	20-28	30	0.3-1.1`	Hickling, 1962
Cyprinus carpio	0-3	20	36	0.2-2.8	Doudoroff and Shumway, 1970
Common Carp, Grass Carp, Bidhead Carp and Silver Carp	21	27-29	32	0.5	Bortz *et al*., 1977

[a] Temperature values are generally for long - term periods (e.g. months), whereas minimum oxygen values are generally for less than one day.

3.5.1. Dissolved oxygen (DO)

Dissolved oxygen concentration is one of the most important water-quality parameters. Oxygen depletion is usually the principle cause of sudden, massive fish kills. Maintaining a 'normal' or desirable oxygen regime in a pond not only helps assure the fish's health, but also indicates that the pond system is functioning suitably. A productive pond will typically have supersaturated DO during the late afternoon, and undersaturated DO at dawn. We can better understand how DO depletion occurs in a fish pond if we describe the interactions between the main variables which result in the daily DO cycle (Figure 4). These variables include photosynthesis, diffusion and respiration

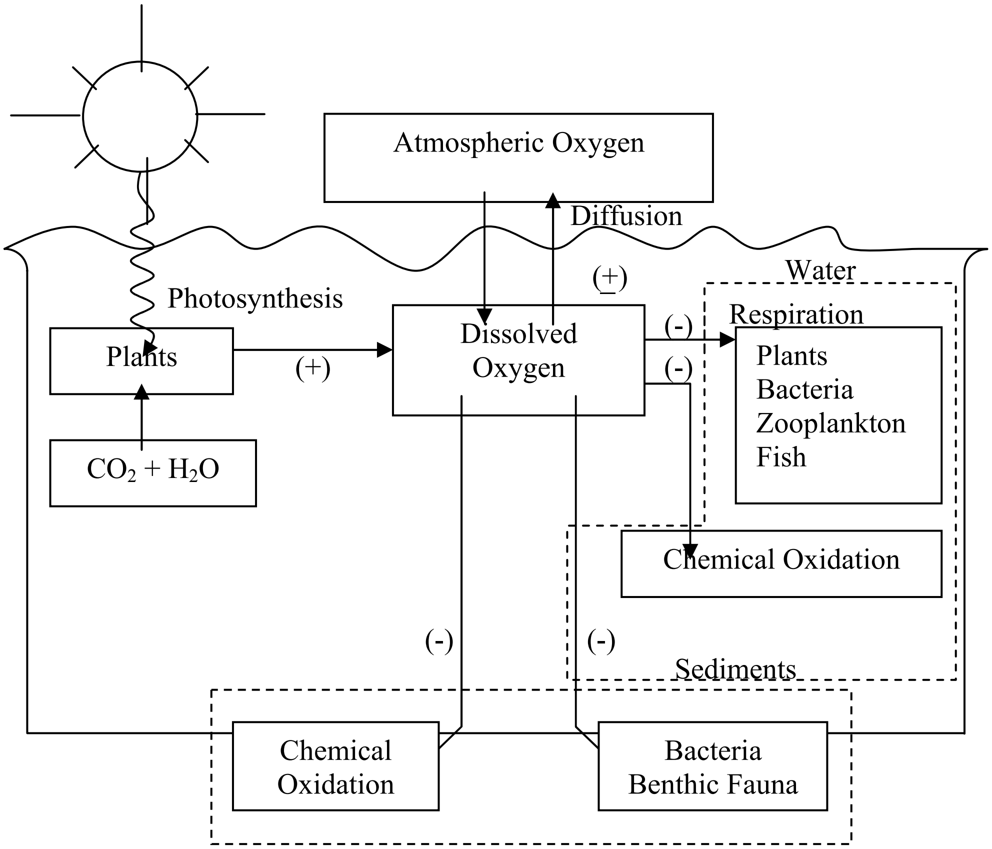

Figure 4. Principle sources and sinks for dissolved oxygen in a fish pond (Fast, 1983)

There are two main sources of oxygen in water; diffusion from the atmosphere and through the photosynthesis of aquatic plants, mostly phytoplankton. The atmosphere contains nearly 21% oxygen gas, but solubility in water is low, so the greater amount of oxygen in the water comes from the process of photosynthesis. There are three main factors that influence the level of dissolved oxygen in the pond; temperature, photosynthesis and respiration. This oxygen is used by plankton, fish and benthic organisms for respiration and for the decomposition of organic material. Oxygen solubility decreases with increasing temperature (Table 4) and increasing salinity. The magnitude of daily changes in oxygen concentration is influenced by phytoplankton density. Oxygen is lowest at sunrise, before photosynthesis becomes active, increases during the daylight hours to peak in late afternoon or early evening, and declines at night. Oxygen consumption rates by fish vary with water temperature, dissolved oxygen concentration, fish size, level of activity, time after feeding, and other factors. Metabolic rates vary by species and are limited by low oxygen conditions; small fish consume more oxygen per unit size than large fish of the same species.

Swingle (1969) developed a dissolved oxygen (DO) scale for warm-water fish:

- DO: < 0.3 mg/litre: Fish die after short-term exposure
- DO: 0.3 mg to 1 mg/litre: Lethal for long- term exposure
- DO: 1mg to 5 mg/litre: Fish survive, but growth is slow for long-term exposure.
- DO ≥ 5mg/litre: minimum for warm water fish (fast growth)

Fish do not grow well when the DO concentration is below 25% of saturation for long periods (Romaire, 1985) and perform better when DO concentrations are near saturation. Some authors recommend that the DO concentration in aquaculture systems be kept at about 90% of saturation, as a minimum at all times, for optimum performance. In our study DO values were low both in the early morning and afternoon, particularly in ponds which loaded raw pig manure, that cause fish gulped air in the early morning (personal observation) (Paper I).

Table 4: The effect of temperature on oxygen saturation of water

Temperature (^oC)	Oxygen saturation (mg/litre)
10	10.9
15	9.76
20	8.84
25	8.11
30	7.53
35	7.04

Source: STOAS 1993

3.5.2. pH

pH is important in aquaculture as a measure of the acidity of the water or soil. Fish can not survive in waters below pH 4 and above pH 11 for long periods. The optimum pH for fish is between 6.5 and 9. Fish will grow poorly and reproduction will be affected at consistently higher or lower pH levels (Table 5).

The pH of most natural waters ranges between 5 and 10 (Boyd, 1990) and it changes according to the influence of many factors such as acid rain, pollution, CO_2 from the atmosphere and fish respiration. The decay of organic matter and oxidation of compounds in bottom sediments also alter pH in water bodies. In ponds, phytoplankton and other organic plants use up CO_2 during photosynthesis, so the pH of a water body rises during the day and drops at night. In poorly buffered pond waters the pH can be as low as 5 to 6 in the morning rising to 9 or more in the afternoon. In waters with high alkalinity, pH typically ranges from 7.5 to 8.0 at daylight and from 9 to 10 in the afternoon. According to Randall (1991), in general, fish are intolerant to pH extremes outside of the range of 5 to 9. In our research (Paper I), the range of pH in the fish ponds was from 6.6 to 7.7 in the morning and from 8.0 to 9.5 in the afternoon, values which are within the desirable range for fish growth and reproduction.

Table 5. The effects of pH on warm-water pond fish

pH	Effect on fish
4	Acid death point
4 to 5	No reproduction
4 to 6.5	Slow growth
6.5 to 9	Desirable range for fish reproduction
9 to 10	Slow growth
11	Alkaline death point

Source: Swingle 1969

3.5.3. Secchi disc (water transparency)

Secchi disc provides crude guidelines for the proper rate and amount of fertilizer treatment. The Secchi disc is the most suitable index of plankton abundance when plankton is the primary source of pond turbidity. Stickney (1979) recommends a depth of 30 cm to achieve and maintain proper fertilization. The mean Secchi disc depth in polyculture ponds in our study was around 20 cm, which is low if compared with the recommendation, but might be suitable in new pond where the bottom soil is clay loam and turbidity is high (Paper I).

3.5.4. Nitrogenous compounds

The major source of nitrogen (up to 90%) in an aquaculture system is from fish feeds and is produced through the normal metabolic processes of the fish. Most of the nitrogen in organic matter exists as the amino acids in protein. The chemistry of nitrogen in ponds is very complex because of the many states in which nitrogen can exist: NH_3, NH_4^+, N_2, N_2O, NO, N_2O_3, N_2O_5, NO_2^-, and NO_3^- (Sawyer and McCarty, 1978). The form of nitrogen will affect the processes in the system (Table 6).

The nitrogen cycle

The nitrogen cycle in aquaculture ponds is the most important biogeochemical cycle of these systems. Nitrogen is essential to sustaining high primary production, but can also be toxic to many organisms in high concentrations. The correct balance of available nutrient and controlled wastes is necessary in any aquaculture system. Bacteria metabolism plays an essential role in controlling unwanted nitrogen as well as maintaining available inorganic nutrient levels. The exact form the nitrogen cycle takes in any pond or lake depends on the location of the majority of nitrogen-associated metabolism (Kuznetsov, 1970). Denitrification occurs in anaerobic sediments and waters, while aerobic waters support nitrification.

Table 6. The major forms of nitrogen in aquaculture systems

Form	Notation	Comments
Nitrogen gas	N_2	Inert gas; transfers in and out from atmosphere; no significance.
Organic nitrogen	Org-N	Decays to release ammonia.
Un-ionized ammonia	NH_3	Highly toxic to aquatic animals; predominates at high pH levels.
Ionized ammonia	NH_4^+	Nontoxic to aquatic animals except at very high concentration; predominates at low pH levels.
Total ammonia	$NH_3{}^{+}$ NH_4^+	Sum of unionized and ionized ammonia; typically measured in the test for ammonia; converted to nitrite by nitrifying bacteria.
Nitrite	NO_2^-	Highly toxic to aquatic animals; converted to nitrate by nitrifying bacteria.
Nitrate	NO_3^-	Nontoxic to aquatic animals except at very high concentrations; readily available to aquatic plants.

Source: Boyd, 1990

In aquaculture ponds, high levels of fish and shellfish metabolism result in the production of various nitrogenous waste products. The major nitrogen containing substance released by these organisms is ammonia (NH_3), which reacts with water to become the soluble ammonium ion NH_4^+. Both NH_3 and NH_4^+ are fairly toxic to aquatic organisms, as is nitrite ion (NO_2^-) and it aqueous counterpart, nitrous acid (HNO_2). Through bacterial activity, ammonia and nitrite are metabolized to yield nitrate (NO_3^-), which is fairly safe for aquatic organisms and useful to phytoplankton as a nutrient source. The biochemical reactions which occur to produce nitrate are given below:

$$2NH_3 + 3.5\ O_2 \xrightarrow{\text{Nitrosomonas}} 2\ NO_2^- + 3\ H_2O$$

$$NO_2^- + 0.5O_2 \xrightarrow{\text{Nitrobacter}} NO_3$$

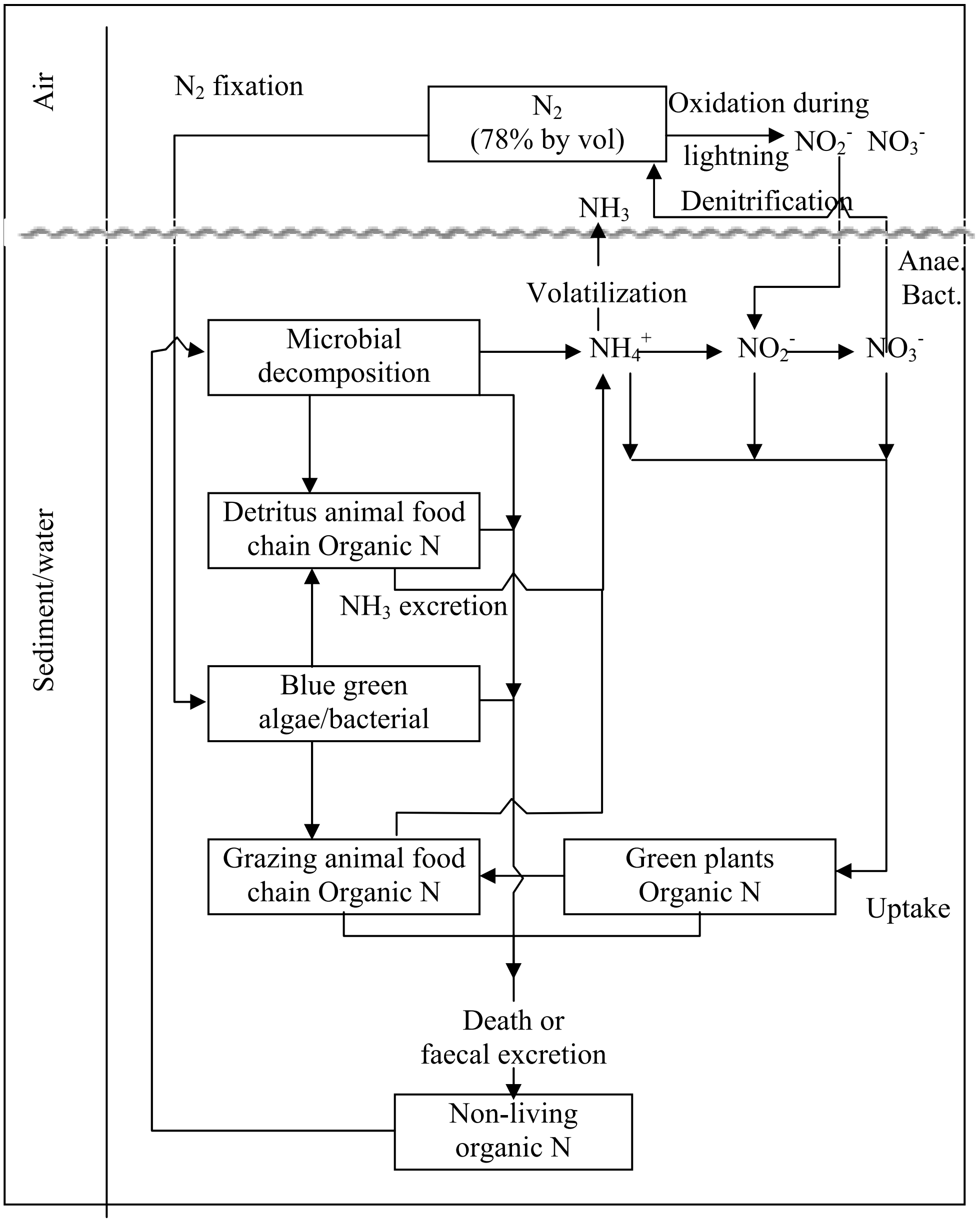

Figure 5: The nitrogen cycle

Ammonia

Ammonia is produced through the biological conversion of organic nitrogen through a process called ammonification (Figure 5). Ammonia is also produced as the major end product of protein catabolism and is excreted by fish and invertebrates (Campbell, 1973). It is excreted primarily as non-ionized ammonia (NH_3) through the gills. Ammonia is also produced through the decomposition of urea, fish faeces, and uneaten feed.

Ammonia can be in two states: ionized ammonia also called the ammonium ion (NH_4^+) and non-ionized ammonia (NH_3). The two sources of ammonia ($NH_4^+ + NH_3$) are called total ammonia or simply ammonia. Total ammonia nitrogen (TAN) = $NH_4^+ + NH_3\text{-N}$. The toxicity of TAN is dependent on what fraction of the total is in non-ionized form since this is by far the more toxic of the two. In most environments $NH_4^+\text{-N}$ predominates, although the fraction present in this form is dependent on pH, temperature and salinity. Water pH has the strongest influence on the direction in which the equilibrium equation will shift. $NH_3 + H_2O = NH_4OH = NH_4^+ + OH^-$

When the pH value is lower, the reaction will shift to the right and as pH is raised the reaction will shift to the left. Toxic concentrations of $NH_3\text{-N}$ for short-term exposure vary between 0.6 and 2 mg/litre for many pond fish, and some effects can be seen at 0.1 to 0.3 mg/litre (Boyd, 1979). Normally warm-water fish are more tolerant to ammonia than cold-water fish. To be safe, ammonia concentrations below 0.05 mg/litre as $NH_3\text{-N}$ and 1.0 mg/litre as TAN are recommended for long-term exposure. In our study mean ammonia concentration were low (0.06 and 0.09 mg/litre in biodigester effluent and raw pig manure ponds, respectively), with that concentrations not affect to fish growth (Boyd,1979).

Nitrite

The nitrite and nitrate concentrations show distinct seasonal patterns in fish ponds because nitrite ($NO_2\text{-N}$) is the ionized form of nitrous acid (HNO_2), and can be as lethal as $NH_3\text{-N}$. Nitrite levels in fish ponds typically range from 0.5 to 5 mg/litre, probably due to the reduction of nitrate in anaerobic mud or water (Boyd, 1982). They both are usually minimal in the summer months and increase in autumn, winter and spring. The toxicity of $NO_2\text{-N}$ is due principally to its effects on oxygen transport and tissue damage.

Nitrate

Nitrate build-up occurs most in pond systems when the water temperature is lower. The nitrosomonas bacteria, which convert ammonia to nitrite, function at cool temperatures (16-20^{o}C), but nitrobacter, which convert nitrite to nitrate, do not function well at temperatures is low, hence nitrite will accumulate. Neither species functions well at temperatures below 16^{o}C. The present study was carried out in summer season with the temperature ranged from 20 to 34°C and so both Nitrite and NH_3- N is low (0.0-0.2mg/litre) (Paper I) which not have negative affect to fish growth. Nitrates are the least toxic of inorganic nitrogen compounds.

3.5.5. Biochemical Oxygen Demand (BOD)

The BOD value, which is waste specific, is highly correlated to the amount of dry matter in the waste. Decomposition is dependent on temperature and thus low temperatures will result in slower breakdown and hence lower BOD values. The rates of decomposition reduce by 50% for every 5^{o}C temperature drop. The use of BOD values in effluents used in aquaculture (Table 7) facilitates estimating safe levels of organic matter that can be added to ponds, ensuring the minimum needs of dissolved oxygen for the fish. The fresh manure has a higher BOD than composted manure, fermented manure and effluent from biodigesters (Han Yujin and Ding Jieyi 1984). For instance, the BOD for biodigester effluent is about 60 to 70% lower than in fresh manures (Bio Cycle 1999; Pich Sophin and Preston 2001) and about 25% lower in biodigester effluent compare to raw pig manure (Paper I). The BOD varies according to the fertilization (Table 7).

Table 7: The 24 hour biochemical oxygen demand (BOD) for various inputs into pond culture of fish

Material	BOD (g O_2/ kg/24 hr) at 30°C
Dry human wastes	35-50
Chicken manure	20-40
Duck manure	20
Terrestrial fodder	13
Pig manure	12
Field day manure	10
Submerged aquatic weeds	8.6
Liquid cowshed manure	7
Floating aquatic weeds	6.3
Emergent aquatic weeds	5.4
Liquid calf manures	5
Human sewage	2.5-3

Source: Edwards, 1982; Schroeder, 1978

3.5.6 Chemical Oxygen Demand (COD)

Whereas the BOD value indicates the oxygen demand for respiration of micro-organisms acting on an organic substrate, the COD measures chemically the amount of oxygen required for the complete oxidation of a particular waste. However, the COD gives no information on the rate of oxidation of a particular waste and will not define, for example, the amount of oxygen taken up in 12 or 24 hours. If the wastes contain fractions that bacteria cannot readily oxidize, the COD value tends to overstate the effect of the wastes in the pond. A ratio of COD/ BOD will however give useful information on the longer term oxygen requirements from waste added to ponds, since it is an indication of the rate of decay and thus oxygen demand over time. Thus if the COD greatly exceeds BOD, the waste will be oxidized slowly and can be expected to take up oxygen beyond 12 or 24 hours. If the values are approximately similar, the waste will have negligible long term effects.

3.6. Protein and amino acid requirements of finfish

Protein is a significant dietary component because of its cost and constraints on growth. The gross protein requirements in fish are higher than in warm-blood animals (Lovell, 1979a). Table 8 summarizes the requirement of certain fish species. Lovell (1979a) states that protein levels

of 30 – 36 percent will probably be adequate for most warmwater fish diets.

For optimal utilization of dietary protein, the amino acid profile of the feed should closely resemble the ten essential amino acid requirements of the fish. As shown in Table 9, real differences exist between species. Thus it is difficult to formulate practical diets for fish whose amino acid requirements are unknown. When a diet is deficient in one or more amino acids, it may be possible to supplement it in the appropriate amounts.

Table 8. Gross protein levels for certain fish species

Species	Crude protein level in diet for optimal growth, g/kg DM
Rainbout trout *(Salmo gairdneri)*	400-460
Carp *(Cyprinus carpio)*	380
Chinook salmon *(Oncorhynchus tschawytscha)*	400
Eel *(Anguilla japonica)*	445
Grass Carp *(Ctenopharyngodon idella)*	410-430
Hybrid Catfish *(Clarias macrocephalus x C. gariepinus)*	200-300 [a]

Source: Cowey, 1979, [a] from Pham Bau, 1996

Table 9. Amino acid requirements of certain fish [a]

Amino acid requirement, g/kg dry diet						
Amino acid	Chinook Salmon	Japanese eel	Carp	Channel Catfish	Gilthead bream	Rainbow trout
Arginine	24	17	16			12
Histidine	7	8	8			
Isoleucine	9	15	9			
Leucine	16	20	13			
Lysine	20	20	22	12.3	20	
Methionine	16[b]	12[b]	12[b]		16[c]	
Phenylalanine	21[d]	22[d]	25[d]			
Threonine	9	15	15			
Tryptophan	2	4	3	2.4		
Valine	13	15	14			

Note: [a] From Cowey 1979
[b] In the absence of cystine
[c] Methionine + cystine
[d] In the absence of tyrosine

3.7. Earthworms as a feed source for livestock and fish production

3.7.1. Role of earthworms in wastes recycling

There is increasing demand for disposal mechanisms to be environmentally compatible and sustainable. Earthworms in dense culture and in large quantities can physically handle most biological waste and potentially at a fraction of the cost of conventional methods of waste management. The earthworm "vermiculture" industry has grown considerably in recent years, particularly in relation to its role in waste management and the production of worms for this purpose has required the development of appropriate production systems. Earthworm meal is a potentially valuable product for use in intensive animal industries.

3.7.2. Nutritive value of earthworms

Earthworms *Perionyx excavatus* sun-dried has 93.62% dry matter, 59.90% crude protein, 402.09 Kcal/100 g gross energy, 7.43% fatty acid, 7.43% crude fibre, 1.73% Ca, and 0.118% P (Bay, 2002). Earthworm meal is very high in protein, and with variable oil content (Table 10).

The dry matter content of earthworms ranges between about 15 and 20%. The fatty acid composition of the lipid extracted from worms is quite similar to the lipid composition of some fish oils, being high in φ-3 polyunsaturated lipids. In our study earthworms had average protein (572 g/kg in dry matter (DM)) and fat (79.4 g/kg in DM) contents, and low fibre (11.2 g/kg in DM). Amino acid content, are shown in Table 11, including the essential amino acids required of fish (Paper II).

Table 10. Gross composition of earthworm meals from 3 species

	Composition range (Fisher, 1988)	L. terrestis	E. foetida	D. veneta
		(Stafford and Tacon, 1988)		
Dry matter g/kg meal	899-966			
Ash g/kg meal	55-245	28.7	17.2	4.3
Crude protein g/kg organic matter (OM)	674-768	787	710	595
Ether Extract g/kg OM	51-130	29	109	200
NFE g/kg OM		200	181	205
Gross energy kJ/g OM	16.3-20.7	-	-	-

Table 11. Amino acid content in earthworms *(P. excavatus)* (g/16 g N)

	Tram *et al*., (Paper II)	Bay, 2002
Aspartic	5.56	6.51
Glutamic	12.47	12.64
Serine	4.28	4.15
Histidine	5.05	5.26
Glycine	3.86	2.55
Threonine	2.18	2.58
Alanine	3.25	2.80
Arginine	6.36	10.83
Tyrosine	4.48	5.96
Valine	4.65	8.62
Methionine	2.10	1.92
Phenylalanine	2.16	2.67
Isoleucine	4.79	8.14
Leucine	6.06	7.72
Lysine	3.58	3.48
4- Hydroxyproline	4.24	-
Proline	3.17	2.65

Note: (-), no determination

According to Edwards, (1983) cited by Bay (2002) vitamin content in earthworm *E.foetida* and some protein source are shown in Table 12.

Table 12. Vitamin content in earthworm meal and some others protein source (mg/kg, except for B12 which is µg/kg)

	Thia-mine	Niaci-ne	B2	Panto-nic acid	B12	Pyrid-oxine	Folic acid	Biotine
Earthworm meal	12.9	567	51	18.4	3760	6.6	1.94	1.53
Fish meal	0.66	55.8	4.84	8.8	184.8	5.94	0.11	0.13
Soya bean meal	2.42	21.5	3.08	13.2	1.98	4.84	7.48	0.33
Blood meal	0.22	31.4	1.54	1.76	44	4.4	0.11	0.09
Meat meal	0.22	59.6	5.28	4.6	6.82	4.84	0.44	0.08

Source: Edwards, 1983 cited by Bay, 2002

3.7.3. Earthworms as a protein feed for livestock and fish production

Earthworm meal as a supplementary in the diet for poultry has been studied by many scientists in the world (Harwood, 1976; Yoshida and Hoshii, 1978; and Mekada *et al.,* 1979; all cited by Bay, (2002). Poultry gave equivalent or better growth as compared with poultry fed conventional protein feed. Worm meal is able to substitute for fishmeal in diets for monogastric animals and and for fish. Worm meal has successfully replaced meat meal in chicken rations (Sabine, 1978). Mekada *et al.,* (1979) cited by Bay (2002) did an experiment in which add 5% worm meal in the diet for chickens, the results indicated that had no effect on growth but improved feed conversion ratio. They were also successful on laying chicken fed diets containing earthworms.

Some studies had done in India (Kale *et al.,* 1982) and Philippines (Guerro, 1983) shown that earthworm *Perionyx excavatus* have successfully converted animals manure into a good source of protein as a supplementary in the diet for animals. Guerro (1983) reported that the weight gain of Tilapia was higher when given a diet with a supplement of earthworms (*P. excavatus)* than when given a fish meal supplement.

In China, earthworm has ability to replace fish meal and improve productivity of meat chickens (Jin-Jo *et al.,* 1982).

In two trials (Harwood and Sabine, 1978; Sabine, 1978, both cited by Edwards, 1988) earthworm protein supplements were fed to starter and grower pigs. Growth rate and feed conversion efficiency were similar to those of pigs fed on commercial rations.

According to Louis (1985) citied by Bay, 2002 earthworm can feed for bird and using a small amount for poultry in order to reduce insufficiently nutrition disease.

According to Chu Thien Nguyen (1997) cited by Bay, 2002, when supplement 5-8% earthworm meal was cook in the diet for mammal have increased milk yield up to 20-40% and can feed earthworm for Eel, frog, Trionychid turtle gave good results.

Earthworms were found to be a good source of protein for growing Hybrid Catfish at levels up to 75 % replacement of trash fish in the diet, which gave the best growth and food conversion ratio (Paper II).

4. Conclusions and recommendations

Based on the results in this thesis, it is concluded that:

- Tilapia, Silver Carp and Hybrid Catfish can be successfully cultured in ponds fertilized with either raw pig manure or biodigester effluent, without supplementary feeding.

- Using Biodigester effluent in fish ponds compared to adding raw pig manure increased the amount of dissolved oxygen in pond water, did not affect water temperature or pH, decreased COD and ammonia, decreased BOD, and reduced the growth rates of Tilapia and Hybrid Catfish but had no effect on the growth of Silver Carp.
- Earthworms contained of 57.2 % crude protein, 7.94 % crude fat, 1.12 % crude fibre, 1.45 % calcium and 0.7% phosphorous on a dry matter basis. No significant difference (P>0.05) was found in water quality when earthworms replaced trash fish in the diet. Fish fed the diet without earthworm inclusion had the lowest growth rate, while the best growth and feed conversion were found when 75% of the trash fish was replaced by earthworms. Survival was not affected by dietary treatment.

However, further research relating to pig manure from different diet on biodigester effluent value and fish production should be considered.

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