UTILIZATION OF EARTHWORMS (*Perionyx excavatus*) AS A PROTEIN SOURCE FOR GROWING FINGERLING MARBLE GOBY (*Oxyeleotris marmoratus*) AND TRA CATFISH (*Pangasius hypophthalmus*)

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MSc. Thesis

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Dedication

To my father (Nguyen Thanh Phat), my mother (Mai Thi The), my father in-law (Nguyen Thanh Tuu), my mother in-law (Nguyen Thi Trang) and my husband (Nguyen Thanh Tam)

Utilization of earthworms (P*erionyx excavatus)* as a protein source for growing fingerling marble goby (*Oxyeleotris marmoratus*) and tra catfish (*Pangasius hypophthalmus*)

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Abstract

Two studies were conducted at the experimental farm of An Giang University, Viet Nam.

The first study on the growth of earthworms (*Perionyx excavates)* was a 2*2 factorial experiment with 4 replicates, in which the treatments were: source of manure (cow or buffalo) and supplementation with water hyacinth at 25% of the weight of manure (DM basis) or none. Adding chopped water hyacinth to buffalo or cattle manure led to a decrease in worm numbers and in productivity per kg DM and crude protein of added substrate. Relative growth in numbers and in weight of the worms was similar on manure derived from buffaloes and cattle. The negative effect of water hyacinth was greater with buffalo than with cattle manure. Residual compost from cattle manure was richer in N and poorer in ash than compost derived from buffalo manure. Water hyacinth added to the substrate resulted in compost with less N but more ash.

The second study included two experiments. The first experiment was on the growth performance of Marble goby and Tra catfish fed diets of trash fish and rice field prawns replaced (on an iso-nitrogenous basis) with 0, 25, 50, 75 and 100% of frozen earthworms (*Perionyx excavates*). The weight gains of Marble goby and Tra catfish, and the survival rate of the Marble Goby, decreased markedly with curvilinear trends as the proportion of frozen earthworms in the diet was increased (Experiment 1). The Tra catfish appeared to adapt better than the Marble goby to the frozen worms. The second experiment, which was designed to test the hypothesis that the poor growth rates with frozen earthworms were due to the low palatability of the earthworms after being frozen, confirmed the negative effects of this method of conservation. Growth rates were 4 and 2 times greater for Marble goby and Tra catfish, respectively, when they were fed fresh rather than frozen earthworms. Survival rate was 100% on the fresh earthworm diet.

Keywords: Ash, chemical composition, compost, earthworms, fermentation, nitrogen, substrate, aquaculture, feed conversion, fresh and frozen earthworms

Table of contents

Appendix

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

I. Nguyen Huu Yen Nhi, Preston T R, Ogle B and Lundh T 2010. Growth of earthworms (*Perionyx excavatus*) on cattle or buffalo manure, with or without water hyacinth.

II. Nguyen Huu Yen Nhi, Preston T R , Ogle B and Lundh T 2010. Effect of earthworms as replacement for trash fish and rice field prawns on growth and survival rate of marble goby (*Oxyeleotris marmoratus*) and Tra catfish (*Pangasius hypophthalmus*).

List of abbreviations

B	Buffalo
C	Cattle
CP	Crude protein
DLG	Daily length gain
DM	Dry matter
DO	Dissolved oxygen
DWG	Daily weight gain
EW	Earthworms
FCR	Feed conversion ratio
H_2O	Water
LC50	50 % Lethal concentration
M	Marble goby
N	Nitrogen
NH_3	Ammonia
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Oxygen
OM	Organic matter
SGR	Specific growth rate
SR	Survival rate
T	Tra catfish
TAN	Total ammonia nitrogen
W/L	Weight : Length
WH	Water hyacinth

1. Introduction

Aquaculture is the growing of aquatic plants or animals for all or part of their life cycles. Primarily, its role is to produce food for human consumption, but it also has other purposes such as ornamental uses and as an aquarium. According to FAO (2008) the total aquaculture production of the world in 2006 reached 51.7 million tones, including 31.6 million tonnes from inland fisheries. The demand is expected to increase, and so aquaculture will become increasingly important in the future. The practice of aquaculture in inland fisheries is by many methods, but most effort is directed to intensive systems. In the past, farmers used the trash fish from inland and coastal water bodies to feed fish which have high economic value. However, the availability of trash fish is decreasing (Supis Thongrod 2005) so the farmers are finding and using other sources of animal protein, like earthworms, which can be produced from organic wastes (Edwards et al 1998).

Presently aquaculture is economically a very important sector in Vietnam. Some species of fish like Marble goby and Catfish have an especially high value. Marble goby (*Oxyeleotris marmorata* Bleeker) is commonly cultured in cages in rivers and reservoirs, ponds and coves in Vietnam (Vu Cam Luong et al 2005). This species is considered as a delicacy in some Asian countries and fetches a high price (> US$17/kg) because of its tender meat and tasty flavor (Cheah et al 1994). Marble goby culture has been developed in ponds and coves since early 2000 (Vu Cam Luong et al 2005). However, lately marble goby culture has decreased due mainly to lack of seed supply and disease outbreaks, with especially high mortality during the larval stages, which is the major problem in mass production of this species. Marble goby is a sit-and-wait predator. However, its feeding behavior has not been fully understood, especially at the young stage. Marble goby larvae that prey on live food had better survival rate and growth rate than larvae fed with artificial feed (Cheah et al 1994). The use of live feed for nursing fingerlings might be a better way to improve seed production. It is important to understand appropriate feeding strategies for marble goby during fingerling stages. Therefore, it is necessary to study the feeding behavior of young marble goby on various live feeds which could be mixed with rice bran. This understanding will provide the basic knowledge to supply live feed to Marble goby during young stages. A successful nursing technique to supply Marble goby seed to farmers would help to conserve natural stocks and enhance marble goby culture in the region.

Tra catfish (*Pangasius hypophthalmus*), a member of the Family Pangasidae, also has high economic value, and has become one of the most important species in Vietnam and other countries in the South East Asia region. This fish is raised in ponds, cages and fence culture (Chau Thi Da et al 2007). However, farmers have changed from the cage to pond culture because the cost of the cage system is higher and the fish easily get diseased. The fingerlings of *Pangasius hypophthalmus* are almost entirely produced by artificial rearing, because the natural fingerling yield from the Mekong River has been reduced (Van Zalinge et al 2002).

The earthworm *Perionyx excavatus* is the most common species in Vietnam. It plays a major role in waste management by converting large amounts of organic matter into rich humus, and thus improving soil fertility. This is achieved by the worm's action of ingesting organic matter deposited on the soil surface, especially manure, which it utilizes as food. Earthworms grow very well on manure, especially that from goats (Nguyen Hieu Phuong 2008). Earthworms have a high protein content with a well-balanced array of essential amino acids http://www.earthwormvietnam.com/Eng/powdereng.htm.

For this reason, earthworms have been shown to be an important feed resource for raising fish (Nguyen Duy Quynh Tram *et al* 2005) and frogs (Latsamy Phounvisouk and Preston 2007). Earthworms offer flexibility since the farmer can raise then without depending on the season, in contrast with freshwater trash fish which are most common in the flood season.

There is a need to develop systems for the production of worms for aquaculture. Earthworms could have a special role in intensive aquaculture industries, especially in raising Marble goby, which can be raised more easily when given live food in the early fingerling stage.

2. Objectives

The experiments were conducted to:

- Record the growth of earthworms raised on manure from cattle or buffaloes with and without addition of water hyacinth
- Study the growth and survival rate of fingerling Marble goby and Tra catfish when fed earthworms, as compared with the traditional feeds of trash fish and rice field prawns.

3. General discussion

3.1. Biological characteristics and feeding behaviors of two fish species

3.1.1. Marble goby (Oxyeleotris marmoratus)

According to the fish-base website, Marble goby, goby or Marbled sleeper goby are common names for the carnivorous fish *Oxyeleotris marmoratus* or *Oxyeleotris marmorata* (Bleeker, 1852) which is a member of the family Eleotridae. This species is one of the largest goby in the world and grows up to 50 cm in total length and reaches maturity at approximately 7 cm (Tan and Lam 1973). The Marble goby is found in natural waters, cages, ponds and coves in Vietnam, Cambodia, Thailand, Indonesia, Singapore and Malaysia (Cheah *et al* 1994; Vu Cam Luong *et al* 2005). The young stage is free-swimming and later on has bottom-dwelling habits (Seenoo *et al* 1994).

Marble goby have an elongated body and no lateral line. They are dark brown on the dorsal side and pale brown on the ventral side. There are long dark blotches on the body. The body color is variable and depends on the environment of the habitat bottom and lighting. The fins have no spines and have black or dusty bands. This species is sluggish and prefers to spend most of the time buried in the habitat substrate with eyes protruding to ambush its prey (Kelvin and Peter 2002).

In nature, Marble goby is a carnivorous fish, considered to be a motionless species that ambush its prey. It usually eats at the bottom and preys on small fish, insects and zoo-benthos, like crustaceans and mollusks which live at the bottom. It seems that the fingerlings and adults of wild Marble goby feed mainly on small fish, small freshwater prawns and benthos. The proportion of feed found in the stomach increases with fish size as well as with seasonal availability of prey (Vu Cam Luong *et al* 2005). The same result was also found by Nguyen Phu Hoa and Yang Yi (2007), in that the number of small shrimp eaten by big Marble goby was much more than in the small Marble goby.

Until recently, Marble goby culture systems did not rely on artificial pelleted feeds. Feeding of this species is still predominantly traditional, usually with trash fish, rice bran, poultry slaughter house by-products or a combination of these (Edwards and Allan 2004; Suchart et al 2005).

Marble goby fry reared in earthen ponds have had a low survival rate (25 -50 %) (Panu et al 1984). The cause of fry mortality was mainly through predators, stress and improper collection. Marble goby have a habit of hiding in muddy water, so it makes fry collection difficult (Rakbankerd 2005). However, juveniles seem to grow faster when supplementary feeds containing high protein animal sources and natural foods are presented in sufficient quantity. Actually, there is a lack of knowledge on the culture requirements of Marble goby for the successful raising of fry up to sub-adult size of 50 to 100g. The sub-adult size is considered an important stage to support Marble goby production because it is the size that is required for growing out in cage culture.

A stocking rate of 100-150 fish/m^2 of 50-100 g in individual weight is suitable for cage culture (Rakbankerd 2005). In this system, harvesting is performed periodically by size selection of between 400 and 1, 200g.

Marble goby of average size of 81 g can live well in water with 4.5-7.8 ppm dissolved oxygen, a PH of 6.6 –7.3 and temperature between 27 and 31.4°C (Vu Cam Luong *et al* 2005). However, there is no information published on the tolerance limits and physiological responses of Marble goby according to changes in water quality.

3.1.2. Tra catfish (Pangasius hypophthalmus)

Tra catfish (*Pangasius hypophthalmus*) is one of 28 species in the family Pangasiidae, of which the majority is in the genera Pangasius. Pangasiidae species are found primarily in the Mekong Delta, Viet Nam. This species also occurs in Cambodia, Laos, and Thailand (http://www.fishbase.org/) (Roberts and Vidthayanon 1991). The Mekong delta in the southern part of Vietnam is known as the main region for catfish farming, where *Pangasianodon hypophthalmus* has been traditionally farmed in small ponds using wild seed. Commercial culture in cages, pens, and ponds commenced with the development of artificial mass seed production in 2000 (Nguyen Anh Tuan *et al* 2003). This fish is very popular and has served as a daily food for many Vietnamese people who live in the southern part of Vietnam, especially in the Mekong Delta.

Tra catfish has a long body, latterly flattened, with no scales, a relatively small head, and broad mouth with small sharp teeth on the jaw and palatal bones. It has relatively large eyes and two pairs of barbels, the upper shorter than the lower. The fins have a dark grey or black color and six branched rays on the dorsal fin. Gill rakers develop normally and small gill rakers are regularly interspersed with larger ones. The fingerlings have a black stripe along the lateral line and another long black stripe below the lateral line. Large adults are uniformly grey but sometimes with greenish tint and silvery sides (FAO 2010).

Tra catfish *(Pangasius hypophthalmus)* is a migratory species. The fish moves upstream of the Mekong River at the end of the flooding season (from October to February) for spawning and the young fish return to the main stream at the beginning of the rainy season (from June to August). Khone Falls on the Lao-Cambodia border is known as the spawning ground of Tra catfish (MRC 2002, cited by Trinh Quoc Trong *et al* 2002). The young fish drift downstream with the water current and are swept into flooded areas in the southern part of Cambodia and the Mekong delta in Vietnam. Tra catfish is omnivorous, feeding on algae, higher plants, zooplankton, and insects, while larger specimens also take fruit, crustaceans and fish. Mature fish peak at a total length of 130 cm and up to 44 kg in weight. This species is typically living within the ranges of pH 6.5-7.5 and temperature 22-26 °C. Females take at least three years to reach sexual maturity in captivity when the weight is over 3 kg. However, males often mature in their second year but probably take about the same time as females in the natural habitat. Natural brood stock typically spawn twice annually, but in cages in Viet Nam have been recorded as spawning a second time 6 to 17 weeks after the first spawning (FAO 2010).

Pangasianodon hypophthalmus is a species which can be air-breathing (Browman and Kramer 1985 cited by Cacot 1999). Thus, this fish can live at low levels of dissolved oxygen. However, it prefers deep and flowing water, and therefore, farming areas of Tra catfish are mostly along the branches of the Mekong River and large canals.

The feed for Tra catfish in the 1990's was farm-made, prepared from various ingredients such as trash fish, rice bran, soybean meal, blood meal, broken rice, cottonseed flour, milk, eggs and vegetables, supplemented with Vitamin C and E premixes. The ingredients were mixed together, cooked and fed in balls or extruded into noodle strands or pellets. However, with food safety concerns and fluctuating farm-made feed quality, there is an increasing trend towards the use of commercial pellets from 2008. The unit cost of farm-made feeds is cheaper but these feeds have feed conversion rate about 2.8-3.0 and cause greater water quality deterioration. In Viet Nam, larger-scale producers only use commercial pellets, while medium-scale producers normally use farm-made feeds for raising table tra catfish (FAO 2010).

3.2. Livestock manures and plants for earthworm cultivation

3.2.1. Animal manure composition

Animal manure is abundant in the countryside and frequently a source of environmental pollution in intensive animal agriculture. By contrast, the efficient recycling of this resource in an integrated farming system can lead to increased profit and decreased environmental damage (Preston 1996). Manure is rich in nutrients (Table 1) and is especially suitable for cultivating earthworms.

Table 1. Chemical composition of some animal manures used for cultivating earthworms

Manure animals	Moisture, %	Crude protein, % in DM	Organic matter, % in DM	References
Cattle solids	80.4	13.8	85.1	Edwards et al 1998
Cattle	78.2	11.3	41.6	Nguyen Hieu Phuong 1908
Cattle	84	15.1	86.1	Chu Manh Thang 1903
Cattle suspension	77.8	12.5	41.9	Nguyen Hieu Phuong 1908
Pig solids	86.5	16.3	83.9	Edwards et al 1998
Pig suspension	77.6	11.1	37.8	Nguyen Hieu Phuong 1908
Poultry	75	15.8	68.4	Chu Manh Thang 1903
Turkey	82.3	16.3	78.1	Edwards et al 1998
Goat suspension	77.8	13.3	38.7	Nguyen Hieu Phuong 1908
Horse solids	78.3	11.2	74.1	Edwards et al 1998

There are many reports on the effects of various animal wastes on growth and reproduction of earthworms. According to Chaudhuri and Gautam Bhattacharjee (2002), biomass production and reproduction of the earthworm *Perionyx excavatus* in four experiments with cow dung alone and in mixtures with straw, bamboo leaf litter or kitchen waste, showed maximum rate of biomass increase and reproduction in the mixtures with straw and bamboo leaf litter. Edwards et al (1998) reported that *P. excavatus* grew at similar rates in cattle manure, pig manure and aerobically digested sewage sludge, but the earthworms did not grow well in horse manure and grew only poorly in turkey excreta. However, the rate of growth and the time of maturation of this species were different under various population densities and temperatures between 15 and 30^0C. The highest rates of reproduction occurred at 25 ^{0}C both in cattle manure and sewage sludge. The species of earthworm also affects biomass production, fecundity and maturation. Surindra Suthar (2009) reported that the mean individual biomass of *P. sansibaricus* (768 mg) was higher than for *P. excavatus* (613 mg).

3.2.2. Water hyacinth

Water hyacinth (*Eichhornia crassipes*) is a fast growing perennial aquatic plant found in wetlands and which prefers nutrient-enriched water (Wilson *et al* 2005). It can cause infestations over large areas of water surfaces and leads to series of problems such as decrease of biodiversity, blockage of rivers and drainage systems, depletion of dissolved oxygen, alterations in water chemistry, environmental pollution, decreased fish population, restricting access to fishing sites and loss of fishing equipment – all of which result in reductions in catch and subsequent loss of livelihoods (Malik 2007). Therefore, many ways have been developed using biological, chemical and mechanical methods for preventing the spread and even eradicating water hyacinth. On the other hand, much attention has been concentrated on the potential of using water hyacinth for a variety of applications (see the review of Gunnarsson and Petersen 2007). For example, production of handicrafts, paper, ropes and furniture have been reported. According to Gunnarsson and Petersen (2007), water hyacinths are rich in nitrogen, (up to 3.2% of DM) and have a C/N ratio around 15. Thus, water hyacinth was proposed as a substrate for compost or biogas production. The sludge from the biogas process contains almost all of the nutrients of the substrate and can be used as a fertilizer for plants. Water hyacinth compost used as fertilizer on different crops has resulted in improved production.

The high protein content makes the water hyacinth a potential feed for livestock such as cows, goats, sheep and chickens. Abdelhamid and Gabr (1991), after chemical analysis of water hyacinths collected from a canal and a ditch, reported them as having 9.5% DM, and in the DM 74.3% organic matter 19% crude protein and 18.9% crude fiber. Poddar et al (1991) reported the chemical composition of water hyacinth as 83.6% organic matter, 16.3% crude protein and 16.4% crude fiber (on DM basis) (cited by Gunnarsson and Petersen 2007). Aboud et al (2005) reported that water hyacinth could provide large quantities of nutritious feed and was a potential source for ruminant nutrition. In addition, it was recently realized that water hyacinth could be a potential biofuel crop and used in biofuel production (Bhattacharya and Kumar 2010). According to Gajalakshmi et al (2001), water hyacinth could be converted to compost by earthworms.

3.3. Water quality in ponds

3.3.1. Temperature

Rowland (1986) indicated that many fish species appropriate for aquaculture will survive and reproduce over a wide temperature range, but the temperature for maximum growth is narrower. For instance, a species can tolerate temperatures of 5 to 36^0C, but the range for maximum growth could be from 25^0C to 30 ^{0}C. However, the fish species in tropical and subtropical latitudes will not growth well when the temperature of the water is lower than 26 - 28 ^{0}C. The temperature in our experiment varied from 28.1 to 29.5^0C and thus was appropriate for normal growth of marble goby and Tra catfish.

3.3.2. Dissolved oxygen (DO)

The oxygen requirement of aquatic animals is quite variable and depends on the species, food intake, activity, size, water temperature and the dissolved oxygen concentration. Dissolved oxygen is probably the most critical water quality variable in freshwater aquaculture ponds. Dissolved oxygen concentrations are different between day and night time. Dissolved oxygen in the water is obtained through diffusion from air into water by mechanical aeration such as wind or aeration systems and biological transformation such as photosynthesis by aquatic plants. According to Swingle (1969), warm water pond fish would die in a short time if dissolved oxygen fell to less than 0.3 mg/liter. A range from 0.3 to 1.0 mg/liter of dissolved oxygen concentration is lethal for fish if exposure is prolonged. The fish survive but growth will be slow from prolonged exposure to dissolved oxygen concentrations from 1.0 to 5.0 mg/liter. More than 5.0 mg/liter of dissolved oxygen is desirable for almost all fish species in warm water ponds. Andrews and Matsuda (1975) showed that the oxygen consumption rates of Channel Catfish one hour after feeding were higher than those taken immediately after feeding or from fasted fish.

3.3.3. pH

The relationship of pH of pond waters to their suitability for fish culture was discussed by Swingle (1961). The acid and alkaline death points are approximately pH 4 and pH 11, respectively. However, the optimum range for fish production is from pH 6.5 to pH 9. A pH range from 4 to 6.5 will result in the fish having a slow growth rate.

In our research (Paper II), the pH did not change so much, and ranged from 7.05 to 7.34 in the morning and from 7.12 to 7.28 in the afternoon. These values are within the desirable range for fish growth and reproduction.

3.3.4. Nitrogen and the nitrification process

Nitrogen in the fish pond comes from waste products such as feed residues and fish excreta. These can be converted into nitrate (NO_3^-), which is non-toxic for fish. This chemical reaction for the nitrification process is:

$$\underset{\text{(Ammonia)}}{2\,NH_3} + \underset{\text{(Oxygen)}}{3.5\,O_2} \xrightarrow{\textit{Nitrosomonas}} \underset{\text{(Nitrite)}}{2NO_2^-} + \underset{\text{(Water)}}{2H_2O}$$

$$\underset{\text{(Nitrite)}}{2NO_2^-} + \underset{\text{(Oxygen)}}{O_2} \xrightarrow{\textit{Nitrobacter}} \underset{\text{(Nitrate)}}{2NO_3^-}$$

In this process, nitrite is an intermediate fish waste compound that is formed when ammonia is transformed to nitrite by *Nitrosomonas* bacteria activity. This particular group of bacteria uses ammonia as their food source, producing a waste product "nitrite". Other groups of bacteria use nitrite as a food resource and produce nitrate as waste. Nitrate is a compound that is not toxic to fish at concentrations typically found in ponds. Another way to reduce ammonia in the pond water is for it to be used directly by phytoplankton (aquatic plants).

3.3.5. Total ammonia nitrogen (TAN)

Total ammonia nitrogen (TAN) is the combination of two forms of ammonia: NH_3^- and NH_4^+. The NH_3^- form is toxic to fish at low levels, while NH_4^+ is relatively non-toxic. Both forms of ammonia are present in fish culture systems. However, the amounts are dependent on temperature and pH. Water pH has the most influence on the direction in which the equilibrium equation will shift: $NH_3 + H_2O = NH_4OH = NH_4^+ + OH^-$. The reaction will shift to the right when the pH value is low; when pH is increased the reaction will shift to the left.

Toxic concentrations of NH_3-N for many pond fish in short-term exposure vary between 0.6 and 2 mg/litre, and some effects can be seen at 0.1 to 0.3 mg/litre (Boyd 1979). The safe levels of ammonia concentrations, recommended for long-term exposure, are below 0.05 mg/litre as NH_3-N and 1.0 mg/litre as TAN. In our study (Paper II), mean total ammonia nitrogen concentrations were low (from 0.158 to 0.206 mg/liter) thus they should not have affected fish growth.

3.3.6. Nitrite

Nitrite (NO_2^-) is toxic for fish when it is absorbed by fish and reacts with hemoglobin to form methemoglobin (Met-Hb). In this reaction, the iron in the "hem" of hemoglobin is oxidized from ferrous to ferric state, so it can not combine with oxygen to bring this compound to the essential organs of the fish. For that reason, reducing activity of hemoglobin or anemia is called nitrite toxicity or metheglobinemia. The blood of fish that contains significant amounts of methehemoglobin is brown in colour, so the common term for this poison is "brown blood disease". According to Greeley (1998), nitrite levels should not exceed 0.10 mg/1iter in pond water for channel catfish or 0.50 mg/1iter for salmonids. However, nitrite levels typically range from 0.5 to 5 mg/litre in fish ponds, probably due to the reduction of nitrate in anaerobic mud or water (Boyd 1982). High ammonia levels can be treated by adding chloride salt (in the form of sodium chloride or calcium chloride) to the water. The level of salt needed is less than 50 mg/liter, which is not toxic to freshwater fish.

3.4. Protein and amino acid requirements of fresh water fish

According to NRC (1993) the protein and amino acid requirements do not differ greatly among fish species. However, the exceptions can often be identified with warm-water or cold-water, finfish or crustacean, omnivorous or carnivorous, and freshwater or marine fish. Therefore, nutrient requirements of these species are not available that can be chose prudence as the nutrient requirement of the other analogy species to using for these species.

Protein in the diet is the component that decides the price of feed. The feed rice is high when the feed has a high protein content. The nutrient requirements of some fish species in the juvenile stage is presented in Table 2.

Although, the protein quantity contributes much more to the growth rate of fish, the protein quality also has very important effects on their performance, and is shown by the amino acid content. For optimal utilization of dietary protein, the amino acid content of the feed should closely resemble the essential amino acid requirements of the fish.

Table 2. Estimated dietary protein requirement for maximal growth of some species of juvenile fish (as fed basis) (cited by NRC 1993)

Species	Protein Source	Estimated protein requirement (% as fed basis)	Reference
Snakehead	Fishmeal	52	Wee and Tacon (1982)
Estuary grouper	Tuna muscle meal	40–50	Teng *et al.* (1978)
Channel catfish	Whole egg protein	32–36	Garling and Wilson (1976)
Grass carp	Casein	41–43	Dabrowski (1977)
Rainbow trout	Fishmeal, casein, gelatin, and amino acids	40	Satia (1974)
Common carp	Casein	31–38	Ogino and Saito 1970; Takeuchi *et al* 1979
Blue tilapia	Casein and egg albumin	34	Winfree and Stickney (1981)
Mossambique tilapia	White fishmeal	40	Jauncey (1982)
Nile tilapia	Casein	30	Wang *et al*. (1985)
Yellowtail	Sand eel and fishmeal	55	Takeda *et al*. (1975)

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

3.5.1. Biology of earthworms

Perionyx excavatus is an earthworm with an iridescent blue or violet sheen on its skin which can be seen clearly under bright light. It is a very small worm so is suitable to use as fishing bait. This species has an impressive growth and reproductive rate, far in excess of the other species grown in bin culture (Darwin (1881) cited by Saheme bin Hashim (2008)).

According to Sherman (2003), the different species of earthworms have similar physical structure. Earthworms belong to the phylum Annelida. They have many segments in their body, and they move by extension and retraction of these segments. The digestive tract of the earthworm extends the whole length of its body. They breathe through their skin and can live in the water for a long time. They die if their skin becomes dry. Earthworms have both male and female sexual organs in their body, but have to find a partner for mating. The sperm is exchanged between two bodies when mating and stored in one of the segments of the worm. The cocoon casing is produced in mature worms. The cocoon is 2 to 4 mm in diameter. The time for cocoons to become the young worm is several months, depending on worm species and the immediate environment. Earthworms can only reproduce using sperm from members of their own species.

3.5.2. Roles of earthworms in waste recycling

Earthworms have been projected as the major organisms to convert organic waste resources into value-added products, i.e., vermicompost and worm biomass. A wide range of organic wastes have been studied as feed material for different species of earthworms: for example, water hyacinth (Gajalakshmi et al 2001), sewage sludge (Benite et al 1999), kitchen waste (Chaudhuri et at 2000), crop residues (Sudha Bansal and Kapoor 2000), cattle manure (Allan Mitchell 1997), neem leaves (Gajalakshmi and Abbasi 2004), straw, bamboo leaf (Chaudhuri and Gautam Bhattacharjee (2002), solid textile mill sludge (Priya Kaushik and

Garg 2003), goat manure (Loh et al 2005), manure from sheep, donkey, buffalo, goat, cow, horse and camel (Garg et al 2005), activated sewage sludge (Ndegwa and Thompson 2001), turkey wastes (Edwards et at 1998), fresh leaves of mucuna and cacahuatillo, litter of macadamia and sawdust (García and Fragoso 2003), pig waste (Nguyen Hieu Phuong 2008) and duckweed (Kostecka and Kaniuczak 2008).

3.5.3. Nutritive value of earthworms

Earthworms have high nutrient value, as described in many studies (Table 3).

Table 3. Chemical composition of earthworms cultivated on animal manure

	Latsamy and Preston (2008)	Earthworms (*Perionyx excavates)* Nguyen Duy Quynh Tram (2005)	Frozen earthworms (*Eisenia foetida*) Pereira and Games (1995)
Dry matter, %	21.1	21.4	7.2
As % in DM			
Organic matter	-	90.5	88
Crude protein	55.1	57.2	65.3
Crude fat	3.26	7.94	5.6

3.6. Earthworms as a protein source for livestock and fish production

A study to evaluate the influence of feed supplemented with worms on the growth and meat quality of broiler chickens indicated that the diets with 2% worms supported the highest live weight at 10 weeks and the highest percentage of breast and leg meat (Vu Dinh Ton et al 2009).

The utilization of earthworm meal as a protein source in aquaculture feeds is poorly studied. Tuan and Focken (2009) reported that fish fed diets contained 30%, 70% and 100% of fish meal protein, replaced by earthworm meal had similar or higher growth rate, protein efficiency, and energy retention than those fed the fish meal based control diet. In the study conducted by Yaqub (1997), the growth performance and feed conversion ratio of catfish (*Heterobranchus isopterus*) fry over 30 days was better on earthworm meal than on fish meal.

Evaluation of earthworm (*Hyperiodrilus euryaulos*) meal as a protein source in diets for *Heterobranchus longifilis* fingerlings under laboratory condition (Sogbesan and Madu 2008) revealed that 25% replacement of fish meal by earthworm meal supported higher net gain in weight and specific growth rate than fish fed 0 (control), 50, 75 or 100% earthworm meal.

According to Pereira and Games (1995), in a study on growth of rainbow trout (average weight1500g), those fed a diet supplemented with frozen earthworms (*Eisenia foetida*) had decreased lipid in the carcass compared with fish fed diets containing 25, 50 and 75% frozen earthworms. Stafford and Tacon (1984) showed that earthworms of the species *Dendrodrilus subrubicundu,* collected from the trickling filter beds of a domestic sewage works and freeze dried, could replace 10, 50 and 100% protein meal in the diets of rainbow trout. There was no loss in fish performance at low levels of dietary inclusion (10% protein replacement) but a decline in fish performance at higher levels (50 and 100% protein replacement).

The experiment reported in Paper II also showed decreased growth rate of Marble goby and Tra catfish when frozen earthworms replaced a mixture trash fish, rice field prawn and rice bran.

4. General conclusions

Earthworms can grow well on animal excreta which have a high nutrient value. However, they do not develop well in mixtures of animal manures and un-decomposed green organic matter like fresh water hyacinth.

Earthworms have a high nutritive value as feed for Tra catfish (*Pangasius hypophthalmus*) and Marble goby (*Oxyeleotris marmoratus*) when fed in the fresh state. However, when the earthworms were frozen, the results were poorer than on the control diet of a mixture of trash fish, rice field prawn and rice bran.

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Growth of earthworms (*Perionyx excavatus*) on cattle or buffalo manure, with or without water hyacinth

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Abstract

The growth of earthworms (*Perionyx excavates)* was studied in a 2*2 factorial experiment with 4 replicates, in which the treatments were: source of manure (cow or buffalo) and supplementation with water hyacinth at 25% of the weight of manure (DM basis) or none.

Adding chopped water hyacinth to buffalo or cattle manure led to a decrease in worm numbers and in productivity per kg DM and crude protein of added substrate. Relative growth in numbers and in weight of the worms was similar on manure derived from buffaloes and cattle. The negative effect of water hyacinth was greater with buffalo than with cattle manure. Residual compost from cattle manure was richer in N and poorer in ash than compost derived from buffalo manure. Water hyacinth added to the substrate resulted in compost with less N but more ash.

Keywords: Ash, chemical composition, compost, earthworms, fermentation, nitrogen, substrate

1. Introduction

The increase in the population in Vietnam and in the standard of living is creating an increased demand for animal products. However, as livestock production increases so does the environmental pollution, through insufficient attention being given to systems of recycling of the excreta.

There are many way to recycle the organic manure from animals. It can be applied to fish ponds (Prowse 1961), used to feed larvae (Mao Zhang 1994; Latsamy Phounvisouk and Preston 2007), applied to the soil to increase organic matter content (Haynes and Naidu 1998), and especially for raising earthworms (Hughes et al 1994; Nguyen Quang Suc et al 2000). According to many studies, the earthworms have a diverse role in agriculture. They can be used in breaking down organic wastes (Phan Phuong Loan et al 2009; Edwards and Arancon 2004; Garg et al 2005), to improve the physical structure of the soil, and to enhance soil fertility by providing organic matter, which leads to better crop growth. Earthworms are rich in high quality protein and can be fed to fish (Yaqub 1997; Phan Phuong Loan et al 2009), frogs (Latsamy Phounvisouk and Preston 2007), chickens (Rodríguez et al 1995; Sorn

Suheang and Preston 2005; Vu Dinh Ton et al 2009), rabbits (Orozco Almanza et al 1988), ducks and turtles (Bui Xuan Men et al 2007). The many opportunities to use earthworms have created a demand to cultivate them, which creates ways in which farmers in the countryside can raise their income. Thus, raising earthworms is one of the ways of making better use of local resources which are abundant in the rural areas (Keo Sath 2005).

There are many ways to raise earthworms. Different sources of manures (from pigs, cows, buffaloes, goats, chickens and rabbits) have been used (Nguyen Quang Suc et al 2000; Chu Manh Thang 2003; Garg et al 2005; Nguyen Hieu Phuong 2008). According to Garg et al (2005) earthworms grew best on sheep manure, while worms grew fastest on goat manure in the experiments of Nguyen Quang Suc et al (2000) and Nguyen Hieu Phuong (2008). Vegetable wastes (Luu Huu Manh et al 2009), maize stover and rice straw (Tian et al 1997) and water spinach and water hyacinth (Kong Saroeun and Khieu Borin 2007) have been mixed with manure for growing earthworms.

Different species of earthworms have been used. The California Red worm *(Eisenia foetida)* has been the subject of most of the studies. However, *Perionyx excavates,* an earthworm adapted to living in environments with high levels of organic matter (Edward et al 1998), has been used in a recent study, with good results (Phan Phuong Loan et al 2009).

For the above reasons, this experiment was conducted to find out if there are advantages from incorporating vegetative matter with livestock manure as substrate for earthworms.

Hypothesis

- The growth rate and conversion rate of earthworms will be better when water hyacinth is mixed with cow or buffalo manure.

Objective

- To record the development of earthworms when using manure from cows or buffaloes supplemented with water hyacinth

2. Materials and methods

2.1. Location and climate

The experiment was conducted in the experimental farm of An Giang University, Long Xuyen City, Vietnam. The climate is tropical monsoon, with a rainy season between May and October and a dry season from November to April. The mean air temperature is 27°C and annual rainfall 1400-1500 mm. The duration of the study was 3 months, from September 2009 to December 2009.

2.2. Experimental treatments and design

The treatments were:

- Source of manure: Cow or buffalo
- Supplementation with water hyacinth or none

The treatments were arranged as a 2*2 factorial with 4 replications in a completely randomized design (CRD) (Table 1). Individual treatments were:

- C100: Cow manure 100% without water hyacinth
- C75: Cow manure 75% and water hyacinth 25% (DM basis)
- B100: Buffalo manure 100% without water hyacinth
- B75: Buffalo manure 75% and water hyacinth 25% (DM basis)

Table 1. Experimental layout

C100	C75	B100	B100
B75	C75	C100	B75
B75	B100	B75	C100
B100	C100	C75	C75

2.3. Experimental feeds

The earthworms (Photo 1) were bought from Cuu Long Delta Rice Research Institute, O Mon District, Can Tho City.

Photo 1. Earthworm (*Perionyx excavatus*)

Photo 2. Water hyacinth (*Eichhornia crassipes*)

Cow and buffalo manure were bought from farmers around Long Xuyen City. Water hyacinth (Photo 2) was collected from the canals and rivers in Long Xuyen City.

The earthworms were raised in baskets lined with plastic sheets (Photo 3). The earthworms (100g) were put in the bottom of each basket followed by the manure. Fresh water hyacinth (aerial parts and roots) was chopped and mixed with the manure. At the beginning a total of 500 g DM of substrate was added to each basket. More substrate was added according to the rate at which it was utilized. Weights of fresh manure and water hyacinth that were added were recorded and samples taken for determination of DM, ash, crude fibre (CF) and crude protein (CP). Clean, fresh water was sprayed on the baskets to keep an appropriate moisture level throughout the experiment.

2.4. Measurements

Before putting the earthworms into the baskets, a random sample of 30 worms was taken to determine the average length and weight of individual worms. A sample of the worms was analyzed for DM, CP and ash.

After 12 weeks the earthworms were separated from the residual substrate (Photo 4). Both worms and substrate were weighed and samples taken for analysis of DM, CP and ash. After harvesting the earthworms, the total weight in each basket was determined. A random sample was chosen of 30 worms for determining average length and weight. From the average weight of the earthworms the total number present in each basket was calculated.

A sample of worms from each treatment was analyzed for DM, CP and ash. The residual quantities of residue in each basket were weighed and a sample analyzed for DM, CP, CF and ash.

Photo 3. Feeding the earthworms

Photo 4. Harvesting the earthworms

2.5. Chemical analysis

Manure, water hyacinth, compost and earthworms were analyzed for DM, CP, CF and ash according to AOAC (1990) procedures.

2.6. Statistical analysis

Data were analyzed using the General Linear Model (GLM) option of the ANOVA program in the Minitab software (Minitab release 13.3, 2000). Sources of variation were: Manure source, water hyacinth, interaction manure*water hyacinth and error.

3. Results and discussion

3.1. Composition of substrate for raising earthworms

The moisture and CP contents of water hyacinth were higher than in the buffalo and cattle manure (Table 2) and were similar to the values reported by Chhay Ty et al (2007) and Nguyen Thi Kim Dong and Nguyen Van Thu (2009). Abdelhamid and Gabr (1991) reported that the water hyacinths collected from a canal had 9.5% DM and 74.3% OM, 20% CP and 18.9% CF in the DM.

Table 2. Composition of manure from cattle and buffalo and water hyacinth

	Buffalo	Cattle	Water hyacinth
DM,%	21.2	16.8	9.16
As % in DM			
CP	9.35	10.4	11.6
Ash	35.1	9.50	31.5
CF	15.6	25.4	17.9

3.2. Earthworm production

Adding chopped water hyacinth to the manure led to a decrease in worm numbers and in productivity per kg total substrate DM and also per kg of manure DM (Table 3). A similar effect was observed for the utilization of the CP. Thus, incorporating water hyacinth in the manure had a detrimental effect on the efficiency of utilization of the manure by the worms. There was an interaction between the source of manure and addition of water hyacinth (Table 5; Figures 1 and 2). The reduction in earthworm numbers and their growth due to the water hyacinth was greater on buffalo manure than on cow manure. According to Sherman (2003), fresh organic matter added to earthworm beds will cause an increase in temperature of the beds, due to the fermentation of readily degradable carbohydrate. This could result in the death of some of the worms. This could be the explanation for the reduction in worm numbers when the water hyacinth was added to the manure.

Table 3. Effect of water hyacinth on body length (cm), weight (g) and number of earthworms

	Manure with water hyacinth	**Manure without water hyacinth**	**SEM**	**Prob.**
Body length, (cm)				
Initial	4.21	4.21		
Final	6.50	5.81	0.25	0.07
Length increase	2.29	1.60	0.25	0.07
Earthworm weight, g				
Initial weight/ basket	100	100		
Final weight/ basket	136	215	5.16	0.001
Weight gain/kg DM of added substrate	15.6	38.3	1.86	0.001
Weight gain/kg of added manure DM	20.8	38.3		
Weight gain/g CP of added substrate	0.15	0.39	0.02	0.001
Weight gain/g CP from manure	0.20	0.39		
Earthworm number				
Initial number/basket	916	916		
Final number/basket	1167	2106	156	0.001
Number increase/kg DM of added substrate	106	389	48.1	0.001
Number increase/g CP of added substrate	1.03	4.00	0.52	0.001

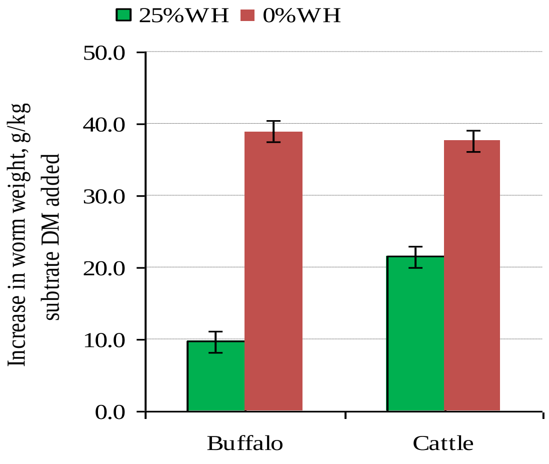

Figure 1. Effect of water hyacinth added to buffalo and cattle manure on earthworm gain in weight per unit added substrate DM

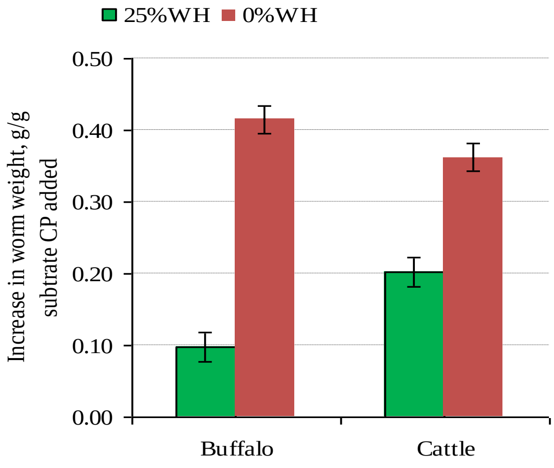

Figure 2. Effect of water hyacinth added to buffalo and cattle manure on earthworm gain in weight per unit added substrate CP

The source of manure had no effect on the growth in numbers and in weight of the worms (Table 4). However, the worms grown on cattle manure were longer than those grown on buffalo manure.

Table 4. Effect of buffalo and cattle manure on the body length (cm), weight (g) and number of earthworms

	Buffalo manure	Cattle manure	SEM	Prob.
Body length, (cm)				
Initial	4.21	4.21		
Final	5.82	6.50	0.09	0.001
Length increase	1.61	2.29	0.25	0.1
Earthworm weight, g				
Initial weight/basket	100	100		
Final weight/basket	175	176	15.7	0.98
Weight gain/kg DM of added substrate	24.3	29.6	4.56	0.43
Weight gain/kg fresh of added substrate	3.70	4.53	0.83	0.49
Weight gain/g CP of added substrate	0.26	0.28	0.05	0.71
Earthworm number				
Initial number/basket	916	916		
Final number/basket	1827	1447	226	0.25
Number increase/kg DM of added substrate	292	203	70.0	0.38
Number increase/g CP of added substrate	3.10	1.94	0.73	0.28

The relative increase in numbers of worms during the trial varied among the treatments, with the highest number on the B100 treatment (Figure 3). At the end of the trial the weights of individual worms on the different treatments did not differ from the weights at the beginning (Table 6). In contrast, the worms at the end were longer than at the beginning, and thus the ratio of weight to length decreased (Figure 4). This was reflected in a tendency (P=0.069) for a decrease in CP content of the worms at the end compared with the beginning. Surprisingly, there were no changes in OM content. There must therefore have been a change in some other component of the body (other than protein and ash) in the worms at the end compared with those at the beginning.

Table 5. Effect of buffalo and cattle manure, with or without water hyacinth, on the growth and reproduction of earthworms

	B75	B100	C75	C100	SEM	Prob.
Earthworm weight, g						
Initial weight/basket	100	100	100	100		
Final weight/basket	125[a]	227[d]	148[b]	204[c]	4.07	0.001
Weight gain/kg of added substrate (fresh)	1.22[a]	6.19[c]	2.77[b]	6.30[c]	0.23	0.001
Weight gain/kg of added substrate (DM)	9.74[a]	38.9[c]	21.6[b]	37.7[c]	1.49	0.001
Weight gain/g CP of added substrate	0.10[a]	0.42[c]	0.20[b]	0.36[c]	0.02	0.001
Earthworm number						
Initial number/basket	916	916	916	916		
Final number/basket	1176[a]	2478[b]	1159[a]	1735[ab]	185	0.001
Number increase/kg DM of added substrate	104[a]	481[b]	108[a]	298[ab]	63.3	0.002
Number increase/g CP of added substrate	1.05[a]	5.14[b]	1.01[a]	2.87[ab]	0.64	0.001

[abc] Means within rows without common letter are different at ***P<0.05***
B75, 75% buffalo manure and 25% water hyacinth (DM basis)
B100, Buffalo manure only (DM basis)
C75, 75% Cattle manure and 25% water hyacinth (DM basis)
C100, Cattle manure only (DM basis)

Table 6. Effect of buffalo and cattle manure, with or without water hyacinth, on the number, mean weight and composition of earthworms

	Initial	B75	B100	C75	C100	SEM	Prob.
No./basket	916[b]	1176[b]	2478[a]	1159[b]	1735[ab]	222	0.001
Weight, g/worm	0.109[ab]	0.106[ab]	0.094[b]	0.129[a]	0.124[a]	0.008	0.003
Length, mm	42.1[c]	63.0[a]	53.4[b]	67.1[a]	62.8[a]	1.50	0.0001
W/L, g/mm	0.259[a]	0.166[b]	0.173[b]	0.189[ab]	0.191[ab]	0.014	0.011
DM, %	18.2[b]	21.0[a]	20.9[a]	20.7[a]	20.5[a]	0.37	0.02
As % of DM							
CP	75.0	66.5	59.1	67.0	65.2	2.88	0.069
OM	92.3	93.6	93.7	93.2	93.2	0.41	0.41

[abc] Means within rows without a common letter are different at ***P<0.05***
B75, 75% buffalo manure and 25% water hyacinth (DM basis)
B100, Buffalo manure only (DM basis)
C75, 75% Cattle manure and 25% water hyacinth (DM basis)
C100, Cattle manure only (DM basis)

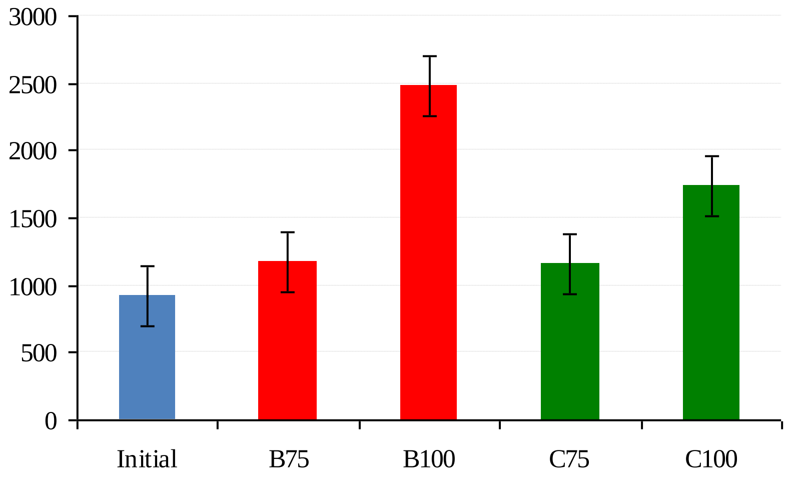

Figure 3. Effect of buffalo and cattle manure, with (B75 and C75) or without water hyacinth, on the number of earthworms per basket at the end compared with initial values

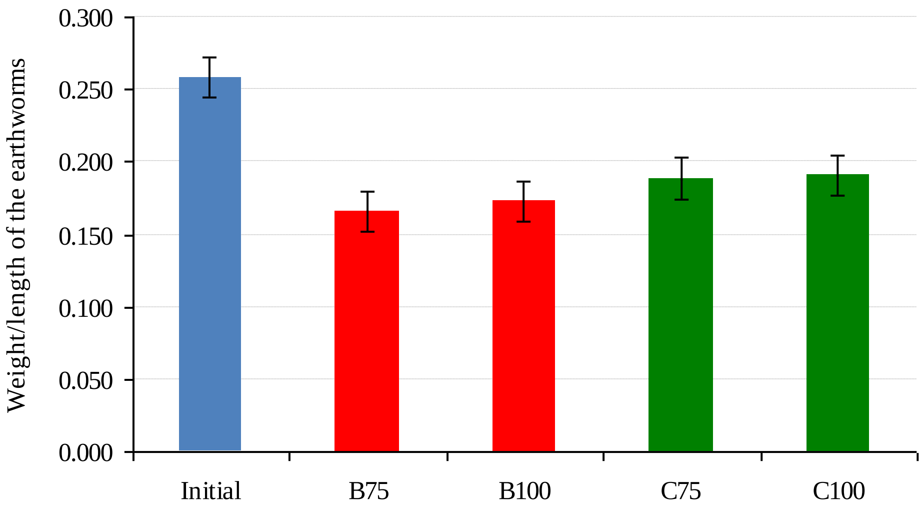

Figure 4. Effect of buffalo and cattle manure, with (B75 and C75) or without water hyacinth, on weight/length ratio of the earthworms at the end compared with initial values

3.3. Characteristics of residual "compost"

Addition of water hyacinth reduced the DM, increased the ash, and decreased the CP and the CF contents (Table 7). Buffalo manure resulted in higher DM, higher ash and lower CP and CF than when cattle manure was the substrate. There were interactions between the main treatments (Table 8), with the differences between buffalo and cattle manures being less pronounced when water hyacinth had been added to the worm beds.

Table 7. Mean values (main effects) for composition of residual compost after earthworm culture on manure from buffalo and cattle, with (25WH) or without (0WH) water hyacinth

	25WH	0WH	Prob.	Buffalo	Cattle	Prob.	SEM
DM, %	16.3	21.5	0.001	22.9	14.9	0.001	0.23
As % in DM							
CP	1.97	2.22	0.001	1.69	2.51	0.001	0.029
Ash	37.5	32.8	0.001	46.6	23.7	0.001	0.19
CF	10.1	11.2	0.001	9.22	12.0	0.001	0.12

Table 8. Composition of residual compost after earthworm culture on manure from buffalo and cattle, with or without water hyacinth

	B75	B100	C75	C100	SEM	Prob.
DM, %	19.2[c]	26.6[d]	13.3[a]	16.5[b]	0.32	0.001
As % in DM						
N	1.65	1.73	2.30	2.72	0.042	0.001
Ash	46.3 [c]	47.0 [c]	28.7 [b]	18.6 [a]	0.27	0.001
CF	9.31[a]	9.14[a]	10.9[b]	13.2[c]	0.17	0.001

[abcd] Means within rows without a common letter are different at P<0.05

The influence of the treatments on the overall fertilizer value of the residual compost will depend mainly on the relative values of the contents of N, ash and organic matter. Compost from cattle manure was richer in N and poorer in ash (but richer in OM) than compost derived from buffalo manure. Water hyacinth added to the substrate resulted in compost with less N but more ash (and less OM). Evaluation of the fertilizer value of the compost, for example by means of the "biotest" (Boonchan Chantaprasarn and Preston 2004; Tran Thi Bich Ngoc and Preston 2006) is a topic meriting future research.

4. Conclusions

- Adding chopped water hyacinth to buffalo and cattle manure led to a decrease in worm numbers and in productivity per kg DM and crude protein of added substrate.
- Relative growth in numbers and in weight of the worms was similar on manure derived from buffaloes and cattle.
- The negative effect of water hyacinth was greater with buffalo than with cattle manure.
- Residual compost from cattle manure was richer in N and poorer in ash than compost derived from buffalo manure. Water hyacinth added to the substrate resulted in compost with less N but more ash.

5. Acknowledgements

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Effect of earthworms as replacement for trash fish and rice field prawns on growth and survival rate of marble goby (*Oxyeleotris marmoratus*) and Tra catfish (*Pangasius hypophthalmus*)

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Abstract

Two experiments were carried out in the research farm of An Giang University. The first experiment was a study on the growth performance of Marble goby and Tra catfish fed diets of trash fish and rice field prawns replaced (on an iso-nitrogenous basis) with 0, 25, 50, 75 and 100% of frozen earthworms (*Perionyx excavates*).

The weight gains of Marble goby and Tra catfish, and the survival rate in the Marble Goby, decreased markedly with curvilinear trends as the proportion of frozen earthworms in the diet was increased (Experiment 1). The Tra catfish appeared to adapt better than the Marble goby to the frozen worms. The second experiment, which was designed to test the hypothesis that the poor growth rates with frozen earthworms was due to the low palatability of the earthworms after being frozen, confirmed the negative effects of this method of conservation. Growth rates were 4 and 2 times greater for Marble goby and Tra catfish, respectively, when they were fed fresh rather than frozen earthworms. Survival rate was 100% on the fresh earthworm diet.

Keywords: Aquaculture, feed conversion, fresh and frozen earthworms

1. Introduction

The population of Vietnam in 2009 was reported as 85.79 million.
http://www.vnnewstime.com/tin-doi-song/vietnam%E2%80%99s-population-hit-858-million/
The need for food for the people is increasing. Fish is the most important source of protein in the national diet, so aquaculture is an important sector in Vietnamese agriculture. Fish are also an important component of the export trade, bringing valuable foreign exchange for the country.

Freshwater aquaculture systems have developed rapidly in Vietnam during recent years. An important feature of these systems is the use of trash fish harvested from coastal areas and also from freshwater surfaces as a feed for culturing fish of economic importance. There are other demands for trash fish, as livestock feed, to make fish sauce and also as human food. As the availability of this natural resource is finite, the increase in demand is resulting in an increase in the price.

An alternative to the use of small shrimp that is now receiving increasing attention is the earthworm. A recent study by Phan Phuong Loan et al (2009) showed that zigzag eel fish grew faster when fed on earthworms than when fed small shrimp. Earthworms can be produced by many methods, but especially interesting is the use of organic matter from agricultural activities (Tian et al 1997; García and Fragoso 2003), animal waste (Edwards et al 1978) and kitchen waste (Luu Huu Manh et al 2009). In a companion paper (Nguyen Huu Yen Nhi 2010) it was shown that the earthworm *Perionyx excavates* grew well on both cattle and buffalo manure.

Presently in Vietnam, the fish species Marble goby and Catfish have especially high value. Marble goby (*Oxyeleotris marmorata*) is commonly cultured in cages in rivers and reservoirs, ponds, and coves in Vietnam (Vu Cam Luong et al 2005). This species is considered as a delicacy in some Asian countries and fetches a high price (> US$17/kg) because of its tender meat and tasty flavor (Cheah et al 1994). However, marble goby culture has decreased due mainly to lack of seed supply and disease outbreaks, with especially high mortality during the larval stages. Marble goby is a "sit-and-wait" predator (Nguyen Phu Hoa and Yang Yi 2007). However, its feeding behavior has not been fully understood, especially at the young stage. Marble goby larvae that preyed on live food had better survival rate and growth rate than larvae fed with artificial food (Cheah et al 1994). The use of live food for nursing fingerlings might be the best way to improve seed production in this species.

Tra catfish (*Pangasius hypophthalmus*), a member of the Family Pangasidae, has high economic value, and has become one of the important species in Vietnam and other countries in the South East Asia region. This fish is raised in ponds, cages and fence culture (Chau Thi Da et al 2007). However, farmers have changed from the cage culture to pond culture because the cost for the cage system is higher and the fish easily become diseased. The fingerlings of *Pangasius hypophthalmus* are almost entirely produced by artificial rearing because of the reduced supply of natural fingerlings from the Mekong River (Van Zalinge et al 2002).

On the basis of the above reports, it was decided to study the role of earthworms in the raising of fingerlings of Marble Goby and Tra catfish, as an alternative to the traditional method of using trash fish and rice field prawns.

The underlying hypothesis of the study was that earthworms would support growth and survival rates in fingerling Marble goby and Tra catfish comparable with use of trash fish and rice field prawns

2. Experiment 1: Replacement of trash fish and rice field prawns with earthworms in diets for Mable goby and Tra catfish

2.1. Materials and methods

2.1.1. Location and climate

The experiment was conducted from August 2009 to October 2009 in the experimental farm of An Giang University, Long Xuyen City, An Giang Province. The climate in this area is tropical monsoon, with a rainy season between May and October and a dry season from November to April. The mean air temperature is 27°C. Average annual rainfall is 1400 -1500 mm.

2.1.2. Treatments and design

The treatments (Table 1) arranged as a 2*5 factorial with 3 replications were:

- Fish species: marble goby and tra catfish
- Protein source: different proportions of earthworms (0, 25, 50, 75 and 100% on crude protein basis) replacing trash fish and rice field prawns (Table 2).

Table 1. Experimental treatments

Protein from earthworms, %	Fish species	
	Marble goby	**Tra catfish**
0	MEW0	TEW0
25	MEW25	TEW25
50	MEW50	TEW50
75	MEW75	TEW75
100	MEW100	TEW100

M: Marble goby (*Oxyeleotris mamoratus*)
T: Tra catfish (*Pangasius hypophthalmus*)

A completely randomized design (Table 2) was used.

Table 2: Experimental layout

MEW100	MEW0	MEW50	MEW100	TEW50	MEW100
TEW75	TEW25	TEW100	TEW0	MEWE50	TEW0
TEW75	MEW0	MEW25	TEW100	MEW75	TEW25
TEW50	TEW75	MEW0	TEW25	TEW50	TEW100
MEW25	TEW0	MEW75	MEW75	MEW25	MEW50

The experimental period was 13 weeks. The first week was for adaptation of the fish to the new diets. Data were recorded over the following 12 weeks.

2.1.3. Fingerlings

Marble goby (Photo 1) and Tra catfish (Photo 2) of around 3-5cm in length (1-2 g) were bought in My Thanh hatchery center, Long Xuyen City. They were kept separately in composite tanks and fed with trash fish (Photo 3) and rice field prawns (Photo 4) mixed with rice bran prior to the experiment. The stocking density was 100 fish/m^3. Thus the 30 tanks (Photo 5), each 0.5 m^3, had 50 fish in each tank.

Photo 1. Marble goby (*Oxyeleotris marmoratus*)

Photo 2. Tra catfish (*Pangasius hypophthalmus*)

Photo 3. Rice field prawn (*Macrobrachium lanchesteri*)

Photo 4. Trash fish

Photo 5. Plastic bins used in the experiment

2.1.4. Management and feeding

The earthworms were purchased from a commercial farm in Long Xuyen City. Rice field prawns and trash fish were bought in a local market. The earthworms, trash fish and rice field prawns were minced and mixed well with rice bran and squid oil (Table 3).

Table 3 Ingredient composition of the experimental diets (% in dry matter)

	Diets				
	EW0	**EW25**	**EW50**	**EW75**	**EW100**
Earthworm	0.0	24.0	47.3	71.4	97.0
Trash fish	39.5	30.0	20.0	10.0	0.0
Rice field prawn	39.5	30.0	20.0	10.0	0.0
Rice bran	17.0	12.8	10.0	7.0	2.4
Squid oil	2.6	2.0	1.5	0.8	0.0
Premix (mineral-vitamin)	1	1	1	1	1

The feeds supplied 50% protein in the diet DM and were fed at 8% of the fish body weight (DM basis). They were given two times per day at 7:00 and 17:00h. The residual feed and fish excreta in the tanks were removed by siphoning, and fresh water was added before feeding in the morning. The residues of feed and feces were filtered and collected separately for Marble goby and Tra catfish, then stored in a freezer (-18^{0}C) until the end of the experiment, when the pooled samples were analyzed for ash, DM and crude protein (CP).

2.1.5. Measurements

Feeds offered were recorded daily and samples collected twice a week for chemical analysis. Before starting the experiment, 100 Marble goby and 100 Tra catfish were randomly chosen to measure the length and weight. At the end of the experiment, the weight, length and numbers of all the fish were recorded.

The following water environment factors were measured every 2 weeks.

- Temperature, pH and dissolved oxygen were measured in the morning and afternoon.
- Samples of the water were taken and kept in a refrigerator until analysis for total ammonia nitrogen (TAN) and NO_2^-.

Specific growth rate (SGR) was calculated as

SGR (%/day) = 100 [Ln(w_f) – Ln(w_i)]/T ….(i)

where:

W_f: Final weight (g)

W_i: Initial weight (g)

T: Number of experimental days

Daily weight gain (DWG) was calculated as:

DWG (g/day) = (w_f - w_i)/T ……………….(ii)

Feed conversion ratio (FCR) was calculated as:

FCR = Feed DM offered/weight gain of fish .....(iii)

Survival rate (SR%) was calculated as:

SR (%) = 100[number of fish harvested/initial number of fish] …….(iv)

2.1.6. Chemical analysis

Feed samples were analyzed for DM, nitrogen (N), ether extract, and organic matter (OM) according to the procedures of AOAC (1990).

2.1.7. Statistical analysis

The data for feed intake, growth rate and feed conversion were analyzed using the General Linear Model (GLM) of the ANOVA program with the Tukey pair-wise comparison in Minitab software (Minitab release 13.3, 2000). Sources of variation were: feed, species, feed*species interaction and error.

2.2. Results and discussion

2.2.1. Water quality

The water quality in all treatments was within the suitable range for the normal growth of fish (Boyd 1990) and was not affected by the experimental treatments (Tables 4 and 5),

Table 4. Mean values for water quality parameters when a trash fish, rice field prawn and rice bran mixture was replaced by earthworms in the diet of Marble goby

	Dietary treatment*						
	MEW0	**MEW25**	**MEW50**	**MEW75**	**MEW100**	**SEM**	**P- value**
DO, mg/litre							
Morning	6.45	7.17	7.36	7.06	6.71	0.258	0.104
Afternoon	6.12	6.86	6.97	6.70	6.19	0.330	0.245
pH							
Morning	7.33	7.39	7.40	7.27	7.24	0.077	0.501
Afternoon	7.24	7.32	7.33	7.23	7.15	0.069	0.376
Water temperature, °C							
Morning	28.3	28.1	28.2	28.2	28.2	0.249	0.995
Afternoon	29.5	29.3	29.4	29.4	29.4	0.406	0.996
TAN, mg/litre	0.195	0.134	0.183	0.098	0.156	0.026	0.068
$NO2^-$, mg/litre	0.691	0.331	0.472	0.357	0.442	0.102	0.114

** M= Marble goby*
EW = Earthworms
EW0= No earthworms as control. EW25, EW50, EW75, EW100: Trash fish, rice field prawn and rice bran mixture replaced on an isonitrogenous basis by earthworms at levels of 25, 50, 75, 100%, respectively

Table 5. Mean values for water quality parameters when a trash fish, rice field prawn and rice bran mixture was replaced by earthworms in the diet of Tra catfish

	Dietary treatment*						
	TEW0	**TEW25**	**TEW50**	**TEW75**	**TEW100**	**SEM**	**P - value**
DO, mg/l							
Morning	6.74	6.44	6.59	6.60	6.69	0.23	0.91
Afternoon	5.97	5.55	5.75	5.78	5.84	0.36	0.95
pH							
Morning	7.26	7.05	7.28	7.34	7.27	0.10	0.37
Afternoon	7.28	7.12	7.24	7.26	7.14	0.11	0.80
Water temperature, °C							
Morning	28.2	28.3	28.3	28.3	28.9	0.38	0.61
Afternoon	29.5	29.6	29.5	29.6	29.5	0.42	1.00
TAN, mg/litre	0.158	0.188	0.206	0.197	0.179	0.03	0.74
NO2-,mg/litre	0.481	0.688	0.603	0.359	0.465	0.16	0.63

**T= Tra catfish; EW = Earthworms*
EW0= No earthworms as control. EW25, EW50, EW75, EW100: Trash fish, rice field prawn and rice bran mixture replaced on an isonitrogenous basis by earthworms at levels of 25, 50, 75, 100%, respectively

2.2.2. Chemical composition of the dietary ingredients

The earthworms had a lower DM content than the trash fish and rice field prawns (Table 6). The CP content was about 15% lower in the earthworms than in the trash fish and rice field prawns. Ether extract in the earthworms (11.1% in DM) was almost 40% higher than in the trash fish and prawns.

Table 6. Chemical composition of the dietary ingredients

	Earthworms	**Trash fish**	**Rice field prawns**	**Rice bran**
DM,%	10.0	15.0	17.6	90.0
As % in DM				
Organic matter	89.2	83.8	82.8	89.9
Crude protein	51.4	60.6	62.4	9.54
Ether extract	11.1	8.84	6.21	16.6

The DM content of the diets decreased linearly as the proportion of earthworms was increased (Table 7). This can be a disadvantage in terms of the ability of the fish to consume the feed, which breaks up easily.

Table 7. Chemical composition of the experimental diets

	EW0	**EW25**	**EW50**	**EW75**	**EW100**
DM,%	23.1	20.8	18.4	17.9	16.7
As % in DM					
Organic matter	85.2	87.1	89.1	91.6	93.8
Crude protein	50.1	50.5	50.0	50.0	50.1
Crude fat	11.5	11.1	11.2	11.4	11.5

EW = Earthworms
EW0= No earthworms as control. EW25, EW50, EW75, EW100: Trash fish, rice field prawn and rice bran mixture replaced on an iso-nitrogenous basis by earthworms at levels of 25, 50, 75, 100%, respectively

2.2.3. Growth performance

The weight gain of both species, and the survival rate in the Marble goby, decreased markedly with curvilinear trends as the proportion of earthworms in the diet was increased (Tables 8 and 9; Figures 1 and 2). The FCR showed similar negative trends as the earthworm content of the diet was increased.

The Tra catfish were more tolerant of the high earthworm diet, as the reduction in growth rate on the 100% earthworm diet was only 36% less than the growth rate on the control diet. The comparable figure for the Marble goby was 73%. Survival rates also differed: on the 100% earthworm diet it was 100% for the Tra catfish but only 63% for the Marble goby.

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Table 8. Effect of replacing a mixture of trash fish and rice bran by earthworms on growth performance and survival rate of Marble goby

	Dietary treatment**						
	MEW0	**MEW25**	**MEW50**	**MEW75**	**MEW100**	**SEM**	**P - value**
Weight, g							
Initial	0.857	0.767	0.760	0.790	0.770	0.028	0.178
Final	4.13[a]	3.59[a]	4.01[a]	2.75[b]	1.85[c]	0.174	0.000
Gain	3.27[a]	2.83[a]	3.25[a]	1.96[b]	1.08[c]	0.170	0.000
DWG, g	0.352[a]	0.296[a]	0.304[ab]	0.196[b]	0.101[b]	0.026	0.000
SGR, %/day	1.87[a]	1.83[a]	1.98[a]	1.48[b]	1.04[c]	0.067	0.000
Length, cm							
Initial	3.52	3.52	3.52	3.52	3.52		
Final	5.41[a]	5.16[ab]	5.40[b]	4.85[c]	4.37[d]	0.064	0.000
Gain	1.89[a]	1.64[a]	1.89[a]	1.33[b]	0.85[c]	0.065	0.000
DLG, cm/day	0.174[a]	0.146[a]	0.145[a]	0.091[b]	0.046[c]	0.010	0.000
SGR_L, %/day	0.190[a]	0.144[a]	0.189[a]	0.081[b]	-0.024[c]	0.013	0.000
FCR#	4.02[a]	3.90[a]	4.02[a]	8.14[b]	19.2[c]	0.54	0.0001
Survival rate, %	93.3[a]	92.7[a]	76.7[b]	69.3[b]	62.7[b]	3.41	0.000

[a,b,c,d] *Means with different superscripts within rows are significantly different (P<0.05)*
 # Calculated from feed offered not feed intake
*** M= Marble goby EW = earthworms*
EW0= No earthworms as control. EW25, EW50, EW75, EW100: Trash fish, rice field prawn and rice bran mixture replaced on an isonitrogenous basis by earthworms at levels of 25, 50, 75, 100%, respectively

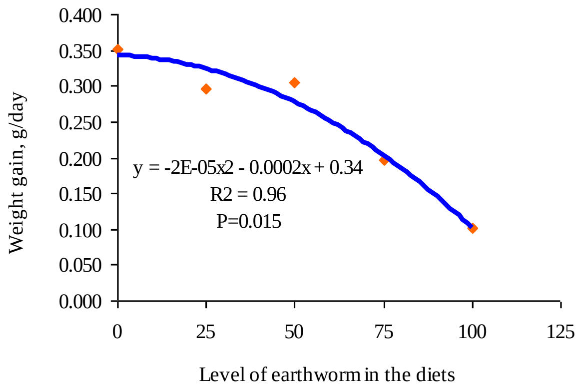

Figure 1. Effect of the percentage of earthworms in the diet on the daily weight gain of Marble goby

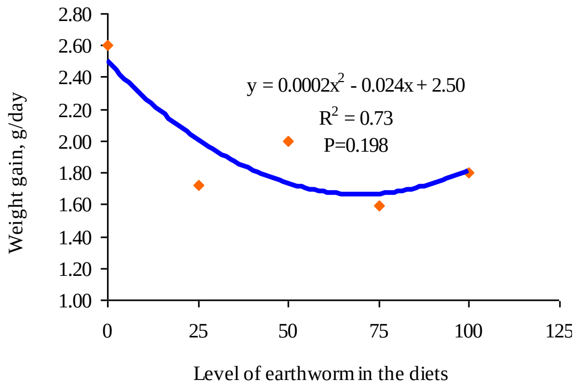

Figure 2. Effect of the percentage of earthworms in the diet on the daily weight gain of Tra catfish

Table 9. Effect of replacing a mixture of trash fish, rice field prawns and rice bran by earthworms on growth performance and survival rate of Tra catfish

	Dietary treatment						
	TEW0	**TEW25**	**TEW50**	**TEW75**	**TEW100**	**SEM**	**P - value**
Weight, g							
Initial	1.19	1.16	1.18	1.20	1.197	0.013	0.305
Final	31.4[a]	22.4[b]	25.2[ab]	20.6[b]	22.5[b]	1.86	0.015
Gain	30.3[a]	21.3[b]	24.0[ab]	19.4[b]	21.3[b]	1.86	0.015
DWG, g/day	2.60[a]	1.72[b]	2.00[ab]	1.60[b]	1.80[b]	0.132	0.002
SGR, %/day	3.90[a]	3.52[ab]	3.64[ab]	3.38[b]	3.49[ab]	0.090	0.02
Length, cm							
Initial	4.48	4.48	4.48	4.48	4.48		
Final	13.3[a]	11.7[b]	12.2[ab]	11.3[b]	11.8[b]	0.315	0.013
Gain	8.80[a]	7.26[b]	7.68[ab]	6.85[b]	7.28[b]	0.315	0.013
DLG, cm/day	0.76[a]	0.62[b]	0.67[ab]	0.59[b]	0.65[ab]	0.023	0.006
SGR_L, %/day	0.80[a]	0.68[b]	0.71[ab]	0.64[b]	0.68[ab]	0.026	0.014
FCR#	1.49	1.16	1.24	1.25	1.18	0.13	0.404
Survival rate, %	88.7	100.0	88.7	100.0	97.3	3.26	0.063

[a,b,c] *Means with different superscripts within rows are significantly different (P<0.05)*
 # Calculated from feed offered not feed intake
T= Tra catfish EW = earthworms
EW0= No earthworms as control. EW25, EW50, EW75, EW100: Trash fish, rice field prawn and rice bran mixture replaced on an isonitrogenous basis by earthworms at levels of 25, 50, 75, 100%, respectively

The different response of the two fish species to the level of earthworms in the diet probably reflects the different eating habits. Marble goby is a pure carnivore (Siriwong et al 2009), and prefers a moving prey, whereas the Tra catfish is an omnivore. If the earthworms had been fed alive (and moving) it is possible the response of both species would not have been different. Support for this explanation is provided by the experiences of Pereira and Gomes (1995) who offered frozen earthworms (*Eisenia foetida*) to replace commercial feed for rainbow trout. The feeding of earthworms depressed the growth rate. In the report of Stafford and Tacon (1984), there was no loss in trout performance when 10% worm meal protein replaced fish meal protein, but at higher levels (50 and 100% protein replacement) a decline in fish performance occurred.

2.2.4. Changes in weight:length ratio

Both species of fish showed an increase in the weight:length ratio as the experiment progressed (Tables 10 and 11). By the end of the experiment, the negative effect of the earthworm content of the diet was more marked in the Tra catfish (60% reduction) than in the Marble goby (42% reduction).

Table 10. Effect of replacing a mixture of trash fish, rice field prawns and rice bran by earthworms on the weight:length ratio of Marble goby (g/cm)

Week of measurement	Dietary treatment						
	MEW0	**MEW25**	**MEW50**	**MEW75**	**MEW100**	**SEM**	**P – value**
0	0.24	0.22	0.22	0.23	0.22	0.008	0.161
2	0.30^{a}	0.28^{ab}	0.25^{b}	0.26^{ab}	0.27^{ab}	0.009	0.03
4	0.40^{a}	0.35^{ab}	0.33^{b}	0.33^{b}	0.32^{b}	0.013	0.009
6	0.56^{a}	0.48^{a}	0.48^{a}	0.50^{a}	0.41^{b}	0.024	0.017
8	0.71^{a}	0.61^{ab}	0.58^{ab}	0.55^{bc}	0.41^{c}	0.032	0.001
10	0.84^{a}	0.75^{a}	0.72^{a}	0.60^{ab}	0.42^{b}	0.060	0.006
12	0.71^{a}	0.64^{ab}	0.71^{a}	0.55^{b}	0.41^{b}	0.021	0.000

^{a,b,c} *Means with different superscripts within rows are significantly different (P<0.05)*
** M= Marble goby EW = earthworms*
EW0= No earthworms as control. EW25, EW50, EW75, EW100: Trash fish, rice field prawn and rice bran mixture replaced on an iso-nitrogenous basis by earthworms at levels of 25, 50, 75, 100%, respectively

Table 11. Effect of replacing a mixture of trash fish, rice field prawns and rice bran by earthworms on the weight:length ratio of Tra catfish (g/cm)

Week of measurement	Dietary treatment						
	TEW0	**TEW25**	**TEW50**	**TEW75**	**TEW100**	**SEM**	**P value**
0	0.26	0.26	0.26	0.27	0.27	0.003	0.264
2	0.48^{a}	0.38^{b}	0.37^{b}	0.34^{b}	0.30^{b}	0.017	0.000
4	0.76^{a}	0.51^{b}	0.48^{b}	0.53^{b}	0.51^{b}	0.036	0.001
6	1.16^{a}	0.88^{b}	0.86^{b}	0.86^{b}	0.93^{b}	0.045	0.004
8	1.71^{a}	1.16^{b}	1.25^{b}	1.12^{b}	1.24^{b}	0.080	0.002
10	1.99^{a}	1.42^{b}	1.63^{b}	1.42^{b}	1.51^{b}	0.070	0.001
12	2.32^{a}	1.89^{ab}	2.05^{ab}	1.80^{b}	1.89^{ab}	0.102	0.032

^{a,b,c} *Means with different superscripts within rows are significantly different (P<0.05)*
** T= Tra catfish EW = earthworms*
EW0= No earthworms as control. EW25, EW50, EW75, EW100: Trash fish, rice field prawn and rice bran mixture replaced on an isonitrogenous basis by earthworms at levels of 25, 50, 75, 100%, respectively

The percentage of offered feed (Table 12) that was consumed was higher for the Tra catfish (93.3%) than for Marble goby (88.9%). The higher content of ether extract in the residues and feces from the Marble goby also indicates that these contained higher proportions of earthworms, which are richer in ether extract than the trash fish and rice field prawns (Table 6). These figures are only approximate, as feces were mixed with feed residues. However, they support the conclusion from the growth and survival data that the Tra catfish adapted better to the earthworm diets than the Marble goby.

Table 12. Total weight and chemical composition of feed residue and feces of Marble goby and Tra catfish

	Tra catfish	**Marble goby**
DM offered, g	20605	7082
DM in feed residues and feces, g	1381	785
As % in DM		
Ash	30.3	29.9
Crude protein	25.1	29.4
Ether extract	4.29	9.33

3. **Experiment 2. Fresh compared with frozen earthworms as feed for Marble goby and Tra catfish**

This experiment was planned to test the hypothesis, derived from the conclusions of Experiment 1, that Marble goby and Tra catfish prefer to eat fresh rather than frozen earthworms.

3.1. Materials and methods

3.1.1. Location

The experiment took place in the dry season, from December 2009 to February 2010, and was conducted in the same location as Experiment 1.

3.1.2. Treatments and design

The treatments, arranged as a 2*2 factorial with 3 replications in a completely randomized design (CRD) (Tables 13 and 14), were:

- Fish species: Marble goby and Tra catfish
- Protein source: fresh and frozen earthworms

Table 13. Experimental treatments

Earthworms	**Fish species**	
	Marble goby	**Tra cat fish**
Fresh	M-fresh	T-fresh
Frozen	M- frozen	T-frozen

M: Marble goby (*Oxyeleotris mamoratus*)
T: Tra catfish (*Pangasius hypophthalmus*)

Table 14: Experimental layout of the fish tanks

M-frozen	T-frozen	M-fresh	T-fresh	T-frozen	T-fresh
M-fresh	M-frozen	T-fresh	T-frozen	M-fresh	M-frozen

The experimental period lasted for 9 weeks. The first week of the experiment was for adaptation of the fish to the new diets, followed by a recording period of 8 weeks.

3.1.3. Experimental procedures

Marble goby (about 4 g in weight and 5.8 cm in length) and Tra catfish (13 g and 10 cm) were bought in My Thanh hatchery center, Long Xuyen City. The stocking density was 100 fish/m^3. Twelve composite tanks (0.1 m^3/each) had 10 fish in each tank (Photo 6).

Photo 6. Plastic bins used in Experiment 2

The earthworms were purchased from a commercial farm in Can Tho City. One part was kept in the freezer to become frozen earthworms, which were thawed before feeding. Another part was kept in a closed shelter together with cattle manure to make sure the earthworms remained alive until feeding time, when they were washed prior to offering them to the fish. The earthworms were fed *ad libitum* two times per day at 7:00 and 17:00h. The residual feed and fish excreta were removed daily by siphoning prior to feeding in the morning. Fresh water was added each 2 days. The residues of feed and feces were filtered and stored in a freezer (-18^{0}C) until the end of the experiment when they were pooled by species and analyzed for ash, DM and CP.

3.1.4. Measurements

The amounts of earthworms offered were recorded daily. Samples were collected every week for chemical analysis. Before starting the experiment, all fish were measured for length and weighed. At the end of the experiment, the weight, length and numbers of all the fish were recorded. Calculations of growth rates and feed conversion were the same as in Experiment 1.

3.1.5. Chemical analysis

This followed the same procedure as in Experiment 1, both for feed (the earthworms), feed residues and feces, and water quality.

3.1.6. Statistical analysis

The data for feed intake, growth rate and feed conversion were analyzed using the General Linear Model (GLM) of the ANOVA program, with the Tukey pair-wise comparison in Minitab software (Minitab release 13.3, 2000). Sources of variation were: feed, species, feed*species interaction and error.

3.2. Results and discussion

Data for water quality (Table 15) showed no differences among the treatments and that all criteria were in the acceptable range for fresh water fish culture (Boyd 1990).

Table 15. Mean values for water quality parameters when fresh and frozen earthworms were fed to Marble goby and Tra catfish

	Marble goby			**Tra catfish**		
	Fresh EW	**Frozen EW**	**SEM/P**	**Fresh EW**	**Frozen EW**	**SEM/P**
DO, mg/litre						
Morning	10.1	10.5	0.30/0.32	10.1	10.0	0.24/0.78
Afternoon	10.3	10.2	0.21/0.79	9.73	9.45	0.26/0.45
pH						
Morning	7.56	7.56	0.06/0.96	7.38	7.37	0.08/0.95
Afternoon	7.61	7.56	0.06/0.577	7.38	7.32	0.097/0.66
Water temperature, °C						
Morning	25.8	25.7	0.15/0.63	25.7	25.7	0.14/0.8
Afternoon	27.2	27.2	0.28/0.95	27.3	27.3	0.27/0.90
TAN, mg/litre	0.055	0.084	0.02/0.30	0.17	0.19	0.04/0.67
NO2-, mg/litre	0.049	0.063	0.02/0.61	0.11	0.098	0.02/.080

Frozen earthworms contained less DM and less CP in the DM than the fresh worms (Table 16). The content of ether extract was lower than was recorded in Experiment 1.

Table 16. Chemical composition of the experimental diets

	Fresh earthworms	**Frozen earthworms**
DM,%	18.2	14.8
As % in DM		
Organic matter	92.1	93.1
Crude protein	75.3	67.5
Ether extract	4.27	5.39

The daily weight gain of Marble goby eating fresh earthworms was four times higher than when the fish were fed frozen earthworms (Table 15; Figure 3). For the Tra catfish the weight gains were two times on fresh compared with frozen worms. Growth rates on the frozen earthworms were lower for the Tra fish in Experiment 2 (0.64 g/day) than in Experiment 1 (1.80 g/day). The opposite occurred with the Marble goby, for which the growth rate was 0.14 g/day in Experiment 2 compared with 0.1 g/day in Experiment 1. However, there were major differences in initial weights, which were 13 and 3.6 g for Tra catfish and Marble goby in Experiment 2 compared with 1.19 and 0.8 g in Experiment 1.

There was no mortality in both fish species when fed fresh earthworms and very little (7 and 3% for Marble Goby and Tra catfish, respectively) on frozen worms.

These results confirm the hypothesis that both Marble goby and Tra catfish grow better when fed fresh compared with frozen earthworms.

Table 17. Mean values for growth performance and survival rate of Marble goby and Tra catfish fed fresh or frozen earthworms (EW)

	Marble goby			Tra catfish		
	Fresh EW	**Frozen EW**	**SEM/P**	**Fresh EW**	**Frozen EW**	**SEM/P**
Weight, g						
Initial	3.58	3.47	0.045/0.158	13.4	13.5	0.085/0.26
Final	8.19	4.64	0.38/0.003	22.3	18.3	0.94/0.039
Gain	4.62	1.18	0.37/0.003	8.89	4.74	0.98/0.04
DWG, g/day	0.55	0.14	0.045/0.003	1.17	0.64	0.11/0.03
SGR, %/day	1.50	0.53	0.083/0.001	0.92	0.54	0.088/0.038
Length, cm						
Initial	5.80	5.80	0.035/0.95	10.1	10.2	0.04/0.146
Final	7.20	6.10	0.12/0.003	12.0	11.5	0.10/0.021
Gain	1.40	0.30	0.14/0.05	1.90	1.30	0.091/0.01
DLG, cm/day	0.17	0.04	0.02/0.01	0.23	0.15	0.013/0.016
SGR, %/day	0.39	0.09	0.038/0.005	0.31	0.22	0.015/0.011
FCR#	1.46	7.69	1.14/0.018	1.98	4.80	1.04/0.127
Survival rate, %	100	93.3	2.38/0.12	100	96.7	2.36/0.37

 # Based on feed offer not feed intake

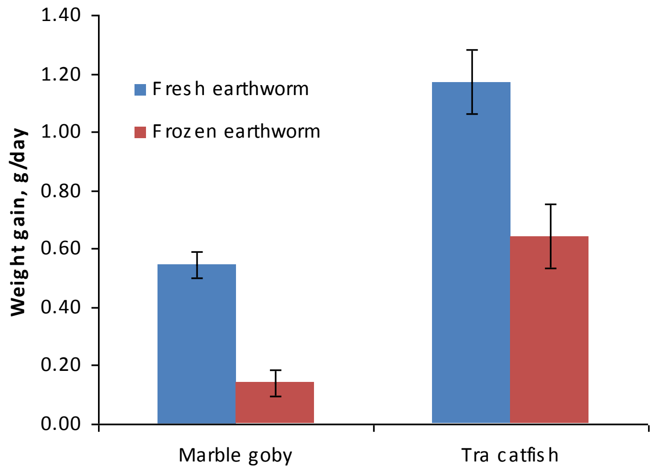

Figure 3. Effect of fresh and frozen earthworms on the weight gain of Marble goby and Tra catfish

On the fresh earthworm diet, the Marble goby increased their weight:length ratio 82% during the 8 week experiment, compared with 40% for the Tra catfish (Table 18). Comparable figures on frozen worms were 27 and 20%, respectively.

Table 18. Effect of fresh and frozen earthworms (EW) on the weight/length ratio of Marble goby and Tra catfish

Week of Measurement	Marble goby			Tra catfish		
	Fresh EW	Frozen EW	SEM/P	Fresh EW	Frozen EW	SEM/P
0	0.62	0.60	0.01/0.3	1.33	1.33	0.01/0.87
2	0.87	0.70	0.02/0.000	1.35	1.28	0.02/0.003
4	0.96	0.70	0.02/0.000	1.40	1.30	0.02/0.000
6	1.03	0.77	0.02/0.000	1.77	1.54	0.02/0.000
8	1.13	0.76	0.03/0.000	1.86	1.60	0.03/0.000

The residual feed and feces accounted for 25.1% of feed offered to the Marble goby but only 5.9% in the case of the Tra catfish (Table 19). The higher value of the CP and lower ash content in the residues from the Marble goby indicate that feed residues were a greater proportion of the total waste for this species compared with the Tra catfish.

Table 19. Total weight and chemical composition of feed residue and feces of Marble goby and Tra catfish

	Tra catfish	Marble goby
DM offered, g	1020	378
DM in residue, g	60.3	94.7
Residue as % of offer	5.9	25.1
As % in DM		
Ash	25.2	21.3
Crude protein	31.2	36.0
Ether extract	6.68	6.90

4. Conclusions

- The weight gains of Marble goby and Tra catfish, and the survival rate in the Marble Goby, decreased markedly with curvilinear trends as the proportion of frozen earthworms in the diet was increased (Experiment 1).

- The negative effects of feeding frozen earthworms was confirmed in Experiment 2, when growth rates were 4 and 2 times greater for Marble goby and Tra catfish fed fresh versus frozen earthworms, respectively.

5. Acknowledgements

The authors wish to acknowledge the Swedish International Development Authority (Sida) for their financial support of this study, through the MEKARN regional project. Thanks are given to the Aquaculture Department, Agriculture and Natural Resources Faculty, An Giang University, Vietnam, for allowing use of their facilities. In particular, the undergraduate students are greatly appreciated for their excellent assistance during the course of the experiment.

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