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Culturing earthworms on pig manure and the effect of replacing trash fish by earthworms on the growth performance of Catfish
(Clarias macrocephalus x Clarias gariepinus)

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Abstract

The purpose of the present study was to evaluate the nutritive value of earthworms *Perionyx excavatus* as protein source feed in growing Catfish *(Clarias macrocephalus x Clarias gariepinus)* rations. Earthworms were cultured on manure from pigs fed a balanced concentrate feed. Their proximate and amino acid composition was determined. Growing Catfish were fed a diet containing 30% crude protein (CP) from trash fish as control diet and with the CP from trash fish (TF) replaced by earthworms (EW) at levels of 25, 50, 75, and 100% giving the five isonitrogenous dietary treatments EW0, EW25, EW50, EW75, and EW100, respectively. Weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), and food conversion ratio (FCR) were measured. Results showed high 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) in earthworm. Amino acid content provided the essential amino acid requirements of the growing fish. There were significant differences in WG, DWG, and SGR among treatments ($P<0.05$), which were highest for treatment EW75 at 41.3 g, 0.69 g/day, and 4.5%/day respectively. The lowest growth parameters were observed in treatment EW0, with value for WG, DWG, and SGR of 23.8 g, 0.39 g/day, and 3.6%/day, respectively. FCR was lowest in treatment EW75 (1.53 g feed/g gain) and highest in treatment EW0 (1.61 g feed/g gain) ($P<0.05$). There was no effect of treatment on water quality and fish survival rate. It was concluded that earthworms is a valuable source of protein for growing Catfish with an optimal replacement level of 75% of the trash fish in the diet.

Key words: Catfish, Earthworms, Glass - tank, Trash fish

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1. Introduction

Earthworms are recognised as an effective and environmentally sound method of increasing the rate of composting of organic matter (Dynes, 2003). Composting by worms decreases the proportion of anaerobic to aerobic decomposition, resulting in a decrease in methane and volatile sulphur compounds (Mitchell *et al.,* 1980), and therefore any composting utilising earthworms may be part of a strategy in greenhouse gas mitigation for the intensive agricultural sector.

The integration of waste utilisation with the production of high quality commercial products is an attractive concept and provides real opportunities to the earthworm and agricultural processing industries, which face increasing waste management issues. This would be a major and sustainable contribution to both urban and rural environmental management. There is thus considerable scope to develop technologies to assist the development of the earthworm industry (Dynes, 2003)

The worms themselves can be fed to chickens as a high quality protein supplement (Rodríguez *et al* 1995). Ravindran and Blair (1993) give an in-depth review of animal protein sources for poultry in which they include earthworm meals. The earthworm is also very important in improving the quality of soil by recycling decaying material as well as providing food for livestock. The cultivation of earthworms using local resources such as livestock manure would seem to be a potential intervention for the improvement of living standards for smallholders in the countryside.

Long-term research studies have shown that species like *Eisenia fetida*, *E Andrei* or *Perionyx excavatus* are most suited to waste management systems that involve composting by earthworms (Dynes, 2003). Of these *P. excavatus* has significantly higher production potential than the other species considered in the evaluation. The high reproductive rate and biomass production of the tropical earthworm *P. excavatus* make it ideally suited to worm meal production (Edwards, 1988).

Earthworms, as any angler knows, are food for fish in their natural habitat, and most fish in captivity prefer live food to the dehydrated type (Philip and Mahan, 1973)

Hybrid Catfish (*Clarias macrocephalus* x *C. gariepinus*) has been one of the most popular cultured freshwater fish in Southeast Asia. As an air breather, Catfish can be grown at extremely high density (100 fish/m^2) with standing crops in pond culture reaching as high as 100 tons/ha (Areerat, 1987). The fish are mainly cultured intensively and fed with trash fish, or pelleted feed. However, those feed are also feed for other animals and high in price. Earthworm can be a solution to overcome those problem in turn of high protein content (40-60% CP), easy to produce by farmer, and recycle waste in agriculture production (Bay, 2002).

Therefore, the purpose of this study was to evaluate the nutritive value of earthworms as a protein source for growing Catfish.

2. Materials and Methods

2.1 Location

This on-station experiment was conducted at Hue University of Agriculture and Forestry (HUAF), from September to November, 2004.

2.2 Fish/fingerling and stocking density

Hybrid Catfish *(Clarias macrocephalus x C. gariepinus)* fingerlings with an average live weight of 3.2 g (0.79) and 7.8 cm (0.59) length were used. Fifteen glass- tanks were used to conduct the experiment, each of 0.9 m length x 0.45 m depth x 0.45 m width, with a total volume of 0.19 m^3; water occupied 70% of the total volume. The fingerlings were randomly distributed to the glass-tanks, with the density of 15 fish/ tank and a density of 113 fish / m^3.

2.3 Experimental design and treatments

The experiment was a Randomized Complete Block design (RCBD) with five treatments and three replicates (Table 1). Three basic feed ingredients kinds of feed were used in the experiment: rice bran, earthworms *(Perionyx excavatus*) and trash fish (Table 2), of which rice bran and trash fish were bought from a local market, and earthworms were cultured on pig manure in the HUAF experimental farm.

Table 1. Experimental layout

	Treatments				
Block 1	EW0	EW25	EW75	EW100	EW50
Block 2	EW75	EW50	EW100	EW25	EW0
Block 3	EW50	EW100	EW0	EW75	EW25

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

The control diet consisted of rice bran and trash fish. The test diets were based on the control diet with replacement of 25, 50, 75, and 100 % of the trash fish (on a crude protein basis) by earthworms, giving treatments EW0, EW25, EW50, EW75, and EW100, respectively. The diets were fed according to a restricted allowance (Ngo Trong Lu and Le Dang Khuyen, 1994) and were distributed equally into 3 meals per day at 06 00, 12 00h and 17 00h. The amount of feed given was 5% of body weight (on dry matter basis) of the fish per day. The following morning before the first meal, all refusals were siphoned off. Water was changed at weekly intervals at 50% of total volume. Oxygen was supplied by an air pump.

Process of culturing earthworms:

The mixture of fresh pig manure, lime, rice hulls and soil without nature earthworms in the propotion of 74, 1, 5, and 20%, respectively, were composted for 20 days then put compose into plot with 30cm high and 1 kg seedling earthworm per m^2 substrate (Ngoan, 2003). After 3 weeks earthworms yield was double and can use as protein source to culture Catfish.

2.4 Data collection and analysis

The chemical composition of dietary ingredients was determined according to standard methods (AOAC, 1990). Dry matter (DM) was measured by drying fresh samples at 100^oC for 24 hours. Total nitrogen (N) was determined on fresh samples by the macro-Kjeldahl method and CP was calculated from total nitrogen (CP=N*6.25). Calcium and phosphorus were determined according to AOAC (1990) using the dry method and the alkali metric ammonium molybdophosphate method, respectively. Amino acids were analyzed according to Spackman *et al* (1958).

Water quality was measured and recorded as: Temperature, Dissolved oxygen (DO), Hydrogen Ion concentration (pH) and ammonia content of water twice a week, once on sampling day at 08 00 h. Water temperatures were measured by thermometer. The pH was measured by a pH meter with a glass electrode. Ammonia was measured by the Aqua Ammonia test kit.

Actual numbers and size of fish present were determined on days 20, 40, and 60 when feeding quantities were correspondingly adjusted. All fish were counted at these times while lengths and weights were measured on samples of ten fish, which were then returned to their respective tanks. The specific growth rate (SGR), was determined by the following equation:

SGR (%/ day) = 100 x (lnWt – lnWo)/t

Where: lnWo: Natural logarithm of initial weight

lnWt: Natural logarithm of final weight

Daily weight gain and daily length gain were measured by the following equations:

DWG (g/day) = (Wt - Wo)/t

Where: Wo: initial weight (g)

Wt: final weight (g)

t: days of Experiment

DLG (mm/day) = (lt - lo)/t

Where: lo: initial length (mm)

lt: final length (mm)

t: days of experiment

The survival rate of the fish was determined by the following equation:

X (%) = (Nt/No) x 100

Where: No: initial number of fish

Nt: final number of fish

Feed conversion ratio, FCR, was determined by the following equation:

FCR = Total feed consumed / live weight gain

2.5 Statistical analysis

The data were subjected to the analysis of variance (ANOVA) procedure for Randomized Complete Block designs using the general linear model (GLM) in MINITAB 13.3 (Minitab, 2003). Pairwise comparisons with a confidence level of 95 were used to determine the effect of dietary treatment.

3. Results

3.1 Chemical composition of the dietary ingredients

The results of composition and chemical analysis of the dietary ingredients are shown in Table 2. The trash fish (TF) and earthworms (EW) had high crude protein (CP) contents of 632 and 572 g/kg on dry matter basis (DM), respectively (Table 2). The total ash, calcium (Ca), and phosphorous (P) contents were markedly different between TF and EW, and were 171, 60.0 and 27.3 g/kg in DM for TF, respectively and 48.1, 14.5 and 7.0 for EW, respectively. The data in Table 3 shows the content of seventeen amino acids in EW and TF Based on the same level of CP (30%) five treatments diets were formulated and are shown in Table 4. All the five dietary treatments had similar DM, and crude fibre (CF) contents. It was found that Ca and P content, ranked from the highest to the lowest were as follows: EW0, EW25, EW50, EW75, and EW100, respectively.

Table 2. Chemical compositions of the dietary ingredients (g/kg DM basis)

	DM	Crude Protein	Organic matter	Crude fat	Crude fibre	Ash	Ca	P
Trash fish	232	632	829	193	9.9	171	60	27.3
Earthworms	214	572	952	79.4	11.2	48.1	14.5	7.0
Rice bran	977	137	871	120	77.7	129	1.7	16.5

DM = dry matter, Ca = calcium, P = phosphorous

Table 3. The concentration of amino acid (g/100g crude protein) in earthworms (EW) and trash fish (TF)

Amino acid	EW	TF
Aspartic	5.56	5.37
Glutamic	12.47	5.57
Serine	4.28	1.91
Histidine	5.05	1.63
Glycine	3.86	4.67
Threonine	2.18	2.89
Alanine	3.25	4.26
Arginine	6.36	3.74
Tyrosine.	4.48	1.80
Valine	4.65	2.81
Methionine	2.10	1.98
Phenylalanine	2.16	1.63
Isoleucine	4.79	2.65
Leucine	6.06	4.22
Lysine	3.58	2.42
4- Hydroxyproline	4.24	1.82
Proline	3.17	2.05

Table 4. Ingredient and chemical composition of the diet *

	Diet**				
	EW0	EW25	EW50	EW75	EW100
Ingredients, g/kg					
Trash fish	329	255	175	90.4	0.0
Earthworms	0.0	85	175	271	374
Rice bran	671	661	650	638	626
Total	1000	1000	1000	1000	1000
Chemical composition, g/kg					
Dry matter	731	722	713	702	691
Crude protein	300	300	300	300	300
Organic matter	857	867	878	889	901
Crude fibre	55.4	54.8	55.2	53.5	52.8
Crude fat	144	135	126	116	105
Ash	143	133	122	111	98.9
Ca	20.9	17.6	14.1	10.4	06.5
P	20.1	18.4	16.7	14.9	13.0
Cost, VND/kg fed basis ***	3347	3193	3039	2885	2731

* Calculated from composition of ingredients

** See Table 1

*** Not include labour cost for earthworms culture

Ca = calcium, P = phosphorous, VND = Vietnamese dong (1 USD = 15800 VND)

3.2. Water quality

There were no effects of treatment on water quality parameters, namely: pH, water temperature, dissolved oxygen (DO) and ammonia (P>0.05, Table 5).

3.3. Growth performance

Results for growth performance are shown in Table 6, and indicated that there were significant differences between treatments for weight gain (WG), daily weight gain (DWG), and specific growth rate (SGR) (P<0.05). The DWG increased from treatment EW0 to EW75 and DWG of EW75 did not differ significantly with EW100. The DWG was highest in treatment EW75 (0.69 g/day) and lowest in EW0 (0.39 g/day) (P<0.05). Similarly, the SGR was highest in EW75 (4.5 %/day) and was lowest in EW0 (3.6 %/day) (P<0.05). SGR was higher for EW25 than EW0 (P<0.05) but was not different to EW50. This means that DWG improved linearly with level of replacement of TF with EW up to a level 75% of CP, the regression equation being:

$$Y = 0.0025X + 0.4491 \ (R^2 = 0.93)$$

Where, Y is average daily weight gain (g), and X is percent CP from TF replaced with EW. The relationship between percent TF replaced with EW on DWG based on the equation is shown in figure 1. There was no significant difference among experimental diets for fish survival rate (P>0.05, Table 6). Feed conversion ratio was lowest on diet EW75 (1.53 g feed/g gain) and highest on the EW0 diet (1.61 g feed/g gain).

Table 5. Effect of replacing a mixture of trash fish and rice bran by earthworms on water quality parameters.

	Dietary treatment*						
	EW0	EW25	EW50	EW75	EW100	SEM	P-value
pH	6.93	6.93	6.93	6.94	6.92	0.05	1.000
Water temperature, °C	24.6	24.7	24.5	24.6	24.9	0.21	0.763
DO**, mg/litre	3.71	3.65	3.63	3.53	3.74	0.10	0.697
Ammonia, mg/litre	0.10	0.09	0.09	0.09	0.09	0.01	0.499

* See Table 1

** Dissolved oxygen

Table 6. Effect of replacing a mixture of trash fish and rice bran by earthworms on growth performance and survival rate of Catfish (60 days of experiment).

	Dietary treatment*						
	EW0	EW25	EW50	EW75	EW100	SEM	P - value
Weight, g							
Initial	3.2	3.2	3.2	3.2	3.2	0.14	1.000
Final	27.0[c]	31.2[bc]	35.5[b]	44.5[a]	43.4[a]	0.98	0.000
Weight gain, g	23.8[c]	28.0[bc]	32.3[b]	41.3[a]	40.2[a]	0.98	0.000
DWG, g/day	0.39[c]	0.47[bc]	0.53[b]	0.69[a]	0.67[a]	0.02	0.000
SGR, %/day	3.6[c]	3.9[b]	4.1[b]	4.5[a]	4.4[a]	0.05	0.000
Length, cm							
Initial	7.8	7.8	7.8	7.8	7.8	0.17	0.505
Final	16.1[b]	16.4[b]	16.3[b]	17.5[a]	17.4[a]	0.26	0.005
Length gain, cm	8.3[b]	8.6[ab]	8.5[ab]	9.7[a]	9.9[a]	0.26	0.005
DLG, cm/day	0.14[b]	0.14[b]	0.14[b]	0.16[a]	0.16[a]	0.01	0.005
SGR, %/day	1.2[b]	1.2[b]	1.2[b]	1.4[a]	1.4[a]	0.03	0.019
FCR, kg DM/kg gain	1.61[a]	1.56[ab]	1.56[ab]	1.53[b]	1.58[ab]	0.01	0.024
Survival rate (%)	85.7	85.7	85.7	87.6	81.0	2.91	0.596

[a,b,c] Means with different superscripts within rows are significantly different (P<0.05)

* See Table 1

DWG = daily weight gain, DLG = daily length gain, SGR = specific growth rate, FCR = food conversion ratio

Table 7. Effect of replacing a mixture of trash fish and rice bran by earthworms on the weight/length ratio of Catfish (0 - 60 days)

	Dietary treatment*						
Day of measurement	EW0	EW25	EW50	EW75	EW100	SEM	P - value
20	0.71[b]	0.78[ab]	0.90[ab]	1.01[a]	0.94[a]	0.05	0.009
40	1.31[b]	1.47[b]	1.65[b]	2.08[a]	2.01[a]	0.06	0.000
60	1.67[c]	1.91[c]	2.24[b]	2.53[a]	2.44[ab]	0.06	0.000

[a,b,c] Means with different superscripts within rows are significantly different (P<0.05)

* See Table 1

Table 9. Ingredient costs (VND/kg, as fed)

Feedstuff	Cost (VND)
Rice bran	2,000
Trash fish	4,000
Earthworm*	3,000

* Not include labour cost for earthworm culture

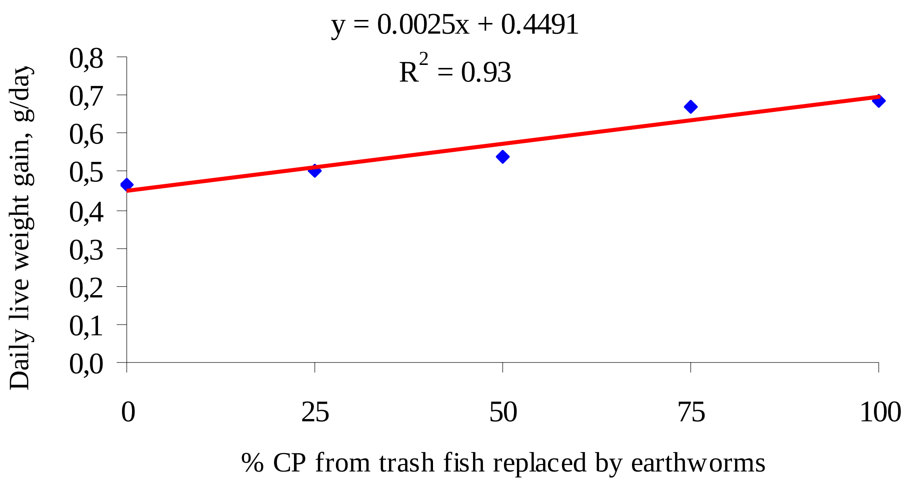

Figure 1. Effect of crude protein (CP) from trash fish replaced by earthworms on daily live weight gain

4. Discussion

The control diet consisted of rice bran and trash fish, which are typical components of diets for Catfish in smallholder farms in Vietnam. According to Edwards and Niederer (1988) meal produced from earthworms is high in protein, and with a very favorable amino acid composition compared to fish meal and with a variable oil content naturally high in omega-3 fatty acids. The proximate analysis of earthworms in the present study showed a high crude protein content (572 g/kg), a fat content of 79.4 g/kg, and crude fibre of 112 g/kg (Table 2), which is similar to values reported by Bay (2002). However in our study EW were higher in calcium and phosphorous than in the report of Bay (2002) (0.11

and 0.12 % for Ca and P, respectively. Orozco *el al*. (1988) also showed that earthworms were high in protein (50.86% CP) and fat (10.16%), and low in crude fibre (2.67%). We also found that the contents of the seventeen amino acids reported in Table 3, are similar to levels reported by Bay (2002). The contents in *P. excavatus* of the nine essential amino acids needed by certain fish species (eg. Chinook salmon, Japanese eel, Carp, Channel Catfish, Gilthead bream and Rainbout trout), met requirements, namely: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine (Cowey, 1979).

Water quality not differs among treatments. pH value ranged from 6.92 to 6.93, and the pH in this experiment would have mainly depended on pH of the inlet water, and was obviously not affected by treatment. The dissolved oxygen was from 3.53 to 3.71 mg/l and ammonia ranged from 0.09 to 0.1, and these values are considered to be suitable for the growth of Catfish (Bau, 1996). In general, there was no treatments effect on water quality, probably because the water was changed twice a week. In addition, the fish were fed the diets following a limited allowance.

Fish growth rate was lowest in EW0 (3.6 %/day) and highest in EW75 (4.5 %/day). Hung (2001) fed Catfish a diet containing a mixture of fish meal, rice bran, cassava root meal, soya bean oil cake, soya bean oil, premix, vitamin and carboxyl methyl cellulose as an agglutinative substance and reported a specific growth rate of 2.58%/day, which was lower than found in our study. The diet EW75 gave the best growth and the lowest food conversion ratio, which was also found by Harwood (1976) and Mekada *et al*. (1979) who carried out the similar experiment on chicken given a dietary supplement of earthworms, which gave a lower feed conversion ratio. Maximum growth was achieved by fish on the EW75 diet, which indicated that the protein and essential amino acid intakes met the fish's requirement. Fosgate and Babb (1972) reported that worm meal is high in crude protein content and had a good balance of amino acids and a reasonably high content of minerals. The growth of fish in the EW100 diet was lower than in the EW75 diet that may due to many reasons with mainly is the content of Ca in the diet (Table 3). In the EW100 diet, without trash fish, Ca content in the diet is lowest and cause calcium deficiency. This was resulted by reductions in growth and ash content of bones.

An experiment also was carried out on fresh water crayfish by Dynes, (2003) which showed that they could be successfully grown on worm meal. Where worm meal replaced fish meal as the dietary protein source there was no reduction in either growth rate or intake. Fisher (1988) reviewed a number of feeding studies and concluded that when included as components of poultry feed, earthworms meal have an excellent chemical composition, and Edwards and Niederer, (1988) also concluded that worms are excellent source of protein and that the amino acid content is comparable to other meals.

A study on chicken in China showed that replacing fish meal with fresh earthworms improved growth performance (Jin-Jou *et al.*, 1982). These authors also carried out an experiment on pigs and concluded that piglets fed a diet with supplemented protein from earthworms had better weight gains compare to piglets given others protein sources. In addition, 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. All these studies support the results of our experiment.

No vitamin/mineral premix was included in the diets and had no health problems or deficiency symptoms were observed. Another interesting observation was the deeper yellow colour as natural colour as fish was cultured in ponds, of the skin of fish fed with earthworms compared to the control diet. This maybe the ability of earthworms to supply vitamins and minerals, it is an important advantage in rural areas where premixes may not be available or expensive. According to Edwards (1983) vitamins content in worm meal was much higher than others sources. The vitamin requirements, which play a major role in fish physiology, most fish have requirements for eleven water-soluble vitamins and at least three of the four fat-soluble vitamins (Halver, 1979).

5. Conclusions

The results from this study indicated that:

- Earthworms are a valuable source of nutrients for growing Catfish, with a content 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 ($P>0.05$) were found in water quality and fish survival rate when earthworms replaced trash fish in the diet.
- Fish fed the diet without earthworms inclusion had the lowest growth rate, and while the best growth and feed conversion were found when 75% of the trash fish was replaced by earthworms.

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