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Earthworm Meal as Fishmeal Replacement in Plant based Feeds for Common Carp in Semi-intensive Aquaculture in Rural Northern Vietnam

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Abstract

It was evaluated whether earthworm meal can fully replace fishmeal in supplemental feeds for common carp (*Cyprinus carpio*) that also feed on natural food resources in semi-intensive aquaculture. A net cage trial (32 nets) was carried out using three iso-nitrogenous feeds fed to common carp either at a level of 10 g/kg$^{0.8}$ metabolic body mass (5 fish per cage) or 20 g/kg$^{0.8}$ metabolic body mass (10 fish per cage). In feeds, fishmeal protein was replaced by 0 %, 50 % or 100 % of protein from sun dried earthworms (*Perionyx excavatus*). At both stocking densities, control groups of fish fed only on natural food resources. The growth rate of fish increased with rising replacement of fishmeal by earthworm meal at both feeding rates. Large zooplankton were the predominant natural food resource. With increasing availability of large zooplankton, sun dried earthworm meal in plant-based supplemental feeds seemed better able to meet the nutritional requirements of common carp than fishmeal. Integration of earthworm production (vermin culture) into small-scale farms in developing countries may open the possibility for farmers in rural areas to engage in semi-intensive aquaculture by using earthworms as a feed ingredient in supplemental feeds for common carp.

Keywords: Natural food, alternative feed ingredient, supplemental feeding, earthworm meal.

Introduction

Nowadays, more than half of the fish being consumed by humans worldwide are produced by aquaculture (Naylor *et al.,* 2009) and in 2012, aquaculture production overtook global beef production in quantity for the first time (Larsen and Roney, 2013). This development in global fish supply is increasing the demand for feed resources, especially for high quality protein and high quality lipid feed resources such as fishmeal and fish oil (Naylor *et al.,* 2009, Tacon and Metian, 2009). The price of fishmeal has increased greatly within the past decade (Hardy, 2010) due to the high demand which inhibits small scale aquaculture enterprises in rural areas from increasing their fish production by using higher quality feed inputs. This, in turn, leads to the search for alternative highly nutritious feed ingredients in aqua feeds (Hardy, 2010).

Rural small-scale aquaculture is very important for food security especially for poor people in developing countries in the tropics and subtropics (Tacon *et al.,* 2010) and is often operated within integrated farming systems under traditional management schemes using low quality on-farm feed resources (e.g. northern Vietnam; Luu, 2001, Dongmeza *et al.,* 2009; Steinbronn, 2009; Pucher *et al.,* 2013; Pucher *et al.,* 2014a). In this region, the introduction of semi-intensive pond aquaculture with omnivorous common carp as the main species in carp and tilapia polycultures led to higher fish production and financial net benefit than traditional aquaculture (Pucher *et al.,* 2013). Semi-intensive aquaculture is based on enhanced natural food productivity within the pond combined with supplemental feeding of limiting nutrients (De Silva, 1993).

In order to introduce semi-intensive pond management into rural areas, supplemental feeds must be developed based on locally available resources. For small-scale farms in northern Vietnam, Tuan (2010) developed such feeds for common carp (*Cyprinus carpio*), which are mainly based on ingredients that are locally available and produced but which still contain expensive, imported fish meal. This makes the application of formulated feeds by farmers impossible as they are often not able or willing to buy them.

Earthworm meal has been investigated as a fishmeal replacement in several studies (Tacon *et al.*, 1983; Stafford and Tacon, 1984; Tuan, 2010). Under laboratory conditions and without access to natural food resources, partial replacement of fish meal protein by earthworm protein in full-feeds for common carp (*Cyprinus carpio*), Rohu (*Labeo rohita*) and Buenos Aires tetra (*Hemigrammus caudovittatus*) had a positive effect on growth performance (Tuan, 2010; Deborah *et al.*, 2011), while total replacement in feeds resulted in growth rates similar to those of fish on test diets that contained fish meal (Tuan, 2010). But it is unknown whether full replacement of fishmeal by on-farm produced earthworm meal may be beneficial for growth under pond conditions where fish also have natural food resources which are known to be of high nutritional value and contain high levels of protein and essential amino acids (Dabrowski and Rusiecki, 1983; De Silva, 1993; Ventura and Catalan, 2010).

The aim of this study was to evaluate the suitability of earthworm meal as a fishmeal replacement in plant-based supplemental feeds for common carp under semi-intensive pond management when natural food resources were also available.

Materials and Methods

Over a period of three months, a net cage trial was conducted in Yen Chau district (Son La province) in mountainous northern Vietnam. In total, 32 net cages (1.5x1.5x2 m, mesh size of 1.5 cm) were installed in the pond of a small-scale farmer. The net cages were covered by mesh to stop the experimental fish from jumping out to prevent natural predators (snakes, kingfishers, egrets) from getting in, and to deter theft by rod fishing which are all common problems in the research area (Steinbronn, 2009). Two walkways were installed into the pond to facilitate feeding and sampling. Common carp for stocking (average body mass 31.0 g±6.8 g standard deviation (SD), N=240)were kept in the net cages for one week for acclimatization before the trial was started by feeding 10 g/kg$^{0.8}$ metabolic body mass.

Feeds and Feeding Rates

Three iso-nitrogenous and iso-lipidic feeds were formulated (Table 1). The feeds were designed to contain 10% crude lipid (CL), 16% crude protein (CP) derived from plant ingredients, and 11% CP derived from animal resources either fishmeal or earthworm meal. In feeds 1, 2 and 3 fishmeal protein was replaced by 0%, 50% and 100% earthworm meal protein (*Perionyx excavatus*), respectively. As earthworm meal and fish meal differed in CP content, rice straw meal was used as a high fibre ingredient to balance the animal derived protein levels without changing the nutritional values of the test feeds.

Eight treatments were applied in four replicates each. The three supplemental feeds were offered with low and high levels of supplement (10 and 20 g/kg$^{0.8}$ metabolic body mass, respectively). Fish receiving a low level of supplementary feed had greater access to natural food resources because of their low stocking density (five fish per net cage). Conversely fish fed a high level of supplemental feed were able to get relatively little natural food due to their higher stocking density (10 fish per net cage). At both stocking densities, there were control groups of fishes that were not fed any supplements in order to determine the nutritional value of the natural food resources alone. The feed amount of 20 g/kg$^{0.8}$ metabolic body mass was split and fed at 11 am and 5 pm but the feed amount of 10 g/kg$^{0.8}$ metabolic body mass was fed only once at 5 pm. Every 10 days, the fish were weighed and the feed amounts were adjusted according to the new weights.

Natural Food Resources

Natural food resources were monitored every 10 days. At three different places in the pond, two fractions of plankton (>60 µm and >200 µm) were sampled by means of pulling nets.

For qualitative determination, the nets were vertically pulled up the water column. The zooplankton samples were filtered through a pre-dried and pre-weighed GF/C filter (Whatman) and dried at

Table 1. Chemical composition and energy content of single feed ingredients and final composition of trial feeds

Feed ingredient	DM [% FM]	CA [% DM]	CP [% DM]	CL [% DM]	GE [MJ/kg DM]	Feed 1 [% DM]	Feed 2 [% DM]	Feed 3 [% DM]
Fishmeal	92.0	29.9	63.4	6.9	16.3	18	9	0
Earthworm meal	89.8	8.0	44.3	8.8	20.8	0	13	26
Heated Soy bean meal	94.6	5.0	40.0	19.4	22.6	32	32	32
Corn Meal	89.9	0.7	9.0	4.5	17.7	30	30	30
Rice bran	90.5	13.0	8.0	2.7	17.6	7	6	5
Cassava meal	90.4	2.0	1.2	0.5	16.8	6	6	4
Vitamin mix	90.0	0.0	0.0	0.0	0.0	1	1	1
Mineral mix	90.0	100.0	0.0	0.0	0.0	1	1	1
Rice straw	93.2	11.7	8.3	1.4	15.5	4	1.5	1
Sunflower oil	98.0	0.0	0.0	100.0	nd	1	0.5	0
Total						100	100	100

CA: crude ash; CL: crude lipid; CP: crude protein; DM: dry matter; FM: fresh matter; GE: gross energy

Table 2. Chemical and essential amino acid composition of trial feeds and requirements of juvenile common carp

Component	Unit	Requirement *	Feed 1	Feed 2	Feed 3
DM	% of FM	na	90.8	90.0	88.4
CA	% of DM	na	10.9	8.5	6.5
CP	% of DM	32°	29.7	30.9	31.0
CL	% of DM	na	12.2	11.9	11.0
P	% of DM	0.70	0.32	0.25	0.17
TI	% of DM	na	0.29	0.24	0.23
GE	MJ/kg DM	13.4°	20.1	20.6	20.7
Thr	% of CP	4.7	3.7	3.7	4.0
Val	% of CP	4.4	4.0	4.2	4.3
Met	% of CP	2.2	1.5	1.5	1.5
Ile	% of CP	3.1	3.4	3.7	3.9
Leu	% of CP	4.4	6.9	7.0	7.6
Phe	% of CP	4.1	4.1	4.2	4.5
His	% of CP	1.6	2.1	4.2	2.3
Lys	% of CP	6.9	4.5	4.6	4.8
Arg	% of CP	5.3	6.0	5.6	5.9
Trp	% of CP	0.9	0.9	1.0	1.1

* (NRC, 2011), ° digestible protein/energy

Arg: Arginine;CA: crude ash; CL: crude lipid; CP: crude protein; DM: dry matter; FM: fresh matter; GE: gross energy; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; P: phosphorus;Phe: Phenylalanine;Thr: Threonine; TI: Trypsin-Inhibitor;Trp: Tryptophan; Val: Valine.

60°C for determination of zooplankton dry matter (DM).

Every 10 days, top pond sediment layers (10 cm) were sampled in five replicates with a PVC corer of diameter 4.5 cm. The sediment samples were carefully washed through a 500 µm mesh. Zoo benthic organisms were segregated and stored in 5% formalin solution. Zoo benthic organisms were identified to family level, divided into body length classes and dry matter (DM) biomass estimated per pond area by using DM-length regressions from the literature (Miserendino, 2001; Miyasaka *et al.,* 2008).

Water Quality Monitoring

During a 10 day cycle, three water samples of 1.5 L were taken at three different sites of the pond at 25 cm water depth. The water samples were transported to the field laboratory immediately (10 minutes' drive). For determination of suspended particle concentration, sample water was sucked under weak vacuum through pre-dried (60°C) and pre-weighed GF/C filters (Whatman). Dry mass (105°C) and ash mass after combustion were used to determine the concentrations of total suspended solids and inorganic suspended solids.

Duplicate analyses were made of the filtrates from the suspended particle determination to measure dissolved total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), and ortho-phosphate phosphorus (PO_4-P) using the Spectroquant Test Kits (Cat. No. 14752, 14776, 09713, and14848, respectively). Un-ionized ammonia nitrogen (UIA-N) concentrations were calculated according to Emerson *et al.* (1975).

Total nitrogen (TN) and total phosphorus (TP) were measured in unfiltered, mixtures of the three samples. In duplicate determinations, 20 ml of mixed pond water were completely oxidized by the addition of two microspoons of Oxisolv® (Merck) and were analysed for dissolved nitrate and ortho-phosphate (described above) being equivalent to TN and TP. Photometric analyses of dissolved TAN, NO_2-N, NO_3-N, PO_4-P, TN and TP were performed using a field photometer (Photolab S12; WTW).

Every 10 days, profiles of dissolved oxygen, pH and temperature were measured at two sites from the walk ways at sunrise, midday and sunset at 5, 25, 50, 75 and 100 cm water depth. Measurements of dissolved oxygen, water temperature, and water pH were measured using a handheld pH/Oxi 340i Meter (WTW) equipped with a CellOx 325 oxygen sensor (WTW), and a SenTix 20 pH probe (WTW).

Chemical Composition

From each net cage, fish were pooled, homogenized and freeze-dried. Samples of fish carcasses and feeds were analysed for DM and crude ash (CA) according to AOAC (1990). Total nitrogen (TN) and total carbon (TC) were determined using a C/N analyser (Vario MAX CN, Elementar Analysen systeme GmbH, Germany, N x 6.25). Crude lipid (CL) was determined by extraction with a Soxlet device, and gross energy (GE) with bomb calorimeter (IKA C 7000, Janke & Kunkel IKA-Analysen technik, Germany) using a benzoic acid standard. TP was analysed by the colorimetric method of Gericke and Kurmies (1952) using a spectrophotometer (U-2000, Hitachi). The trypsin inhibitor activity was determined essentially according to Smith *et al.* (1980).The amino acid analysis of feeds was made by the State Institute for Agriculture Chemistry (Hohenheim, Germany) following EU standard methods 98/64/EG and 2000/45/EG.

Statistical Analysis

Differences in proximate fish carcass composition were tested by one-factorial ANOVAs using STATISTICA 8 (StatSoft®, USA). Data were graphed by SigmaPlot 11.0.

Results

Water parameters of the trial pond over the experimental period are shown in Table 3. NO_2-N, TAN and PO_4-P were detected near the detection limit of the method used. Dissolved oxygen concentration was lowest in the early morning before sunrise (1.9±0.5 mg L^{-1}).

Three potential natural food resources of common carp are shown in Table 4. Over the trial period, natural food availability showed temporary changes in all sampled fractions but zoo benthos showed the greatest variability with time. It had on average the lowest DM of all the natural food species in the pond and showed poor correlation with the growth of common carp. DM of zooplankton larger than 200 µm correlated best with the growth of common carp.

The abundance and availability of natural food resources in the pond were not sufficient for culturing common carp without supplemental feeding. Un-supplemented fish suffered a steady loss of body mass at both stocking densities. No fish died during the trial except for two in the unfed groups. The growth of fish under the different treatments is shown in Figure 1 as specific growth rates (SGR). In general, the SGRs were rather low, probably because the fish were stressed by the sight of predators (esp. snakes) and by farmers fishing in the ponds outside the cages. Similarly, small tilapia were observed to enter the net cages through the large mesh holes and compete with common carp for the supplemental feeds. On average, growth of common carp improved with increasing fishmeal replacement by earthworm meal under both feeding schemes (10 and 20 g/$kg^{0.8}$metabolic body mass), but these were not significantly different.

The interactions of the test diets and the availability of zooplankton DM (> 200 µm in size) is depicted in Figure 2. The linear regressions show that when fish have access to natural food, they grow better when they are given a supplemental diet in which the animal protein comes from earthworm than when they are given one in which the animal protein source is fishmeal.

The feeding schemes differed significantly in their effects on the proximate body composition of the fish (see Table 5). Fish without supplemental feed had significantly lower DM, CL and GE but higher CA content than supplemented ones. Fish receiving double supplementation had even higher contents of CL and GE.

Table 3. Water quality parameters (mean±SD) over the experimental period

Parameter	Unit	Mean±SD
NO_2-N	mg L^{-1}	0.04±0.01
NO_3-N	mg L^{-1}	0.43±0.15
TAN	mg L^{-1}	0.15 ±0.07
UIA-N	µg L^{-1}	6.2±1.8
TN	mg L^{-1}	1.81 ±0.29
PO_4-P	mg L^{-1}	0.01±0.01
TP	mg L^{-1}	0.16±0.11
SDD	cm	34.7±2.0
pH		7.7±0.1
Temperature	°C	31.9±0.9
$O^2_{6\ am}$	mg L^{-1}	1.9±0.5
$O^2_{12\ am}$	mg L^{-1}	6.3±1.7
$O^2_{6\ pm}$	mg L^{-1}	7.9±1.2
TSS	mg L^{-1}	32.4±5.9
TSS_{org}	mg L^{-1}	14.6±1.7
TSS_{inorg}	mg L^{-1}	17.8±5.1

NH_3-N: un-ionized ammonia nitrogen; NO_2-N: nitrite nitrogen; NO_3-N: nitrate nitrogen; $O^2_{6\ am,\ 12\ am,\ 6\ pm}$: oxygen averaged over all measured depths at the respective day time; PO_4-P: ortho-phosphate phosphorus; SDD: Secchi disk depth; TAN: total ammonia nitrogen; TN: total nitrogen; TP: total phosphorus; TSS: total suspended solids; TSS_{org}: organic fraction of TSS; TSS_{inorg}: inorganic fraction of TSS

Table 4. Dry matter (DM) of different size groups of zooplankton and zoobenthos (mean± SD) per unit pond area as an estimate of the natural food available for stocked common carp

	Size class	DM per unit pond area [mg/m^2]
Zooplankton	> 60 µm	772±187
	> 200 µm	364±88
Zoobenthos	> 0.5 cm	52±53

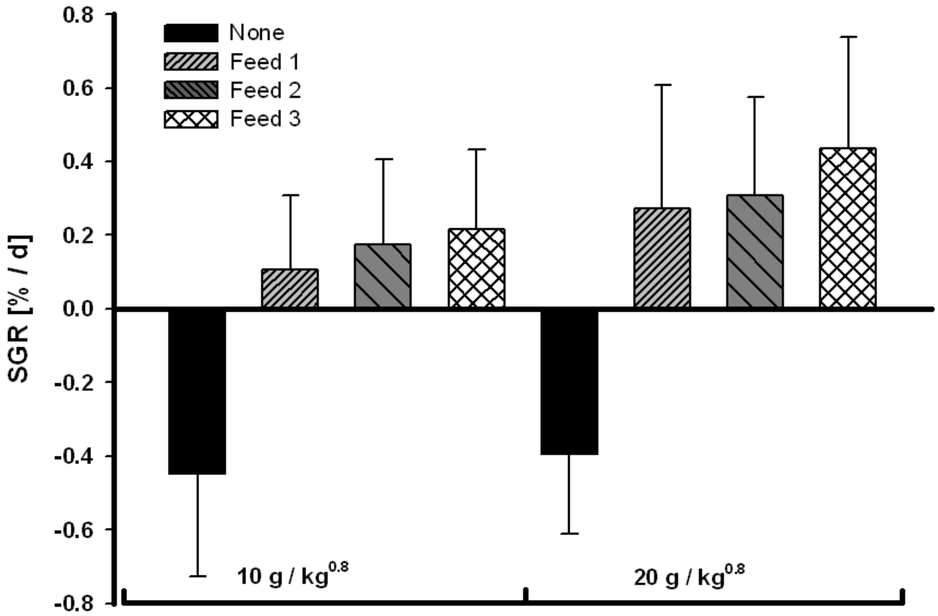

Figure 1. Specific growth rate (SGR; y-axis) of common carp receiving four feeds (None: no supplemental feeding and supplemental feeds 1, 2, and 3 in which fishmeal derived protein is replaced by 0%, 50%, and 100% earthworm meal protein, respectively) plotted against level of supplemental feeding (none, 10 g/kg$^{0.8}$ metabolic body mass, and 20 g/kg$^{0.8}$ metabolic body mass) and stocking density (5 and 10 fish per net cage).

SGR [% / day] = 100 * ((ln $_{\text{final body mass}}$ (g) – ln $_{\text{initial body mass}}$ (g)) / time (days))

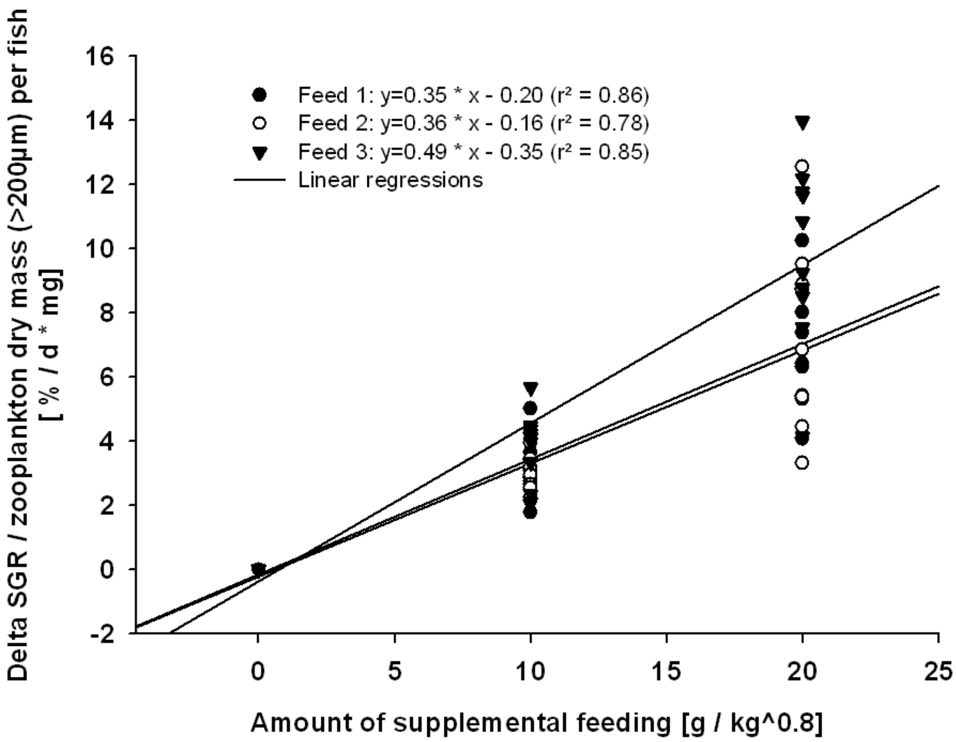

Figure 2. Delta SGR per zooplankton dry mass per fish (%/ d * mg; y-axis) of common carp receiving three supplemental feeds (1, 2, and 3 in which fishmeal derived protein is replaced by 0%, 50%, and 100% earthworm meal protein, respectively) plotted against level of supplemental feeding (none, 10 g/kg$^{0.8}$ metabolic body mass, and 20 g/kg$^{0.8}$ metabolic body mass). Equations and r^2 given in the legend are describing the linear regression and the fitting accuracy of the respective feed.

Delta SGR = $SGR_{\text{fed feed x}}$ - SGR_{unfed}

Discussion

Earthworms as a Feed Resource

In the literature, earthworms are generally described as resources with high protein content and protein quality but these parameters have been shown to differ according to earthworm species and, to a lesser extent, the feed substrate (Tacon *et al.,* 1983; Stafford and Tacon, 1984; Sun *et al.,* 1997; Changguo *et al.,* 2006; Sogbesan *et al.,* 2007; Dong *et al.,* 2010; Tuan, 2010). There are several reports that demonstrate the suitability of various earthworm species as components of aqua feeds while others suggest that single earthworm species can have negative effects on the growth and health of fish. In salmonids, low levels of fishmeal replacement by earthworm meal (*Dendrodrilus subrubicundus*, *Allolophora foetida*) produced enhanced growth performance (Akiyama *et al.,* 1984, Stafford and Tacon, 1984) but higher levels had increasingly negative effects(Stafford and Tacon, 1984). In common carp without free access to abundant natural food resources, the replacement of fishmeal by dried earthworm meal (*Eudrilus eugeniae*, Kinberg, 1867) resulted in reduced growth (Nandeesha *et al.,* 1988). Feeds in which there was incremental replacement of fishmeal by frozen *Perionyx excavates* caused reduced growth in Marble goby(*Oyxerlotris marmorata*) and pangasius catfish (*Pangasius hypophthalmus*), but fish in a trial by Nguyen *et al.* (2010) had manifestly higher growth rates when fedfresh earthworms rather than frozen ones. Tacon *et al.* (1983) reported several earthworm species (*Allolobophora foetida*, *Lumbricus terrestris*)as being suitable for feeds for rainbow trout. In contrast, frozen or freeze dried *Eisena foetida* was found to be unpalatable to trout due to haemolytic factors in the coelom fluid but the isolated protein of this earthworm species seems to be suitable as a food and feed resource (Medina *et al.,* 2003; Kostecka and Paczka, 2006). Coelomic fluid of earthworms was shown to play an important role in their immune reactions (Kauschke *et al.*, 2007) and also to affect the immune reactions in other animals treated with coelomic fluid. The coelomic fluid of *Eisena foetida* had toxic effects in fishes and other vertebrates (Kobayashi *et al.,* 2001; Ohta *et al.,* 2003) but had less effect on invertebrate species (Kobayashi *et al.,* 2001). Lysenin, a component of the coelomic fluid, was reported to produce toxic effects by binding to sphingomyelin (Yamaji *et al.,* 1998, Kobayashi *et al.,* 2001, Kobayashi *et al.,* 2004), but heat-treatment of coelomic fluid caused a reduction in toxic effects suggesting lysenin and other haemolytic factors may be heat labile proteins (Tacon *et al.*, 1983; Kauschke *et al.,* 2007). Thus the preservation and processing methods used when some species of earthworms are incorporated into fish feed can greatly affect its palatability. Tacon *et al.* (1983) suggested pre-treatment of earthworm by removal of coelomic fluid, heat treatment in the course of drying, or blanching with hot water. However, toxic coelomic fluid was not detected in all earthworm species (Kauschke *et al.,* 2007). *Perionyx excavatus* (used in this study) were found to negatively affect fish growth depending on the preservation methods used (Nguyen *et al.,* 2010) suggesting that there are anti-nutritional factors in this earthworm species too. In the present study, earthworm meal from *Perionyx excavates* hardly elicited any negative effects on growth in common carp probably because the worms underwent a form of heat treatment during sun drying and the percentage of earthworm in the diets were low.

The protein quality of earthworm meal was reported to be comparable to that of fishmeal with high levels of essential amino acids for fish (Tacon *et al.,* 1983; Tacon and Metian, 2009; Dong *et al.,* 2010; Tuan, 2010; NRC, 2011). As in full feeds, earthworm meal was suitable as a partial replacement for fishmeal with similar or beneficial effects on fish growth (described above). In this study growth increased as increasing levels of earthworm were used to replace fishmeal. Under semi-intensive pond management, there is a strong interaction between supplemental feed composition and the availability and nutritional quality of the natural food resources. Natural food resources differ in nutritional quality (De Silva, 1993) although zooplankton and macro-zoo benthos, which are the main natural food sources of common carp (Kolar and Rahel, 1993; Milstein *et al.,* 2002; Rahman *et al.,* 2008b), generally have a high

Table 5. Proximate body composition of trial fish fed the three test diets 1, 2 and 3 in which fishmeal protein was replaced by 0%, 50% and 100% earthworm meal protein, respectively. In four replicate each, these test diets were fed to groups of common carp at 0, 10 and 20 g/kg$^{0.8}$ metabolic body mass at stocking densities of five and ten fish per net cage

Daily feeding	[g kg$^{-0.8}$]	0		10			20		
Stocking	[Fish cage^{-1}]	5	10	5	5	5	10	10	10
Feed		No	No	1	2	3	1	2	3
DM	% of FM	13.6±1.7^{e}	17.4±1.0^{d}	23.5±0.1^{c}	24.7±1.4^{b}	24.2±0.5^{b}	28.8±2.8^{a,b}	27.6±4.7^{a,b}	28.8±1.2^{a}
CA	% of DM	30.1±5.2^{a}	24.2±3.5^{a}	15.3±1.1^{b}	14.9±1.3^{b}	14.5±1.0^{b}	11.8±0.9^{b}	11.6±0.6^{b}	10.8±0.5^{b}
CP	% of DM	67.1±5.3^{a,c,d}	73.9±2.6^{a}	71.1±1.2^{a}	71.5±2.8^{a,c}	69.3±2.1^{a}	62.8±1.6^{b,c}	61.0±2.0^{b,d}	60.1±1.6^{b,d}
CL	% of DM	0.9±0.3^{c}	1.3±1.0^{c}	9.8±1.5^{b}	11.4±3.5^{b}	12.6±2.2^{b}	23.4±2.7^{a}	25.2±2.9^{a}	24.6±1.7^{a}
GE	MJ kg^{-1}	15.3±1.4^{c}	16.9±1.3^{c}	21.5±0.6^{a}	21.7±0.6^{a}	22.0±0.4^{a,d}	24.4±0.7^{a,b}	24.8±1.1^{b,d}	24.8±0.2^{b,d}

Mean values in the rows that do not share the same superscript(s) differ significantly in the pond management effect at $P \leq 0.05$.
CA: crude ash; CL: crude lipid; CP: crude protein; DM: dry matter; FM: fresh matter; GE: gross energy

protein content and high levels of essential amino acids (Dabrowski and Rusiecki, 1983; De Silva, 1993; Ventura and Catalan, 2010). The availability and nutritional quality of natural food resources determines the nutritional quality of supplemental feeds needed to fully supply the requirements of cultured fish(Viola, 1989; De Silva, 1993). In the present study, the amounts of natural food resources available were not sufficient to support fish growth without supplemental feeding (negative growth of control groups). With increasing levels of supplemental feeding and reduction of natural food per fish (higher stocking density), the increasing effect of higher natural food availability on growth performance showed that protein was the limiting factor. This is not surprising as the test feeds were formulated below the protein requirements of common carp (NRC, 2011) as suggested by De Silva (1993) for supplemental feeding at low stocking densities. Even so, the combination of earthworm meal and natural food seemed to meet the nutritional requirements of common carp more efficiently than fishmeal in the test feeds.

The SGRs of common carp correlated most closely with the abundance of zooplankton DM larger than 200 µm in size, which implies that large zooplankton are the most important natural food resources for juvenile common carp under these conditions. This feeding habit was also found by Pucher and Focken (2013) in other net cage trials designed to evaluate plant based supplemental feeds under local conditions in the research area. Rahman *et al.* (2008a) named large zooplankton as the most important natural food resources for common carp as soon as the supply of the preferred zoo benthos in the pond becomes limited. An increase in production of natural food should be aimed by changing the water management and fertilization management (Pucher *et al.*, 2014a; Pucher *et al.*, 2014b) to increase the financial benefit and growth of common carp fed on supplementary on plant-based feeds with earthworm as the animal protein resource. In the research area, the increase of natural food availability may be promoted by improving methods of fertilization as well as water flow management. For a detailed description of techniques for achieving these ends see Pucher *et al.* (2013, 2014a, 2014b).

Vermi Culture as Additional Farming Activity in Integrated Small-Scale Farms

As shown in this study, earthworms might be used by small-scale farmers in rural areas to produce supplemental feeds for omnivorous fish like common carp. This necessitates the introduction of earthworm production technologies (vermin culture) into rural areas of developing countries as an additional farming activity in integrated farming systems. Vermi culture accelerates nutrient cycling within the farms and generates additional financial benefit due to utilization of underutilized wastes and by-products of low quality (Sinha *et al.*, 2002, Sogbesan *et al.*, 2007) by producing a high qualitative feed resources for fish (or other animals) and highly fertile soil (called vermin compost)for gardening or pond fertilization (Ghosh, 2004, Chakrabarty *et al.*, 2009, Chakrabarty *et al.*, 2010).In a case study performed in small scale farms in northern Vietnam, Müller *et al.* (2012) calculated that vermin culture based on underutilized on-farm wastes (ruminant manure) produces 6 - 36 kg of earthworm DM per year and farm. Using this amount of earthworm meal in plant based supplemental feeds could produce 18-112 kg of additional common carp per farm, raising the financial yield by 12-75% per year compared to the traditional aquaculture applied by small scale farmers in the research area(Steinbronn, 2009; Pucher *et al.*, 2013).

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References

Akiyama, T., Murai, T., Hirasawa, Y. and Nose, T. 1984. Supplementation of various meals to fish meal diet for salmon fry. Aquaculture, 37: 217-222. doi: 10.1016/0044-8486(84)90154-6

AOAC 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Chakrabarty, D., Das, S.K., Das, K.M. and Biswas, P.K. 2009. Application of Vermitechnology in Aquaculture. Dynamic Soil, Dynamic Plant, 3: 41-44.

Chakrabarty, D., Das, S.K., Das, M.K. and Bag, M.P. 2010. Assessment of Vermicompost as Direct Application Manure in Fish Farming Ponds. Turkish Journal of Fisheries and Aquatic Sciences, 10: 47-52. doi: 10.4194/trjfas.2010.0107

Changguo, X., Pingjiu, Z., Genxing, P., Duosheng, Q. and Qiuhua, C. 2006. Changes in diversity, protein content, and amino acid composition of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, China. Acta Ecologica Sinica, 26: 1667-1674. doi: 10.1016/S1872-2032(06)60030-9

Dabrowski, K. and Rusiecki, M. 1983. Content of total and free amino acids in zooplanktonic food of fish larvae. Aquaculture, 30: 31-42. doi: 10.1016/0044-8486(83)90149-7

De Silva SS (1993) Supplemental feeding in semi-intensive aquaculture systems. In: New MB, Tacon AGJ, Csavas I (eds.) Farm-made aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consultation on

Farm-Made Aquafeeds, Bangkok, Thailand, 14-18 December 1992. FAO-RAPA/AADCP, Bangkok, Thailand, 434p., pp. 24-60

Deborah, P.T., Divya, V.V., Ulaganathan, P., Balamurugan, V. and Umamaheswari, S. 2011. Replacing fish meal with earthworm and mushroom meals in practical diets of *Labeo rohita* and *Hemigrammus caudovittatus* fingerlings. Indian Journal of Animal Research, 45: 115-119.

Dong, X.-H., Guo, Y.-X., Ji-DanYe, Song, W.-D., Huang, X.-H. and Wang, H. 2010. Apparent digestibility of selected feed ingredients in diets for juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. Aquaculture Research, 41: 1356-1364. doi: 10.1111/j.1365-2109.2009.02424.x

Dongmeza, E., Steinbronn, S., Francis, G., Focken, U. and Becker, K. 2009. Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the moutainous regions of Northern Vietnam. Animal Feed Science and Technology, 149: 162-178. doi: 10.1016/j.anifeedsci.2008.04.012

Emerson, K., Russo, R.C., Lund, R.E. and Thurston, R.V. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board of Canada, 32: 2379-2383. doi: 10.1139/f75-274

Gericke, S. and Kurmies, B. 1952. Colometric determination of orthophosphorc acid using vanadate molybdate (German). Fresenius´ Journal of Analytical Chemistry, 137: 15-22.

Ghosh, C. 2004. Integrated vermi-pisciculture - An alternative option for recycling of solid municipal waste in rural India. Bioresource Technology, 93: 71-75. doi: 10.1016/j.biortech.2003.09.014

Hardy, R.W. 2010. Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. Aquaculture Research, 41: 770-776. doi: 10.1111/j.1365-2109.2009.02349.x

Kauschke, E., Mohrig, W. and Cooper, E.L. 2007. Coelomic fluid proteins as basic components of innate immunity in earthworms. European Journal of Soil Biology, 43: 110-115. doi: 10.1016/j.ejsobi.2007.08.043

Kobayashi, H., Ohta, N. and Umeda, M. 2004. Biology of lysenin, a protein in the coelomic fluid of the earthworm *Eisenia foetida*. International Review of Cytology, 236: 45-99. doi: 10.1016/S0074-7696(04)36002-X

Kobayashi, H., Ohtomi, M., Sekizawa, Y. and Ohta, N. 2001. Toxicity of coelomic fluid of the earthworm Eisenia foetida to vertebrates but not invertebrates: Probable role of sphingomyelin. Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, 128: 401-411. doi: 10.1016/S1532-0456(00)00213-1

Kolar, C.S. and Rahel, F.J. 1993. Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia, 95: 210-219. doi: 10.1007/BF00323492

Kostecka, J. and Paczka, G. 2006. Possible use of earthworm Eisenia fetida (Sav.) biomass for breeding aquarium fish. European Journal of Soil Biology, 42: S231-S233. doi: 10.1016/j.ejsobi.2006.07.029

Larsen, J. and Roney, J.M., 2013. Farmed Fish Production Overtakes Beef. http://grist.org/article/farmed-fish-production-overtakes-beef/, Accessed 11. July.

Luu, L.T. 2001. The VAC system in Northern Vietnam. In: Integrated agricaulture-aquaculture. FAO, Rome:29-32.

Medina, A.L., Cova, J.A., Vielma, R.A., Pujic, P., Carlos, M.P. and Torres, J.V. 2003. Immunological and chemical analysis of proteins from *Eisenia foetida* earthworm. Food and Agricultural Immunology, 15: 255-263. doi: 10.1080/09540100400010084

Milstein, A., Wahab, M.A. and Rahman, M.M. 2002. Environmental effects of common carp *Cyprinus carpio* (L.) and mrigal *Cirrhinus mrigala* (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research, 33: 1103-1117. doi: 10.1046/j.1365-2109.2002.00753.x

Miserendino, M.L. 2001. Length-mass relationships for macroinvertebrates in freshwater environments of Patagonia (Argentina). Ecologia Austral, 11: 3-8.

Miyasaka, H., Genkai-Kato, M., Miyake, Y., Kishi, D., Katano, I., Doi, H., Ohba, S.-y. and Kuhara, N. 2008. Relationships between length and weight of freshwater macroinvertebrates in Japan. Limnology, 9: doi: 10.1007/s10201-008-0238-4

Müller, J.L., Pucher, J., Tran, T.N.T., Focken, U. and Kreuzer, M. 2012. The potential of vermiculture to produce on-farm feed resources for aquaculture in mountainous areas of North Vietnam. In: Proceedings of the International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April 2012, pp. 113-114

Nandeesha, M.C., Srikanth, G.K., Basavaraja, N., Keshavanath, P., Varghese, T.J., Bano, K., Ray, A.K. and Kale, R.D. 1988. Influence of earthworm meal on the growth and flesh quality of common carp. Biological Wastes, 26: 189-198. doi: 10.1016/0269-7483(88)90165-6

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K. and Nichols, P.D. 2009. Feeding aquaculture in an era of finite resources. PNAS, 106: 15103-15110. doi: 10.1073/pnas.0905235106

Nguyen, H.Y.N., 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*). Livestock Research for Rural Development, 22: 1-12.

NRC (National Research Council) 2011. Nutrient Requirements of Fish and Shrimp. National Research Council of the National Academics,The National Academies Press, Washington D.C.

Ohta, N., Aizu, M., Kaneko, T., Sato, T. and Kobayashi, H. 2003. Damage to the Gills, Skin and Other Tissues by Lysenin and the Coelomic Fluid of the Earthworm *Eisenia foetida* in Two Teleosts, *Tanichthys albonubes* and *Oreochromis mossambicus*. Journal of Experimental Zoology Part A: Comparative Experimental Biology, 295: 117-126. doi: 10.1002/jez.a.10193

Pucher, J. and Focken, U. 2013. Intensification of common carp culture in rural areas of northern Vietnam by plant-based feeds with protein sources of different qualities. In: Poleksic, V. (Ed), Proceedings of the 6th International Conference "Water & Fish", Belgrad-Zemun, Serbia, 12-14 June 2013. University of

Belgrad, Faculty of Agriculture, pp. 44-49

Pucher, J., Steinbronn, S., Mayrhofer, R., Schad, I., El-Matbouli, M. and Focken, U. 2013. Improved Sustainable Aquaculture Systems for Small-Scale Farmers in Northern Vietnam (Chapter 8). In: H.L. Fröhlich, P. Schreinemachers, G. Clemens and K. Stahr (Eds), Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag 281-317.

Pucher, J., Mayrhofer, R., El-Matbouli, M. and Focken, U. 2014a. Effects of modified pond management on limnological parameters in small scale aquaculture ponds in mountainous Northern Vietnam. Aquaculture Research, (in press), pp. 1-15, doi:10.1111/are.12465

Pucher, J., Mayrhofer, R., El-Matbouli, M. and Focken, U. 2014b. 15N Tracer Application to evaluate Nitrogen Dynamics of Food Webs in two subtropical small-scale Aquaculture Ponds under different Managements. Isotopes in Environmental and Health Studies, (in press), 1-14 pp., doi:10.1080/10256016.2014.922963

Rahman, M.M., Joa, Q., Gong, Y.G., Miller, S.A. and Hossain, M.Y. 2008a. A comparative study of common carp (*Cyprinus carpio* L.) and calbasu (*Labeo calbasu* Hamilton) on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated rohu (*Labeo rohita* Hamilton) ponds. Aquaculture, 285: 78-83. doi: 10.1016/j.aquaculture.2008.08.002

Rahman, M.M., Nagelkerke, L.A.J., Verdegem, M.C.J., Wahab, M.A. and Verreth, J.A.J. 2008b. Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach. Aquaculture, 275: 108-115. doi: 10.1016/j.aquaculture.2008.01.027

Sinha, R.K., Herat, S., Agarwal, S., Asadi, R. and Carretero, E. 2002. Vermiculture and waste management: study of action of earthworms *Elsinia foetida*, *Eudrilus euginae* and *Perionyx excavatus* on biodegradation of some community wastes in India and Australia. The Environmentalist, 22: 261-268. doi: 10.1023/A:1016583929723

Smith, C., VanMegen, W., Twaalfhoven, L. and Hitchcook, C. 1980. The determinations of trypsin inhibitor levels in foodstuffs. Journal of the Science of Food and Agriculture, 31: 341–350. doi: 10.1002/jsfa.2740310403

Sogbesan, A.O., Ugwumba, A.A.A. and Madu, C.T. 2007. Productivity potentials and nutritional values of semi-arid zone earthworm (*Hyperiodrilus euryaulos*; Clausen, 1967) cultured in organic wastes as fish meal supplement. Pakistan Journal of Biological Sciences, 10: 2992-2997.

Stafford, E.A. and Tacon, A.G.J. 1984. Nutritive value of the earthworm, *Dendrodrilus subrubicundus*, grown on domestic sewage, in trout diets. Agricultural Wastes, 9: 249-266. doi: 10.1016/0141-4607(84)90084-2

Steinbronn, S. 2009. A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam - Current state and potential (PhD thesis). Ph.D.-Thesis, Hohenheim, Stuttgart, Germany, University of Hohenheim.

Sun, Z., Liu, X., Sun, L. and Song, C. 1997. Earthworm as a potential protein resource. Ecology of Food Nutrition, 36: 221-236. doi: 10.1080/03670244.1997.9991517

Tacon, A.G.J. and Metian, M. 2009. Fishing for aquaculture: Non-food use of small pelagic forage fish-a global perspective. Reviews in Fisheries Science, 17: 305-317. doi: 10.1080/10641260802677074

Tacon, A.G.J., Metian, M., Turchini, G.M. and de Silva, S.S. 2010. Responsible aquaculture and trophic level implications to global fish supply. Reviews in Fisheries Science, 18: 94-105. doi: 10.1080/10641260903325680

Tacon, A.G.J., Stafford, E.A. and Edwards, C.A. 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture, 35: 187-199. doi: 10.1016/0044-8486(83)90090-X

Tuan, N.N. 2010. Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources (PhD thesis). PhD in Agriculture Sciences, Stuttgart, University of Hohenheim.

Ventura, M. and Catalan, J. 2010. Variability in amino acid composition of alpine crustacean zooplankton and its relationship with nitrogen-15 fractionation. Journal of Plankton Research, 32: 1583-1597. doi: 10.1093/plankt/fbq066

Viola, S. 1989. Production of commercial feeds for warm water fish. In: M. Shilo and S. Sarig (Eds), Fish Culture in Warm Water Systems: Problems and Trends. CRC Press Inc., Boca Raton, Florida:143-162.

Yamaji, A., Sekizawa, Y., Emoto, K., Sakuraba, H., Inoue, K., Kobayashi, H. and Umeda, M. 1998. Lysenin, a Novel Sphingomyelin-specific Binding Protein. The Journal of Biological Chemistry, 273: 5300-5306. doi: 10.1074/jbc.273.9.5300