Effects of earthworm, *Perionyx excavatus* meal in practical diets on growth and body composition of common carp, *Cyprinus carpio*

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Abstract. Invertebrates including poly- and oligochaete worms are often part of the natural diet of fish. Previous studies on the applicability of earth worm meal (EWM) as a protein source for fish have produced contradictory results. In this trial, we designed four isonitrogeneous practical diets (32% protein) containing 12% fishmeal with 0%, 30%, 70% and 100% of the fishmeal replaced by EWM (*Perionyx excavatus*). Other protein sources were soybean meal, rice bran and wheat meal while amino acids were supplemented where required. All diets were fed to common carp *Cyprinus carpio* of 11-12 g for eight weeks in 5 replicates to investigate the influence on growth and body composition of carp. Feed palatability was good and all diets were consumed at all times. Fish fed the fishmeal control diet grew significantly better than those fed diets containing EWM. There were no differences between the three EWM diets. Significantly higher protein efficiency ratio (PER) and protein productive value (PPV) were observed in fish fed the control diet. This suggested that a so far unknown factor in EWM impairs protein metabolism in carp independent of its concentration in the diet. Further research on processing of EWM is required in order to make full use of this bioresource.

Key Words: *Cyprinus carpio, Perionyx excavatus,* growth, protein productive value, protein efficiency ratio.

Introduction. Price of fishmeal has increased over the past decades and this has led to efforts in looking for alternative sources of protein. Despite plant-based feedstuffs taking up an ever larger role in supplying the necessary protein (Hardy 2010), absolute need for fishmeal is still on the rise as the global aquaculture sector is rapidly expanding. Plant-based feedstuffs are good alternatives to fishmeal. However, they may also have some significant disadvantages over animal-based protein, such as poor palatability, low protein content or imbalanced amino acid composition. Moreover, anti-nutritional factors in plant-based feedstuffs, such as phytate, may reduce digestibility of nutrients (Francis et al 2001). In contrast, invertebrates including poly- and oligochaete worms are often part of the natural diet of fish and may therefore suit their nutritional requirements.

Vermiculture, the process of breaking down organic wastes by earthworms, has been on the rise in tropical countries around the world (Edwards & Niederer 2011). Considering essential amino acid composition, apparent metabolizable energy and fatty acid composition, earthworm meal (EWM) has been identified to be similar to fish meal (Ebadi et al 2008; Tuan 2010). In terms of fish development and growth, previous experiments on the applicability of EWM as a protein source for fish have produced contradictory results (Unpublished data). So far, experiments concerning the suitability of EWM have mostly been conducted by replacing different percentages of fishmeal with EWM and comparing these to a fish meal control diet. However, present practical diets for carp contain only between 5-10% of fishmeal with the rest of the protein supplied through plant meals. In this trial, we designed a practical diet containing 12% fishmeal which was replaced with various percentages with EWM and investigated its effects on growth and body composition of common carp (*Cyprinus caprio*).

Material and Method

Experimental procedure. Four isonitrogenous (32% protein) and isoenergetic diets were produced, in which 0% (Control), 30%, 70% and 100% of the fishmeal were replaced by EWM (*Perionyx excavatus*). Protein sources aside fishmeal or EWM respectively were soybean meal, rice bran and wheat meal. Amino acids were supplemented according to the requirements established by the NRC (2011). Twenty aquaria (5 replicates per treatment) each containing 5 fish with body weight between 11-12 g were set up. For the period of 8 weeks, fish were fed three times a day 5 times their maintenance requirement (Becker et al 1983). All fish were weighed individually in biweekly intervals to adjust the feed allowance. At the end of the experiment, fish were sacrificed, autoclaved, homogenized and freeze-dried prior to analysis of proximate composition. The feeding trial was carried out from April to June 2012 at the Aquaculture Laboratory of Thuenen Institute of Fisheries Ecology, Ahrensburg, Germany.

Growth of the fish was expressed as specific growth rate (SGR), feed conversion ratio (FCR), body mass gain (BMG), metabolic growth rate (MGR), protein efficiency ratio (PER), protein productive value (PPV), lipid productive value (LPV) and energy productive value (EPV). These were calculated as follows:

$$SGR\ (\%\ d^{-1}) = [(\ln \text{final body mass in g}) – (\ln \text{initial body mass in g})/\text{number of trial days}] \times 100;$$

$$FCR = \text{dry feed fed (g)}/\text{body mass gain (g)};$$

$$BMG = \text{final body mass (g)} – \text{initial body mass (g)};$$

$$MGR\ (g * kg^{0.8}\ d^{-1}) = (\text{body mass gain in g})/[\{(\text{initial body mass in g}/1000)^{0.8} + (\text{final body mass in g}/1000)^{0.8}\}/2]/\text{number of trial days};$$

$$PER = \text{fresh body mass gain (g)}/\text{crude protein fed (g)};$$

$$PPV\ (\%) = [(\text{final fish body protein in g} – \text{initial fish protein in g})/\text{total protein consumed in g}] \times 100;$$

$$LPV\ (\%) = [(\text{final fish body lipid in g} – \text{initial fish body lipid in g})/\text{total crude lipid consumed in g}] \times 100.$$

Statistics. Statistic ver. 7 software was used for data analysis. Results were checked for normality and analysis of variance was conducted to test for significant differences in treatments. Tukey post-hoc test was applied to test for differences between specific treatments. Percentages were arcsine transformed before analysis. Results are depicted as mean ± standard deviation.

Results and Discussion. Feed palatability was good and all diets were consumed at all times. Growth development and parameters are shown in Figure 1 and Table 1, respectively. The fish fed fishmeal control diet grew significantly better than those given diets containing EWM. There were no differences in growth between fish fed EWM diets. Significantly lower protein and ash content and higher fat were observed in body carcasses of fish fed the control diet (Table 2), but due to the higher growth rate in fish fed the control diet, PER and PPV were higher than those in the EWM groups (Table 1).

This study showed a significantly lower BMG when fish were fed diets containing EWM. The inclusion level of EWM did not seem to affect the fish growth. Specifically, protein utilization was impaired by EWM as seen by the significantly lower PER and PPV values. Hilton (1983) observed a gradual decline of growth rates of trout fed 50 to 100% of *Eudrilus eugeniae* meal. Similarly, earthworm meal from *Eisenia foetida* could not compete against a variety of alternative protein sources in diets for blue gourami *Trichogaster trichopterus* (Mohanta et al 2013) and a diet in which fish meal was partially replaced by earthworm meal from *E. eugeniae* performed worse than the control

(Nandeesha et al 1988). On the other hand, a diet with EWM from *P. excavatus* as 70% of fishmeal protein replacement gave a better growth of carp than the fish meal based control diet (Tuan 2010). SGR and PER on an absolute scale are higher for all treatments compared to those in our trial.

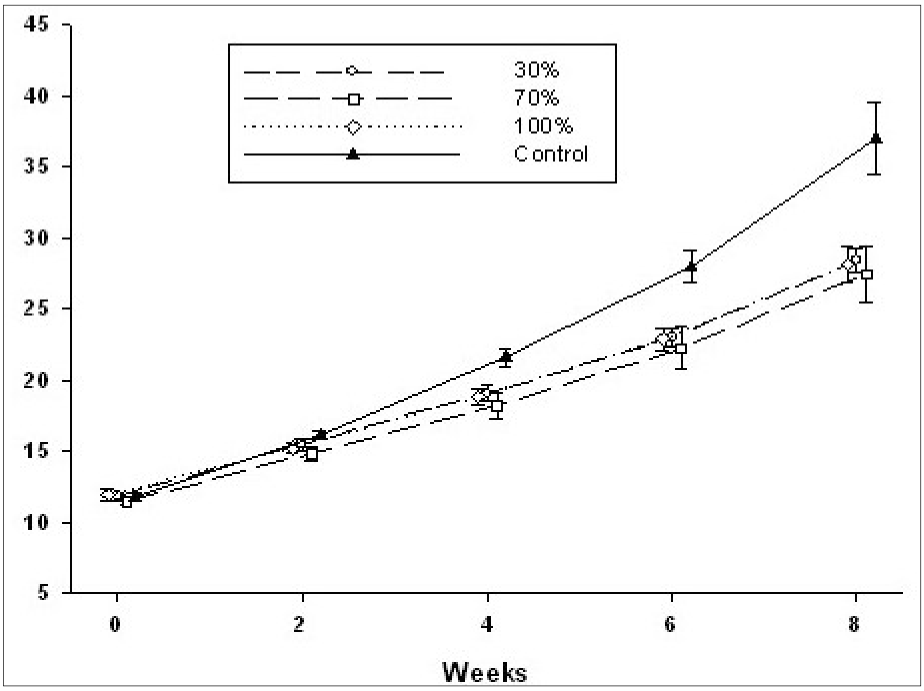

Figure 1. Body mass gain of experimental fish.

Carcass lipid content was lower in treatments containing EWM. This is different to a previous study by Stafford & Tacon (1985) who observed an increase in lipid content of fish fed 5, 10 and 20% (by weight) of *E. foetida* meal.

Differences in the performances between EWM seem to be dependent on genus, species and probably also be related to earthworm processing. Differences in fish performance could also be based on anti-nutritional factors that exist in some earthworm species, however, no study could be found, in which antinutritional factors were analyzed in EWM. Based on the results of our trial, the potential of EWM to improve the nutritional quality of a plant-based diet for common carp seems to be lower than that of fishmeal, which was in contrast to previous studies using the same earthworm and fish species (Pucher et al 2014; Monebi & Ugwumba 2016; Tuan et al 2015). This situation may be due to differences in the culture conditions or processing of the EWM. Steam processing, for example, has shown to have a positive effect on protein leaching and pellet integrity of EWM based diets (Liam et al 2014). Still, when considering the fact that local farmers in tropical countries often encounter problems to get affordable access to fishmeal or fishmeal-based diets, it may still be viable to replace fishmeal with EWM and further research should be conducted to optimize the efficiency of EWM as an alternative protein source.

Table 1

Growth performance parameters and nutrient utilization of fish fed experimental diets for 8 weeks

Parameter	*Treatment*			
	Control	*30%*	*70%*	*100%*
BMG	25.2±2.63[b]	16.6±0.85[a]	16.0±1.83[a]	16.2±1.20[a]
SGR	2.04±0.15[b]	1.57±0.06[a]	1.56±0.11[a]	1.53±0.12[a]
FCR	1.41±0.14[b]	1.95±0.09[a]	1.98±0.16[a]	1.99±0.16[a]
MGR	8.98±0.63[b]	6.84±0.27[a]	6.75±0.49[a]	6.67±0.54[a]
PER	2.27±0.20[b]	1.65±0.08[a]	1.61±0.13[a]	1.64±0.13[a]
PPV	34.5±3.24[b]	25.2±1.47[a]	25.0±2.91[a]	24.9±1.29[a]
LPV	30.6±8.52	31.0±3.73	34.2±7.66	24.9±8.43

Mean values with different superscript differ significantly from one another ($p \leq 0.05$). Mean values with same superscript differ significantly from one another ($p = 0.05$). BMG (g) = body mass gain; SGR (%/day) = specific growth rate; FCR = feed conversation ratio; MGR (g x $kg^{0.8}$ day^{-1}) = metabolic growth rate; PER = protein efficiency ratio; PPV (%) = protein productive value; LPV (%) = lipid productive value.

Table 2

Body composition of experimental fish

Treatment	*Dry matter (%)*	*Crude protein (%)*	*Crude lipid (%)*	*Ash (%)*
Initial fish	20.9±1.36	66.0±2.36	11.6±5.08	17.1±4.13
Control	23.3±1.14[a]	63.4±3.01[a]	23.6±5.51	10.1±0.93[a]
30%	21.5±0.49[b]	68.0±2.05[b]	19.0±1.56	11.9±0.80[b]
70%	21.9±0.87[a,b]	67.5±1.41[b]	19.8±2.37	12.2±0.67[b]
100%	21.4±0.32[b]	68.4±1.17[b]	18.8±0.90	11.4±0.32[b]

Mean values with different superscript differ significantly from one another ($p \leq 0.05$).

Conclusions. Earthworm produced on farm from resources such as leave material and ruminant manure or in industrial scale from organic wastes can be utilized as fish feed ingredient replacing fishmeal, which is a limited resource. However, in this experiment a growth-retarding effect was observed irrespective of the inclusion level, suggesting some anti-nutritional effect, specifically on protein metabolism. Further research on the reasons for inconsistent results of different trials and recommendable processing techniques to eliminate anti-nutrients from the earthworm biomass is required.

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