Black Soldier Fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients' class in aquaculture diets

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Abstract

Insect meals as ingredients for animal feeds had been rediscovered recently. The potential of using insect meals to replace fishmeal seems to show promising perspectives, especially for organic aquaculture feeds. In the present study, different levels of fishmeal replacement (0, 50, 75 %) by using Black Soldiers Fly larvae meal had been tested on rainbow trout. Also growth responses and carcass compositions were significantly influenced by the content of BSF meal in the diet with a negative trend from the control to the highest substitution level; body weight gain, feed conversion ratio and protein efficiency ratio were comparable among the BSF 50 and the control group. As neither signs of nutrient deficiencies nor of higher mortalities have been observed, it could be concluded that BSF-meal can substitute fish meal up to an extent of 50 % in trout feeds.

Introduction

Regarding the growing demand for fish- and soybean meal in livestock- and aquaculture feeding, alternative protein carriers are of increasing concern to the animal feed industry. Beside the possibility to use animal by-products of non-ruminant origin in aquafeeds (EU-regulation (EC) 56/2013), the use of insect-based feeds is progressively discussed among fish-feed producers, scientists and policy makers. The black soldier fly, BSF (*Hermetia illucens*) occurring circumpolar in warm and temperate climates proved to be an ideal candidate (Bondary and Sheppard, 1981). Its larvae can be reared on a wide range of organic (waste)-material, reduce the volume of this waste by up to 50%, producing biomass with a protein content of about 42% and a fat content of up to 35 % (Sheppard et al., 1994). The suitability of the BSF larvae meal as a protein source in feedstuff had been proven in some warm water fish species (Bondari, Sheppard, 1981) but regarding carnivorous cold water species only limited data is available; e. g. on turbot and trout (Kroeckel et al. 2012; St-Hilaire et al. 2007). This study shows results of a feeding-trial on rainbow trout fed with two BSF-larvae meal containing organic diets.

Material and methods

Experimental design

The trial was conducted at the experimental station of the University of Goettingen, Germany. Rainbow trout of 120g (± 13g) were fattened for a total duration of 8 weeks in triplicates of 200 fish. Three diets with different fish meal substitution levels (0, 50, 75 % BSF meal) were tested. Initial stocking density in the concrete tanks was 6 kg/m^3

Prior to the experiment, the fish were fed a conventional pelleted trout feed (42 % crude protein, 22 % crude fat and 17.4 MJkg-1 digestible energy). At stocking, two fish per replicate were sacrificed and stored at -20 °C for whole body analysis. A random sample of 20 fish was taken to assess the carcass traits body weight, body length, body height, body width, filet-, gut- and liver weight. During the experiment, dead fish were replaced by tagged ones to assure equal stocking densities. The fish were hand fed twice a day according to body weight and water temperatures. At the end of the feeding trial the fish were starved 24 hours. All fish were weighed and a sample of 20 fish per replicate was slaughtered and gutted in order to assess the carcass traits. Ten fish, showing a representative weight within the range of one standard deviation of the replicate's mean, were stored at -20 °C until they were used for whole body and sensory analysis.

Diet Formulation

Pre-pupae of the BSF were obtained from a German producer rearing the larvae on tomato plant compost as a substrate. The pre-pupae were freeze-dried and ground prior to blending to the experimental diets. The main ingredients of the diet were soy bean-, fish- and BSF-meal (except control), wheat, cholinchlorid and a mineral/vitamin premix. All feeds were pelleted (size 4 mm). Three rations with 0% (control), 50 % (BSF 50)

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or 75 % (BSF 75) fish meal substitution were designed by a German feedmill, using the Winfumi-software (Hybrimin®, Germany). The diets were iso-nitrogenous and met the energy and nutrient requirements of rainbow trout. The plant material used was certified organic according to Bioland and/or EU-standards.

Table 1: Proximate feed composition of the experimental diets

	Control	BSF 50	BSF 75
Moisture (%)	9.07	9.04	9.06
Crude protein (%)	47.54	47.53	47.51
Crude fat (%)	13.4	15.6	20.1
Crude fibre (%)	0.76	3.46	4.69
Ash (%)	16.3	12.9	11.2
Phosphorus (%)	2.02	1.14	0.7
Gross Energy (MJ/kg)	19.5	20.4	21.6

Sensory evaluation

Two fish from each replicate were filleted; fillets were washed, vacuum-packed and frozen at -20 °C till the sensory evaluation. Twelve hours prior to the test, the fillets were thawed at 4 °C. The evacuated fillets were poached in a water bath at 90 °C for 5 minutes. Each fillet was then divided into three equal parts. The sensory test was conducted in two replicates with 18 untrained panellists. Each panellist was served an equal piece of the fillet for all diets tested in order to carry out a ranking test (DIN 10963, ISO 8587).

Analytical methods

Proximate compositions of the ingredients were either given by suppliers or by Winfumi software and were considered for the diet formulation. Proximate analysis of fish carcasses was carried out according to German standard methods (Naumann & Bassler, 1976-1997). Based on the measurements taken before (body weight, length, thickness, height, fillet- and gut weight), during and at the end of the experiment the following parameters were calculated for each replicate:

- weight gain (g) = mean initial body weight (g) – mean final body weight (g)
- specific growth rate (SGR) = 100 x (ln final body weight (g) – ln initial body weight (g))/days of trial
- condition factor = final body weight (g) / body length (cm)^3 x 100
- hepatosomatic index = liver weight (g) / final body weight (g) x 100
- net carcass weight (g) = final body weight (g) – head weight (g) – fin weight (g) – gut weight (g)
- gutted weight (g) = final body weight (g) – offals (g)
- dressing percentage = gutted weight (g) / final body weight (g) x 100
- feed conversion ratio (FCR) = dry feed intake (g) /wet weight gain (g)
- protein efficiency ratio (PER) = weight gain (g) / protein intake (g)

Statistical analysis

The data were analysed using the SAS software package (SAS 9.1). The initial and final body weights were log-transformed. All variables were considered to be normally distributed. To compare differences between groups the Duncan's multiple range test was applied to compare the means given by Proc GLM. The following model was used $y = \mu + Di + Tj + Di * Tj + e$, where D is a fixed effect of the ith diet, T is a fixed effect of the jth tank, D*T a fixed effect of the interaction between the ith diet and the jth tank and e the random error term. Differences between means were considered to be significant at a level of $P \leq 0.05$, indicated by different indices in the results' table.

Results

The initial body weight was similar for all replicates. Growth responses and carcass compositions observed from the experiment were significantly influenced by the content of black soldier fly meal in the diet. The final body weights from those fish fed the BSF 50 diet were found to be 13,4% lower than those from the control fish and the fish fed the BSF 75 diet showed a 15% lower final body weight. Also for specific growth rate, and net carcass weight, marked differences were found between the experimental groups with a negative trend from the control to the BSF 75 group.

Table 2: **Effects of different levels of BSF meal in the diet of rainbow trout**

Trait	Control	BSF 50	BSF 75
Specific growth rate (%)	1.12 ± 0.27 [a]	0.89± 0.19 [ab]	081± 0.25 [b]
Body weight gain (g)	92.8 [a]	80.5 [ab]	62.2 [b]
Feed conversion ratio	1.22 ± 0.11 [a]	1.31 ± 0.35 [a]	1.68 ± 021 [b]
Protein efficiency ratio	1.74 ± 0.17 [a]	1.70 ± 0.50 [a]	1.27 ± 0.17 [b]
Final body weight (g)	227.3 ± 31.88 [a]	196.8 ± 31.11 [b]	193.3 ± 33.30 [b]
Net carcass weight (g)	160.5 ± 24.5 [a]	137.6 ± 26.8 [b]	133.3 ± 25.5 [b]
Dress out percentage (%)	70.5 ± 2.15 [a]	69.4 ± 6.28 [ab]	68.9 ± 2.10 [b]
Corpulence factor (g/cm^3)	1.09 ± 0.07 [a]	1.03 ± 0.12 [b]	1.04 ± 0.08 [b]
Hepatosomatic index (%)	2.7 ± 03 [a]	3.3 ± 0.6 [b]	3.3 ± 0.4 [b]

Nevertheless, body weight gain, feed conversion ratio and protein efficiency ratio were comparable among the BSF 50 and the control. Carcass quality was not significantly different among the different treatments. Fish from the experimental groups (BSF 50, BSF 75) were leaner, the higher the level of fish meal substitution had been. Concurrently the crude protein and ash content increased with an increasing share of black soldier fly meal in the diet, whereas total phosphorus was equal among all groups. Also through the sensory evaluation of the rainbow trout fillets no significant preference for one of the diets was detected. However, the three diets were by trend ranked according to the fish meal substitution level, where BSF 50 and BSF 75 showed a 2.8 % and 21.6% lower mean grade compared to control, respectively.

Discussion

From the present results it could be concluded that BSF-meal might substitute fish meal without severe losses in body weight gain, FCR and protein retention ratio, up to an extent of 50 %. Although the growth performance of rainbow trout fed the highest level of BSF-meal (BSF 75) was poorest, neither signs of nutrient deficiencies nor of higher mortalities were observed. According to Rust (2002) a reason for declining performance could be the chitin content of the pre-pupae as this component of the invertebrate exoskeleton might have adverse effects on the digestibility of nutrients. In order to obtain iso-nitrogenous rations in the present study, soybean meal was added when fish meal was replaced by black soldier fly meal. Glencross et al. (2008) postulated that increasing amounts of soy beans lead to reduced growth in salmonid species. The high content of saturated fatty acids could also have negative effects on the performance of the feed. A reduced fat and energy digestibility of the BSF-component could be the reason for the observed decreasing carcass-fat content of the fish which were fed the BSF-diet. Fasakin, et al. (2003) attributed lower growth in Clarias gariepinus fingerlings fed a full-fat maggot meal to a reduced protein digestibility. Overall performance and digestibility of diets containing BSF-meals should increase if the feed is extruded and the level of lipids is reduced by mechanically defatting of the BSF raw-material.

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