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When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*)

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ABSTRACT

Larvae of black soldier flies (*Hermetia illucens*) are commercially produced on agricultural waste streams and convert these into animal body protein and fat. A feeding trial was carried out for 56 days in a recirculating aquaculture system (RAS) by replacing fish meal protein subsequently by *Hermetia* meal (HM) protein. Six diets were formulated for the replacement and contained 0%, 17%, 33%, 49%, 64%, and 76% of HM (54.1 ± 1.1% crude protein, 13.4 ± 0.7% crude lipid, dry matter basis). The diets were fed to triplicate groups of turbot 54.9 ± 0.9 g once a day by hand until apparent satiation. Feed intake was affected by dietary HM inclusion and decreased with increasing HM incorporation due to low palatability. Growth performance was high, but affected by dietary HM inclusion. SGR was lower in all treatments containing HM whereas FCR was significantly higher at HM inclusion levels > 33%. Protein retention was highest at HM inclusion ≤ 33% and decreased significantly with increasing HM supplementation. Whole-body protein content was not affected by treatment, while body lipid decreased with increasing HM inclusion levels. The apparent digestibility coefficients (ADC) of HM were low for organic matter, crude protein, crude lipid, and gross energy. Chitinase activity or chitinolytic active bacteria were not detected in the mid gut of turbot. The presence of chitin might have influenced the feed intake, availability, and digestibility of the nutrients and therefore growth performance. In general, our study shows that the incorporation of HM protein in fish diets is possible, but limited by its low nutritive value. Considering that HM is produced on local greenhouse waste streams, HM might be a feasible alternative protein source for the partial replacement of fish meal. Further research on HM meal processing to increase nutrient utilization is needed.

1. Introduction

Shortages in fish meal and oil supply resulted in rising prices of formulated diets, while they are the main expenditure in intensive aquaculture production (40–70% of production costs) (Wilson, 2002). Especially, carnivorous flatfish e.g. turbot (*Psetta maxima*), plaice (*Pleuronectes platessa*), common sole (*Solea solea*), and Atlantic halibut (*Hippoglossus hippoglossus*) have high dietary protein demands of 500 up to 650 g kg^{-1} DM, thus the aquaculture industry has to face this problem and identify alternative feed sources in order to decrease dietary costs (Lee et al., 2003). The production of flatfish in aquaculture systems in Europe, headed by turbot, is growing steadily in the last years and profound knowledge on the physiology of turbot is needed to meet the dietary requirement of this aquaculture fish species under the fluctuating feed source supply (Brown, 2002; Person-Le Ruyet, 2002). Several studies have assessed the use of alternative plant based proteins including their nutritional value in flat fish (Bonaldo et al., 2011; Burel et al., 2000a,b; Fournier et al., 2004; Nagel et al., 2012b; Regost et al., 1999; Slawski et al., 2011). Nevertheless, these plant materials are often deficient in essential amino acids, especially lysine or methione, and therefore their utilization is limited as observed in turbot (Regost et al., 1999; Slawski et al., 2011), Japanese flounder (*Paralichthys olivaceus* ; Deng et al., 2006) and halibut (Helland and Grisdale-Helland, 2006). Still, the quality of these products is high and utilization as animal feed might thus compete with its exploration for human nutrition.

Only some studies were investigated with the usage of alternative animal proteins in nutrition for turbot. The replacement of 250 g kg^{-1} FM protein by poultry by-product meal was successful in diets for Black Sea turbot (*Psetta maeotica*) (Yigit et al., 2006). However, determined ADC's

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for feather meals, poultry meat meal and hemoglobin meal were rather low in turbot when compared to gilthead sea bream (*Sparus aurata*) or European sea bass (*Dicentrarchus labrax*) (Davies et al., 2009). In Germany, commercial production of the black soldier fly was developed in order to provide an alternative protein source for feed purposes. Applied research is now needed to fill the knowledge gaps by utilizing the pre-pupae of *Hermetia illucens* in fish diets. Larvae of *Hermetia* fly feed on animal manure and plant material and are capable to convert low valued organic waste into protein rich biomass (Diener et al., 2009). Due to the amount of protein (476 g kg^{-1}, dry matter DM) and fat (118 g kg^{-1}, DM) and the well balanced essential amino acid (EAA) profile, the pre-pupae meal might be a suitable alternative for fish meal. HM meets macronutrient requirements of terrestric animals and fish; former studies showed suitability of HM as ingredient in diets for swine and broiler chickens as well as in nutrition of rainbow trout (*Oncorhynchus mykiss*), or as pre-pupae in channel catfish (*Ictalurus punctatus*) and blue tilapia (*Tilapia aurea*) (Bondari and Sheppard, 1981; Elwert et al., 2010; Newton et al., 1977; Stamer et al., 2008; St-Hilaire et al., 2007). However, the exoskeleton of the black soldier fly pre-pupae contains the polysaccharide chitin (approximately 87.0 g kg^{-1}, DM) which might affect the digestibility and the utilization of other nutrients (Diener et al., 2009; Shiau and Yu, 1999). If chitinolytic activity is observed in fish, the ingested chitin might have a substantial nutritive function of energy intake (Fines and Holt, 2010; Goodrich and Morita, 1977a,b). Chitinase activity has been detected in blood, plasma and intestinal tract of fishes, but not yet proven to actually break down the chitin fraction in 6 marine teleosts (Fänge et al., 1976, 1979) and rainbow trout (*Salmo gairdneri*, Lindsay, 1984; Lindsay et al., 1984). However, it has been documented that the marine species cobia (*Rachycentron canadum*) is capable to digest chitin from either shrimp or crab meal revealing high endogenous chitinolytic activity in the stomach (Fines and Holt, 2010). In cod (*Gadus morhua*) the enzyme chitinase was found in stomach and intestinal tract (Danulat, 1986a,b; Danulat and Kausch, 1984; Lindsay, 1987), but no chitinolytic bacteria activity was reported. No research was realized on digestibility of chitin in turbot. But due to its natural feeding profile, the ability to digest chitin might be possible. Thus, aim of this study was to elucidate the nutritional value of *Hermetia* meal for juvenile turbot by determining the growth potential, feed intake and nutrient retention efficiencies. Special emphasize was given to digestibility of the HM itself and the capability of turbot to break down chitin by the endogenous enzyme chitinase or by the activity of chitinolytic bacteria.

2. Materials and methods

2.1. Diet formulation and diet preparation

Hermetia meal (HM) was obtained from a commercial producer (Hermetia Futtermittel GbR, Baruth/Mark, Germany). For the production of HM, the pre-pupae of the black soldier fly were collected from the substrate and freezed at −24 °C. The frozen pupae were cut to enable the leakage of intracellular fat from the larvae. This material was transferred into a tincture press (Fischer Maschinenfabrik GmbH, HP-5MT-VA, Neuss, Germany) and pressed at 450 bar at 60 °C for

Table 1
Formulation (g kg^{-1} feed), proximate composition (g kg^{-1} dry matter), and amino acid composition (g kg^{-1} crude protein) of the raw materials (HM = *Hermetia* meal, FM = fish meal) and experimental diets used in the feeding trial.

	Raw material		Diets					
	FM	HM	HM%0	HM%17	HM%33	HM%49	HM%64	HM%76
Ingredients (g kg^{-1}, dm)								
Herring meal[a]			687	550	422	305	180	80
Hermetia meal[b]			0	165	332	486	640	756
Wheat protein[c]			20	20	20	20	20	20
Blood meal[d]			50	50	50	50	50	50
Wheat starch[c]			148	130	102	75	57	50
Fish oil[a]			90	80	69	59	49	39
Vit/min. mix[e]			5	5	5	5	5	5
Proximate composition (g kg^{-1}dry matter)								
Moisture	73	44	78	64	59	62	52	47
Crude lipid	98	118	128	130	142	142	132	128
Crude protein [f]	641	476	548	549	537	539	533	527
Chitin	0	96	0	16	32	47	61	73
NfE [g]	95	151	194	166	148	138	128	120
Crude ash	166	159	130	139	141	134	146	152
Calcium	21	65	31	35	39	44	48	51
Phosphorus	29	7	17	15	13	11	9	7
Gross energy (MJ kg^{-1})	22	21.1	21.6	21.5	21.8	21.9	21.5	21.3
Essential amino acids (g kg^{-1} crude protein)								
Arginine	59.3	57.1	61.2	58.2	53.4	56.1	51.2	49.2
Cystine	8.7	8.2	9.0	8.7	8.2	8.6	8.0	7.6
Histidine	20.9	33.4	26.4	27.0	29.0	28.1	29.4	30.4
Isoleucine	39.8	51.9	37.3	37.6	39.7	39.5	40.3	40.5
Leucine	71.0	86.3	76.8	76.2	77.3	77.9	77.2	77.6
Lysine	71.6	71.2	66.9	64.9	62.1	64.5	60.5	59.2
Methionine	26.4	21.8	23.3	21.9	19.9	21.3	19.1	18.1
Phenylalanine	39.9	38.9	42.1	42.6	45.2	45.7	46.3	48.3
Threonine	41.2	46.4	38.7	38.7	38.3	38.0	37.4	37.8
Valine	48.1	72.1	53.3	54.5	59.2	57.5	60.9	61.9

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[f] Protein content calculated by nitrogen∗6.25; protein content was corrected for chitin.
[g] NfE, nitrogen-free extract = 1000 − (crude protein + crude lipid + crude ash + chitin).