

***Hermetia illucens* meal as fish meal replacement for rainbow trout on farm**

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Abstract

In a 7-week on-farm feeding trial rainbow trout (*Oncorhynchus mykiss*) were provided with a diet containing 28% mechanically de-fatted insect meal prepared from larvae of the black soldier fly, *Hermetia illucens* (HIM) and compared to a control that received a certified organic and fishmeal based diet. In the test diet insect meal replaced almost 50% of the fishmeal. The whole experiment was conducted under practical conditions on an organically certified rainbow trout farm in Switzerland. Fish of initially 66.5 ± 2.3 g body weight were grown to 125 ± 4.5 g and assessed for their growth performance, as well as analysed for their proximate composition, feed conversion ratio, fatty acid contents and organoleptic properties. Improved lipid utilisation and decreased protein utilisation were observed in fish fed the HIM diet. Furthermore, in a controlled degustation no differences except a slightly darker coloration of fish fed HIM were observed. The experiment demonstrated that substantial replacement of fishmeal by insect meal is possible without compromising growth, feed conversion and product quality. However, the decreased protein utilisation efficiency in HIM fed fish might lower production efficiency when applied over a whole production cycle and not only over 7 weeks.

Keywords: insect meal, fishmeal replacement, growth performance, feed conversion, organoleptic properties

1. Introduction

Production of fish in aquaculture is increasingly contributing to global food fish supply. Already in 2006 almost every second food fish was aquaculture produced (Cressy, 2009). The global finfish production increased by 90% during the last decade (2004-2014) (FAO, 2016). Thus, demands for high quality and protein rich feed ingredients is growing, too. This is especially the case for organic aquaculture as all feed ingredients must be organically or sustainably certified, thus strongly limiting the availability of feed ingredients and influencing the prices. Traditionally, fishmeal is the most important protein source for aqua feeds but fishmeal supply from targeted fishery is limited to around 5.5-6.5 million metric tons annually (Hardy, 2010) resulting in continuously decreasing fishmeal levels in fish feeds (Deutsch *et al.*, 2007; Naylor *et al.*, 2009). Furthermore, some of the fish currently used for fishmeal production could directly be consumed by humans (Fréon *et al.*, 2014). For organic aquaculture the situation is even more complicated as the implementation of conventionally produced fishmeal, i.e. fishmeal from targeted reduction fishery, is prohibited

according to all organic and almost all sustainability directives or standards, such as the standard for organic aquaculture of the European Union (EC, 2009), Naturland (Naturland, 2014), Soil Association (Soil Association, 2016), Bio Suisse (2015) and the Aquaculture Stewardship Council (ASC, 2012). Instead, fishmeal produced from trimmings of food fish or from fish caught under a sustainability scheme (e.g. Marine Stewardship Council) needs to be used which, in case of fishmeal from trimmings, contains relatively more Phosphorous than other fishmeal. As a consequence of the limited fishmeal resources, plant derived protein sources have been increasingly utilised for aquaculture feeds in the last decade, with soy beans and soy protein concentrate, wheat and wheat gluten, corn, canola, cottonseed, peas/lupines and barley being the most important alternatives (Naylor *et al.*, 2009; Olsen and Hasan, 2012). Still, in terms of land use efficiency, the direct competition between plants being produced as animal feed, versus plants produced as human food remains an unsolved issue (Cassidy *et al.*, 2013; Schader *et al.*, 2015).

Besides plant based protein sources several animal based feedstuffs are readily available and utilised too (Naylor *et al.*, 2009). The interest in insects as feed for a variety of livestock species is strongly increasing (Barroso *et al.*, 2014; Henry *et al.*, 2015; Makkar *et al.*, 2014; Sánchez-Muros *et al.*, 2014). One particularly promising candidate insect species is the black soldier fly, *Hermetia illucens*, because it can be employed to convert food waste material or manure into high quality insect protein highly suitable to be implemented in animal feed (Sheppard *et al.*, 1994). It is native to Central and Latin America and large parts of the USA, yet secondarily established in virtually all (sub-) tropical regions worldwide (Sheppard *et al.*, 1994). The protein and lipid content of *H. illucens* meal (HIM) is highly variable; based on dry matter, protein and lipid contents reported for de-fatted HIM were 47.2 and 11.8% (Kroeckel *et al.*, 2012) and 51.8 and 14.8%, respectively (Cullere *et al.*, 2016), whereas protein and lipid content reported for full-fat HIM were 36.2 and 18.0% (Barroso *et al.*, 2014). Its potential as a valuable feed ingredient has been reported for several livestock species, such as poultry (cockerels, Hale, 1973; layer hens, Maurer *et al.*, 2016; broilers, Leiber *et al.*, 2017; Cullere *et al.*, 2016), pigs (Newton *et al.*, 1977) and a number of commercially important cultured fish species like Atlantic salmon (*Salmo salar*, Lock *et al.*, 2015), channel catfish (*Ictalurus punctatus*) and blue tilapia (*Oreochromis aureus*, Bondari and Sheppard, 1981, 1987), Nile tilapia, (*Oreochromis niloticus*, Hem *et al.*, 2008; Webster *et al.*, 2015), rainbow trout (*Oncorhynchus mykiss*, Gasco *et al.*, 2015; Sealey *et al.*, 2011; St-Hilaire *et al.*, 2007) and turbot (*Psetta maxima*, Kroeckel *et al.*, 2012).

The rainbow trout is the most important freshwater aquaculture species in central Europe with a total production volume of 294,000 metric tons (mt) in 2014 (FAO, 2016). In Switzerland the production volume was 1,100 mt out of 1,393 mt total volume in 2014, thus, making it by far the most important cultured species of which around 30% are organically certified (FAO, 2016; Stadtlander and Gerber, 2014).

The aim of this experiment was to evaluate de-fatted HIM as replacement of approximately half of the fishmeal in extrusion cooked and organically certified trout feed. In a seven week experiment the growth performance, feed and nutrient conversion and organoleptic properties of rainbow trout fed with either a commercial control feed or a feed with high fishmeal replacement by HIM were compared.

2. Materials and methods

Production of insect meal

Freshly emerged *H. illucens* larvae were fed with chicken feed (Demeter layer hen crumble) for 10 days until reaching the second larval stage. Later on they were fed

with vegetarian preconsumer food waste supplied by convenience industry. The feeding substrate for *H. illucens* larvae was basically composed of pasta, spent brewer grains and fruit and vegetable leftovers. When the majority of the larvae reached the prepupal stage, they were separated from debris, killed by freezing at -20 °C and stored frozen until further processing. The prepupae were washed in water and cleaned from debris, oven dried for 24–34 hours at 60 °C and coarsely ground. Afterwards the insects were mechanically defatted with a small scale commercial oil press (KK 20 F Universal; Screw Press, Reut, Germany) and the press cake was milled in order to obtain a homogenous meal. The defatted HIM was stored frozen until fish feed production.

Experimental diets

Two different diets, one commercial and one experimental diet, were applied. Manufacturing of both diets using extrusion-cooking in a commercial-scale extruder was commissioned to Hofmann Nutrition AG (Bützberg, Switzerland). The standard organic grow-out feed 'Natura Trout' (certified under the Bio Suisse regulation) served as control diet (diet C) and contained the ingredients fishmeal, wheat flour, soy meal, blood meal, vitamin and mineral premixes and the immunostimulant Immuguard®. The same ingredients with the same proportions have been used for the *H. illucens* meal diet (diet HIM) with the only difference that 46% of the fishmeal has been substituted by HIM, which in turn corresponded to 28.1% of the final diet. The pellet size was 3 mm; the proximate composition of both diets is presented in Table 1. The formulation details are confidential property of Hofmann Nutrition AG and have not been cleared for publication. The digestible energy was estimated by caloric equivalents of 16.7 MJ/kg for nitrogen free extracts (NFE), 33.5 MJ/kg for crude lipids (CL) and 19.6 MJ/kg for crude protein (CP) according to Brett and

Table 1. Proximate composition of the control (C) and the *Hermetia illucens* meal (HIM) diets. Values derived from one pooled sample per feed type.

Proximate analyses	Diet C	Diet HIM
Crude protein (g/kg DM)	457	491 (477) ¹
Crude lipids (g/kg DM)	151	126
Ash (g/kg DM)	134	126
Crude fibre (g/kg DM)	13	44
Nitrogen free extract (g/kg DM)	189	164
Digestible energy (MJ/kg DM)	17.2	17.0 (16.3) ¹
DP:DE ratio (g/MJ) ²	23.5	25.9 (25.5) ¹

¹ Crude protein corrected for crude fibre (presumably corresponding to chitin in the HIM; see Lovell *et al.*, 1968).

² DP:DE = estimated digestible protein to digestible energy ratio in g digestible protein per MJ digestible energy.

Groves (1979). The digestibility of CP in both diets was estimated to be 83.1% (based upon an average gross energy content in CP of 23.6 MJ/kg (NRC, 2011) and the digestible energy content of 19.6 MJ/kg (Brett and Groves, 1979)).

Experimental setup and fish

The experiment was conducted on an organically certified trout farm in Switzerland under practice conditions. The farm is designed as water re-use system comprising of three channels running in a circle with seven 65.1 m³ compartments per side (14 per channel), a fluid bed bio filter at each end and a drum filter at the distal end. The water in each channel is flowing circularly through all compartments and the respective water flow rate is approximately 200 l/s. Around 10% of the total water volume was exchanged per day. For the experiment the two compartments directly downstream of one of the bio filters were used. This setup was replicated in each of the three channels resulting in three independent replicates for each treatment. At the beginning of the experiment each compartment was stocked with a total of 191.3 kg rainbow trout with an average individual weight of 66.5±2.3 g (mean ± standard deviation), corresponding to an initial stocking density of 2.91 kg per m³ and 2,874±100 fish in each compartment, respectively.

The fish were hand fed four times per day according to the water temperature and their biomass under a restrictive scheme adapted from the feed manufacturer (Table 2). The water temperature was ambient and ranged between 8.7 and 12.1 °C. Every second week around 100 fish were weighed in each compartment to adjust feeding rations. When the water was too turbid, for instance after heavy rainfall, no feed was provided (this happened on 2 days).

In order to evaluate the growth performance and nutrient utilisation the following parameters were calculated:

$$\text{Percent weight gain (PWG; \%)} = (\text{final body weight (g)} - \text{initial body weight (g)}) / \text{initial body weight (g)} \times 100$$

$$\text{Specific growth rate (SGR; \% / day)} = (\ln \text{final body weight (g)} - \ln \text{initial body weight (g)}) / \text{days of experiment} \times 100$$

Table 2. Feeding table adapted from the feed manufacturer showing daily feeding allowance (as % of body weight per day).

Weight of fish (g)	Water temperature (°C)				
	8	9	10	11	12
50-100	1.28	1.36	1.44	1.52	1.59
100-200	1.13	1.20	1.27	1.34	1.40

$$\text{Feed conversion ratio (FCR)} = \text{total dry feed intake (g)} / (\text{final body weight (g)} - \text{initial body weight (g)})$$

$$\text{Protein efficiency ratio (PER)} = (\text{final body weight (g)} - \text{initial body weight (g)}) / \text{total protein intake (g)} \times 100$$

$$\text{Protein productive value (PPV; \%)} = (\text{final fish protein content (g)} - \text{initial fish protein content (g)}) / \text{total protein intake (g)} \times 100$$

$$\text{Lipid efficiency ratio (LER; \%)} = (\text{final body weight (g)} - \text{initial body weight (g)}) / \text{total lipid intake (g)} \times 100$$

$$\text{Lipid productive value (LPV)} = (\text{final fish lipid content (g)} - \text{initial fish lipid content (g)}) / \text{total lipid intake (g)}$$

$$\text{Viscerosomatic index (VSI; \%)} = \text{viscera weight (g)} / \text{final body weight (g)} \times 100$$

$$\text{Hepatosomatic index (HSI; \%)} = \text{liver weight (g)} / \text{final body mass (g)} \times 100$$

Water quality

Oxygen content, pH and temperature of the water were measured daily with a hand-held Hach HQ 40d multi (Hach Company, Loveland, CO, USA). Once a week ammonia-N, nitrite-N and nitrate-N were measured spectrophotometrically with test-kits from Hach Company and calculated as ammonia, nitrite and nitrate.

Sampling

Prior to each sampling occasion, all fish were starved for one day in order to ensure that digestive tracts have been cleared. Before the experimental feeding started, 10 fish from the initial stock were randomly sampled as a reference for proximate composition. At the end of the 7-week feeding trial, 10 fish per replicate (30 per treatment) were randomly sampled for determination of proximate composition and morphometric characteristics. Collections of morphometric data included body weight and fork length, and the visceral fat coverage estimated on a scale of 0 to 4, corresponding to 0-100% coverage, respectively. Further, the viscera were dissected and weighed before dissecting and weighing the liver. Afterwards all viscera, including the liver, were frozen together with the rest of the respective fish until further analysis. All fish intended for morphometric and proximate composition analysis were euthanized using 150 mg/l MS-222 buffered with 300 mg/l sodiumhydrogencarbonate, and then frozen at -18 °C until further analysis. Also at the end of the experiment, another 10 fish per replicate were starved for five days, electrically stunned, exsanguinated and kept on crushed ice for the organoleptic test the next day.

Chemical analysis

For chemical analyses, the fish sampled initially before the feeding trial started and at the end of the experiment were homogenised and then pooled for each replicate per treatment, resulting in one initial sample and six samples taken at the end of the experiment. For homogenisation, they were cut into small pieces while still frozen, autoclaved for 15 minutes at 121 °C and turraxed with an Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany). The homogenate was frozen again and lyophilised subsequently using a model Beta 1-16 (Christ, Osterode am Harz, Germany) in order to determine the water contents of each sample before they were finely ground and subjected to further analyses. For amino (AA) and fatty acid (FA) determination, aliquots of the three replicated samples per treatment were again pooled resulting in one sample per treatment. Feed pellets of both feeds were ground into a fine powder prior to analysis.

Chemical analyses of CP, CL, crude fibre (CF) and ash (CA) was conducted for fish and feed samples according to standards defined by the Association of German Agricultural and Analytic Research Institutes (VDLUFA, 2017). NFE has been calculated by difference (100 – CP + CL + CA + CF). AA and FA determination have been conducted by chromatographical methods according to standard methods of the German Society for Fat Science (DGF, 2015). The FA concentrations are presented as fatty acid methyl esters.

Organoleptic test

For the organoleptic test the fish were filleted, the fillets cut into three equally large pieces, wrapped in aluminium-foil and steam-cooked without pressure for 8–10 minutes at 100 °C. The organoleptic test was conducted according to DIN EN ISO 5495. Fifteen untrained panellists were offered double blind testing samples of the filet for differences in odour, colour, texture and taste, each in 5 (texture in 6) different traits. The panellists then rated the different characteristics on a scale between 0 (does not apply) to 9 (applies fully).

Statistical analysis

All data are presented as mean ± standard deviation ($n=3$) if not indicated otherwise. Statistical analyses were performed using IBM SPSS vers. 21 (IBM Corporation, Armonk, NY, USA). Normal distribution of the data was assessed using Kolmogorov-Smirnov and homogeneity of variance was assessed using Levene tests. In case of normal distribution and homogeneity of variance, treatment means of the C and HIM fed fish were compared using t-tests with compartment as individual unit and three replicates per treatment, accordingly. In case criteria of

normal distribution or homogeneity of variance were not met non-parametric Mann-Whitney-U tests were applied. Alpha-levels were set to 0.05.

3. Results

Chemical analyses revealed differences in the proximate composition of the two different feeds. As compared to diet C, diet HIM contained higher levels of CP and CF and lower levels of CL and CA (Table 1). The AA profiles of the diets and the requirements of the essential AA or rainbow trout is presented in Table 3.

Diet C was deficient in threonine and isoleucine, diet HIM was deficient in methionine and both diets were almost equally deficient in lysine (Table 3). The fatty acid profiles of both diets are presented in Table 4. Diet HIM had a considerably higher overall level of saturated fatty acids (SFA) and a lower level of unsaturated fatty acids (UFA) in comparison to diet C. This is also reflected in the ratio of UFA to SFA which was considerably higher in diet C compared to diet HIM. The main differences in UFA content are caused by oleic acid, eicosanoic acid and erucic acid, but also the level of the important poly-

Table 3. Whole amino acid profiles of the experimental diets (control; C, and *Hermetia illucens* meal; HIM) (g/100 g DM) and the requirements of essential amino acids for rainbow trout (g/100 g DM, NRC, 2011). Dietary amino acid concentrations not reaching the requirements are shown in bold. Values derived from pooled samples of the three replicates per treatment.

Amino acid	Diet C	Diet HIM	Requirements ¹
Aspartic acid	2.46	2.80	
Threonine	1.03	1.17	1.1
Serine	0.96	1.21	
Glutamic acid	3.21	3.57	
Proline	1.71	1.78	
Glycine	1.99	2.34	
Alanine	1.96	2.46	
Cysteine	0.30	0.33	
Valine	1.50	1.78	1.2
Methionine	0.70	0.66	0.7
Methionine + cysteine	1.00	0.99	1.1
Isoleucine	1.01	1.17	1.1
Leucine	2.23	2.36	1.5
Tyrosine	0.77	1.18	
Phenylalanine	1.22	1.35	0.9
Phenylalanine + tyrosine	1.99	2.53	1.8
Histidine	0.96	1.32	0.8
Lysine	1.94	1.99	2.4
Arginine	1.50	1.66	1.5

¹ Requirements according to NRC (2011).

unsaturated fatty acids (PUFA) arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were reduced by one third in diet HIM compared to diet C. The SFA lauric acid was only present in diet HIM and contributed considerably to the low UFA:SFA ratio in diet HIM (Table 4).

Mortality, final body weight, growth (relative weight gain and specific growth rate), feed conversion, viscerosomatic and hepatosomatic indices and intraperitoneal fat were comparable for fish of both groups (Table 5).

Protein utilisation, however, was significantly higher, even when corrected for assumed chitin content, in fish fed diet C compared to fish fed diet HIM, as indicated by PER and PPV (Table 5). Contrary to the protein utilisation, the apparent lipid utilisation (LER and LPV, Table 5) was significantly improved in fish fed diet HIM compared to fish fed diet C. Carcass composition revealed no significant differences in protein, lipid and ash content between treatments (Table 6).

Table 4. Fatty acid profiles of the two experimental diets (control; C, and *Hermetia illucens* meal; HIM) (g/100 g fatty acid methyl esters). Values derived from pooled samples of the three replicates per treatment.¹

Fatty acid	Trivial name	Diet C	Diet HIM
C12:0	lauric acid	—	14.8
C14:0	myristic acid	4.30	5.70
C14:1	myristoleic acid	0.12	0.15
C15:0	pentadecyclic acid	0.35	0.30
C16:0	palmitic acid	14.0	13.5
C16:1 n-7	palmitoleic acid	4.60	3.91
C18:0	stearic acid	2.70	2.38
C18:1 n-9	oleic acid	28.6	25.7
C18:2 n-6	linoleic acid	10.9	10.3
C18:3 n-3	linolenic acid	2.75	2.28
C18:4 n-3	stearidonic acid	0.56	0.45
C20:0	arachidic acid	0.24	0.20
C20:1 n-9	eicosenoic acid	8.98	6.06
C20:4 n-6	arachidonic acid	1.59	1.06
C20:5 n-3	eicosapentaenoic acid	3.83	2.59
C22:0	behenic acid	0.22	0.15
C22:1 n-9	erucic acid	9.99	6.35
C22:5 n-3	sardine acid	0.98	0.68
C22:6 n-3	docosahexaenoic acid	4.55	3.06
C24:0	lignoceric acid	0.68	0.38
Σ SFA	Σ SFA	22.5	37.4
Σ UFA	Σ UFA	77.5	62.6
UFA:SFA		3.44	1.67

¹ SFA = saturated fatty acids; UFA = unsaturated fatty acids.

Table 5. Growth and nutrient utilisation of fish fed with either control (C) or *Hermetia* (HIM) diets (data = mean \pm standard deviation; n=3).

Parameter ¹	C fed fish	HIM fed fish ²
Initial body mass (g)	67.0 \pm 2.5	66.2 \pm 1.9
Final body mass (g)	125.3 \pm 4.8	125.5 \pm 4.4
PWG (%)	87.8 \pm 1.0	89.6 \pm 1.7
SGR (%/day)	1.43 \pm 0.05	1.45 \pm 0.05
FCR	0.80 \pm 0.07	0.81 \pm 0.04
PER	2.77 \pm 0.30	2.51 \pm 0.12*
PPV (%)	60.6 \pm 6.6	50.9 \pm 1.8* (52.4 \pm 1.8*) ³
LER	8.39 \pm 0.90	9.77 \pm 0.47*
LPV (%)	47.5 \pm 7.3	60.2 \pm 4.5*
VSI (%)	10.1 \pm 0.8	10.5 \pm 0.2
HSI (%)	1.36 \pm 0.10	1.40 \pm 0.03
Mortality (%)	0.15 \pm 0.07	0.22 \pm 0.07
Intraperitoneal fat	1.40 \pm 0.26	1.43 \pm 0.15

¹ FCR = feed conversion ratio; HSI = hepatosomatic index; LER = lipid efficiency ratio; LPV = lipid productive value; PER = protein efficiency ratio; PPV = protein productive value; PWG = percentage weight gain; SGR = specific growth rate; VSI = viscerosomatic index.

²* = significant difference (P<0.05).

³ Corrected for estimated chitin.

Table 6. Proximate composition of the fish at the beginning and after 7 weeks of feeding. Values = mean \pm standard deviation (n=3 for the control diet and *Hermetia illucens* meal, and a single pooled sample of 10 fish for initial analysis).¹

	Initial	C fed fish	HIM fed fish
Moisture (%)	71.3	73.0 \pm 0.5	72.9 \pm 0.3
Crude protein (% FM)	10.8	16.2 \pm 0.9	15.2 \pm 0.3
Crude lipids (% FM)	6.73	6.30 \pm 0.36	6.46 \pm 0.22
Ash (% FM)	nd	2.20 \pm 0.04	2.28 \pm 0.05
Gross energy (kJ/g FM)	7.93	6.95 \pm 0.18	6.92 \pm 0.11

¹ FM = fresh matter; nd = not determined.

At the end of the experiment, fish fed with HIM had a lower content of UFA and a higher content of SFA compared to the initial fish and those fed with diet C. The relative increase in SFA of HIM fed fish, as compared to the initial fish, is caused by increasing levels of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acid contents (Table 7). In conjunction with a decrease of the content of linoleic acid (C18:2 n-6) also a reduction of the UFA:SFA ratio of HIM fed fish was observed over the experiment while the C fed fish showed an increase of UFA:SFA ratio (Table 7). The UFA:SFA ratio in C fed fish increased, as compared to the initial fish, mainly due to an increase in oleic acid and decreases of levels of myristic and palmitic acid. Levels of linoleic acid decreased in both treatments, though less pronounced in fish receiving diet C. Compared to initial fish, both treatments exhibited reductions in the levels of ARA, EPA and DHA, whereas those in the two latter FAs were more pronounced in HIM fed fish (Table 7).

Table 7. Fatty acids of the initial fish and the fish fed with the two different experimental diets (control; C, and *Hermetia illucens* meal; HIM (g/100 g fatty acid methyl esters).¹

Fatty acid	Trivial name	Initial fish	C fed fish	HIM fed fish
C12:0	lauric acid	<0.1	<0.1	4.68
C14:0	myristic acid	3.39	2.72	4.98
C15:0	pentadecyclic acid	0.17	0.23	0.21
C16:0	palmitic acid	18.9	17.2	20.0
C16:1 n-7	palmitoleic acid	5.17	5.64	5.65
C18:0	stearic acid	1.70	1.75	1.81
C18:1 n-9	oleic acid	39.6	47.4	42.2
C18:2 n-6	linoleic acid	17.0	13.4	11.3
C18:3 n-3	linolenic acid	0.77	0.60	0.46
C18:4 n-3	stearidonic acid	4.09	4.10	3.53
C20:0	arachidic acid	0.29	0.33	0.20
C20:1 n-9	eicosanoic acid	0.86	0.73	0.68
C20:3 n-6	dihomo-γ-linolenic acid	0.58	0.71	0.34
C20:4 n-6	arachidonic acid	1.84	1.25	1.20
C20:5 n-3	eicosapentaenoic acid	0.66	0.43	0.22
C22:0	behenic acid	<0.1	<0.1	<0.1
C22:1 n-9	erucic acid	2.37	1.64	1.43
C22:5 n-3	sardine acid	0.32	0.21	0.14
C22:6 n-3	docosahexaenoic acid	1.82	1.11	0.79
C24:0	lignoceric acid	<0.1	<0.1	<0.1
Σ SFA	Σ SFA	24.5	22.3	31.8
Σ UFA	Σ UFA	75.5	77.7	68.2
UFA:SFA		3.09	3.50	2.14

¹ SFA = saturated fatty acids; UFA = unsaturated fatty acids.

The panellists in the organoleptic trial did not detect any differences in any of the evaluated traits of odour, texture or taste between fish originating from different feeding regimes. The sole difference perceived was a slightly darker colour of filets ($P=0.048$) when fish were fed with HIM (Figure 1).

4. Discussion

Both diets were readily accepted by the fish throughout the entire experiment and all feed was taken up within 5 minutes after feeding. The overall weight gain, final weight and SGs after the seven week experiment did not differ between C and HIM fed fish (Table 5). Similarly the FCRs did not differ between both treatments, and, reaching values around 0.8, were well below the estimated global average FCR of 1.3 for trout in 2015 (Tacon and Metian, 2008). Previous studies incorporating HIM in fish diets reported partly conflicting results compared to our study. Rainbow trout fed with 15% HIM in the diet were reported to show no differences in growth performance and feed conversion but had a reduced lipid content. In the same study, inclusion of 30%, however, resulted in significantly reduced growth, feed conversion and lipid content (St.-Hilaire *et al.*, 2007). Different results were obtained in a study of Sealey *et al.* (2011) who found that 'nutritionally enriched' (due to feeding of fish offal to the insect larvae) HIM in levels up to 36% in rainbow trout diets did not result in statistically reduced growth. In the same study, however, non-'enriched' HIM, resulted in significantly negative effects even at 16% inclusion (Sealey *et al.*, 2011).

The growth during our experiment was comparable to that reported by Sealey *et al.* (2011) who observed 93% weight gain of fish fed with a diet containing 32% non-enriched HIM over eight weeks. Though growth rates in this range appear very low compared to those reported by St.-Hilaire *et al.* (2007) (530-621% relative weight gain over a 9-week period). The most important difference between those studies and our study was the feeding regime which was restricted in our study while Sealey *et al.* (2011) and St.-Hilaire *et al.* (2007) fed 2 and 3 times per day, respectively, until apparent satiation. Our feeding levels were chosen to be comparatively low (Table 2) on purpose in order to have good feed conversion ratios rather than to optimise growth response. This is in accordance with organic regulations of Bio Suisse for Switzerland (Bio Suisse, 2015) as the total production period for organic trout must be a minimum of 18 months resulting in slow growth but high feed utilisation efficiency. Besides that, the temperatures reported by St.-Hilaire *et al.* (2007) and Sealey *et al.* (2011) were 14.3 and 14.5 °C, respectively, while the average temperature in our study was only 10.0 °C. Furthermore the starting weights of the fish differed significantly between the studies (~25 g in St.-Hilaire *et al.*, 2007, 145 g in Sealey *et al.*, 2011 and 66.5 g, this study). The last but not least important difference

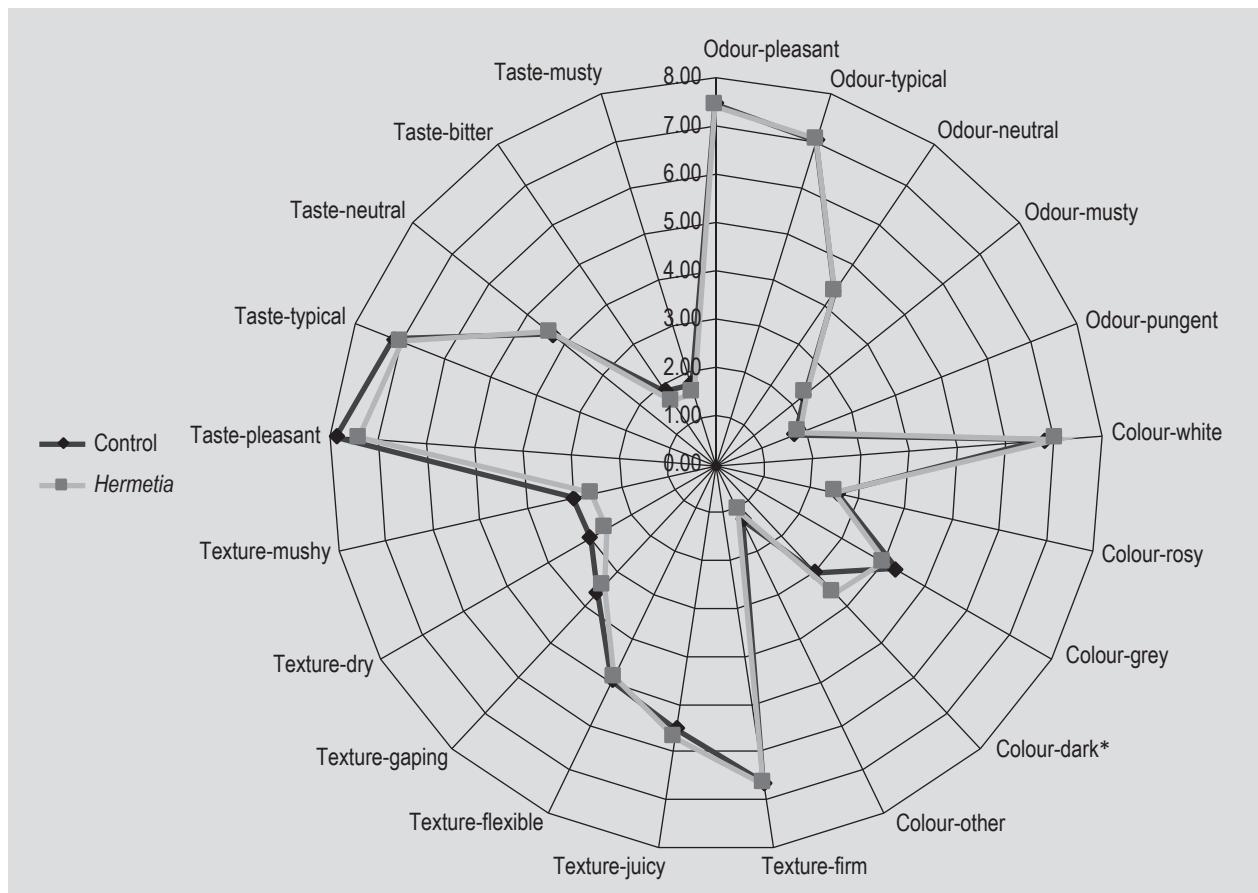


Figure 1. Results of the double-blinded organoleptic test. The higher the value, the more the panellists agreed with that characteristic.
* = significant difference, Student's t-test, $P=0.048$; $n=15$.

between the studies was the manufacturing of the feeds, which in our case was extrusion-cooked while compression-pelleting was implemented in the studies of St.-Hilaire *et al.* (2007) and Sealey *et al.* (2011). Extrusion generally increases the digestibility and absorption of macro- and micro-nutrients (Sørensen, 2012), thus, likely resulting in improved feed utilisation which may in turn diminish differences between C and HIM fed fish that have been observed elsewhere.

In Atlantic salmon (*S. salar*), Lock *et al.* (2015) reported growth rates between 44–132% for fish fed different levels of two different HIM. They also found that the FCR of fish fed one type of HIM decreased with increasing HIM content, while a second type of HIM had significantly negative effects on growth and FCR only at 100% of fish meal replacement (Lock *et al.*, 2015). Insect meal inclusion had no impact on FCR in our study but we observed a generally lower FCR which can partly be explained by the restricted feeding regime and the extrusion processing compared to St.-Hilaire *et al.* (2007), Sealey *et al.* (2011) and Lock *et al.* (2015) who reported higher FCRs of 1.0 to 1.5. However, due to the very low stocking density, a certain impact of natural food items (e.g. *Gammarus* spp.) cannot be excluded, as

no stomach content analysis was conducted and the farm is certified organic. In our study, inclusion of HIM lead to a significantly reduced protein utilisation while the lipid utilisation was significantly improved. This pattern may have several reasons, among which the most plausible appears to be linked to protein and lipid digestibility, which has not been evaluated in our study. The apparent digestibility of HIM crude protein and crude lipid were 63.1 and 78.0%, respectively, in turbot (Kroeckel *et al.*, 2012). That was considerably lower than that typically observed for fishmeal (86–97%) (NRC, 2011). Contrary to the study with turbot, digestion of FA and AA in HIM containing diets was very high in Atlantic salmon (FA between 82.5 and 100% and AA similar to control diets, i.e. 85–95%) (Lock *et al.*, 2015). The digestive system of rainbow trout is more closely related to that of Atlantic salmon, both being salmonids. Therefore it may be assumed that the digestibility of HIM in our study is closer to the values obtained by Lock *et al.* (2015) than to the value obtained by Kroeckel *et al.* (2012).

Lock *et al.* (2015) noted that especially C12:0, which is one of the major FA in HIM (Ushakova *et al.*, 2016), remained highly digestible over all HIM inclusion levels. That could, at least partly, be responsible for the significantly improved

lipid utilisation in our experiment which may also be a sign of improved energy saving or excess dietary digestible energy but not necessarily for improved lipid utilisation. The significantly improved protein utilisation in fish fed diet C is possibly related to an improved digestible protein to digestible energy (DP:DE) ratio compared to fish fed diet HIM. According to NRC (2011) the optimal DP:DE ratio for rainbow trout is 21.6 g/MJ. Both diets in our study had unfavourably high DP:DE ratios (Table 1). It is generally acknowledged that increasing dietary digestible energy at constant protein content, to a certain point, leads to an improved protein utilisation, the so-called protein sparing effect (Cho and Bureau, 2001; De Silva *et al.*, 1990; Einen and Roem, 1997; Steffens, 1981). In accordance with the Swiss organic regulation, allowing only up to 15% of lipids in the diet (Bio Suisse, 2015), both our diets contained very low lipid contents for a trout diet. That leads to an increased utilisation of proteins for energetic demands and less protein for somatic growth. However, the lipid level was 25 g/kg higher in diet C compared to diet HIM which results in a lower DP:DE ratio supporting the observed protein sparing effect in fish fed diet C and possibly leading to increased lipid or energy utilisation in diet HIM due to a very high DP:DE ratio. The protein content difference, on the other side, might not be as great as it seems because of the nitrogen content in chitin. The exoskeleton of insects, also of *H. illucens* larvae, contains chitin with around 69 g/kg nitrogen on a molecular weight basis. Ignoring the chitin would apparently result in overestimating CP contents. In our study, diet HIM is higher in CP, which is partly explained by the high inclusion level of the HIM. In a study comparing different analytical methods to determine the chitin content in crayfish meals, Lovell *et al.* (1968) reported that the CF content is a good estimator for chitin. A chitin content in *H. illucens* larvae of 21 g/kg fresh matter corresponding to 33.9 g/kg DM was estimated by Finke (2013), which nearly matches the difference in CF between diet C (13 g/kg DM) and diet HIM (44 g/kg DM) in our study. Assuming that the difference in CF between diets C and HIM is related to chitin, the corrected protein content of diet HIM would be 477 g/kg DM instead of 491 g/kg DM. Accurate measurement of the chitin-content is necessary for future investigations as chitin may not only hamper precise estimates of protein contents but possibly triggers advantageous or disadvantageous side-effects like immunostimulation (Sakai, 1999) or, potentially acting as a non-starch polysaccharide, anti-nutritional effects (Sinha *et al.*, 2011).

The protein utilisation in our study was exceptionally high (PPV around 50% for HIM and around 60% for C fed fish) compared to the PPV of other studies utilising HIM meal (Atlantic salmon: around 40%, Lock *et al.*, 2015; turbot: between 27 and 37%, Kroekel *et al.*, 2012). Given the unfavourably high DP:DE ratios, the high protein utilisation may point towards a very high protein

digestibility and thus high protein quality or is, similarly to the low FCRs, supporting a higher proportion of natural food than assumed. However, without further information on digestibility and true protein content, our data suggest that the protein of diet C was utilised significantly better compared to that of diet HIM due to a higher dietary energy to protein ratio content of the former, while opposing patterns were found for lipids.

The control diet contained comparatively higher levels of UFA than the HIM diet. Although the FA profiles of the fish could only be analysed in a single sample, they are qualitatively discussed here because they show a potential constraint of feeding HIM to fish. Cold- and freshwater fish including salmonids, can cover requirements of essential fatty acids (EFA) by biosynthesising physiologically important PUFA through chain-elongation of linolenic acid as a precursor (Tocher, 2010). The quantitative requirement for linolenic acid was described for rainbow trout as 0.7-1.0% of dietary dry matter by Castell *et al.* (1972). However, for several fish species, including salmonids, n-3 PUFA (EPA and DHA) are more effective in meeting the EFA requirements than linolenic acid alone (Tocher, 2010). In the present study, none of the diets appeared to be deficient in EFA although diet HIM contained lower amounts of linolenic acid, EPA and DHA. Thus, our feeding trial suggests a reduction of all UFA, in particular of the n-3 fraction, when HIM is incorporated into fish diets in considerable proportions. This may point to a negative side-effect of HIM, as the high supply of n-3 FA from marine animals is an important issue in the nutritional importance of fish (Astorg *et al.*, 2004; Koussoroplis *et al.*, 2008)

The estimated intraperitoneal fat content as well as HSI and VSI were not influenced by the different dietary fatty acid profiles. Lock *et al.* (2015) argue on the contrary that in Atlantic salmon HSI and VSI were higher in HIM fed fish because of increased hepatic and intraperitoneal fat content which they relate to the comparatively high content of C12:0 in HIMs and the better utilisation of this short chain FA compared to others.

Aside from the nutrient content and nutritional value of a new feed ingredient set to replace fishmeal, also its effects on product quality, i.e. fish fillets, are of importance. Fat is largely responsible for taste in food and, therefore, a better knowledge on the organoleptic properties that different FA profiles may cause is critical. The degustation conducted in our trial revealed solely a significantly darker filet colour of fish fed with HIM compared to control fed fish, while the test panel perceived no significant differences for any of the other selected parameters of taste and odour. This is in accordance with other studies in which HIM fed rainbow trout (Sealey *et al.*, 2011) or Atlantic salmon (Lock *et al.*, 2015) have been tested for their organoleptic properties. This is particularly interesting since these studies reported

differences in FA profiles of the HIM fed fish compared to control fed fish that were similar to those detected here.

In conclusion, our on-farm experiment shows that a rainbow trout diet in which almost 50% of the fishmeal has been replaced by HIM performs similar compared to a commercial diet in terms of growth and feed conversion and does not negatively influence product quality. The protein utilisation, however, was decreased in the HIM containing diet and putative reasons deserve further research. In organic aqua feeds fishmeal levels are often, mainly because availability of other organic protein sources is limited, considerably higher (up to 50%) than in conventional feeds for salmonids (around 15%, Olsen and Hasan, 2012). Replacement of a substantial amount of that fishmeal component could reduce the fishmeal content in organic aqua feeds substantially but also improve sustainability of aquaculture feeds in general.

Despite occasionally divergent findings, the present study supports the feasibility of replacing fishmeal in practical aquaculture feeds by insect meal, derived from black soldier fly larvae. Fish of both treatments were growing equally well and showed no differences in feed conversion, chemical composition, morphometric parameters and organoleptic properties. Also, further research is needed in order to enhance the knowledge base on which factors are most important, such as feeding and procession history of the insects, inclusion levels of insect meal, feed production technologies and developmental stage of the fish, just to mention a few.

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