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INSECTS IN THE FEED OF RAINBOW TROUT, *ONCORHYNCHUS MYKISS* (ACTINOPTERYGII, SALMONIDAE): EFFECT ON GROWTH, FATTY ACID COMPOSITION, AND SENSORY ATTRIBUTES

Jan TUREK*[1], Sabine SAMPELS[1, 2], Sarvenaz KHALILI TILAMI[1], Daniel ČERVENÝ[1], Jitka KOLÁŘOVÁ[1], Tomáš RANDÁK[1], Jan MRÁZ[1], Jan MÁSÍLKO[1], Christoph STEINBACH[1], Viktoria BURKINA[1], Pavel KOZÁK[1], and Vladimír ŽLÁBEK[1]

[1] *Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodnany, Czech Republic*

[2] *Swedish University of Agricultural Sciences, Department of Molecular Sciences, Uppsala, Sweden*

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Background. An ongoing quest for alternative feed sources in global aquaculture includes insect breeding for feed and food production. The aim of this study was to evaluate the effect of partial to full replacement of commercial diets with live insects on growth and health parameters of rainbow trout, as well as on sensory and texture attributes and fatty acid composition of fish muscle.

Material and methods. Five isocaloric diets containing commercial pellets and live insects were evaluated in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), in a 60-day feeding trial. Control Group (K) was fed commercial pellets only. In other groups, 25% gross energy of pellets was replaced by live adult house cricket, *Acheta domestica* (Group C), live superworm, *Zophobas morio* larvae (Group L), or a combination of 12.5% crude energy of each (group LC). The insect-only group (I) was fed live cricket and superworm only (50% by 50% crude energy).

Results. No significant differences were found in growth, survival, feed conversion ratio (dry basis), or energy utilization between groups. The protein efficiency ratio was highest in Group K and decreased with increasing cricket proportion. Insect inclusion was associated with lower content of nutritionally valuable *n*-3 fatty acid in fish muscle. Subjective sensory evaluation of cooked fillets revealed significantly less acceptable taste, aroma, and aftertaste in Group I than for Groups K, L, and LC. Some differences were found in the whiteness and redness of fillets between groups. The control group had significantly lower hardness compared to those receiving insect diets. No gross morphological or histopathological anomalies of viscera in any group and no significant differences in 7-ethoxyresorufin-*O*-deethylase activity were observed.

Conclusions. Live insects replaced the commercial diet of the equivalent caloric level without negative effects on the growth or health of rainbow trout. The lower content of *n*-3 fatty acids and differences in color and texture of fillets from fish fed insects may influence acceptability to consumers. The high cost of insects compared to commercial feed currently limits their widespread use in trout production.

Keywords: rainbow trout, fillet quality, insect feed, growth performance, fatty acids

INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), is widespread in temperate inland waters and represents a large share of global salmonid production. Aquaculture is a rapidly growing industry with production increasing at an average annual rate of 5.8 percentage points reaching 73.8 million t in 2014. Salmon and trout represented about 17% of the total value of internationally traded fish products in 2014 (Anonymous 2016). The increasing production has led to an increased demand for quality feed, the biggest component of the production costs. Despite their declining proportion in aquafeeds, fish meal (FM) and fish oil (FO) remain major

* *Correspondence: Ing. Jan Turek PhD, Fakulta rybářství a ochrany vod, Jihočeská univerzita v Českých Budějovicích, Jihočeské výzkumné centrum akvakultury a biodiverzity hydrocenóz, Zátiší 728/II, 389 25, Vodňany, Czech Republic, phone: +420 387 774 755, e-mail: (JT) *turek@frov.jcu.cz*, (SS) *sabine.sampels@slu.se*, (SKT) *sarvenaz.khalili84@gmail.com*, (DČ) *cerveny@frov.jcu.cz*, (JK) *kolarova@frov.jcu.cz*, (TR) *trandak@frov.jcu.cz*, (JM) *jmraz@frov.jcu.cz*, (JMá) *masilko@frov.jcu.cz*, (CS) *steinbach@frov.jcu.cz*, (VB) *vburkina@frov.jcu.cz*, (PK) *kozak@frov.jcu.cz*, (VŽ) *vzlabek@frov.jcu.cz*, ORCID: (JT) 0000-0003-1937-6932, (SS) 0000-0003-1695-5939, (SKT) 0000-0001-8521-3483, (DČ) 0000-0003-1491-309X, (JK) 0000-0001-5879-4497, (TR) 0000-0003-0462-266X, (JM) 0000-0003-3545-1269, (JMá) 0000-0003-0060-1044, (CS) 0000-0001-6450-3414, (VB) 0000-0003-3015-9306, (PK) 0000-0003-2764-4757, (VŽ) 0000-0002-8857-4353.

dietary components, especially for carnivorous finfish, including salmonids (Tacon and Metian 2008). Decreasing availability, rising prices, and the negative environmental impact of FM and FO use have intensified the search for alternative protein and lipid sources.

Insects at various life stages constitute the major part of the natural salmonid diet either throughout their life or in the juvenile stage and show potential for inclusion in formulated feeds. They have been evaluated for potential FM replacement in aquafeeds with varying results (Makkar et al. 2014, Henry et al. 2015). In temperate regions, large quantities of crickets, mealworms, locusts, and housefly maggots are commercially produced for pet food and fish bait (van Huis et al. 2013). Insects can be cultured on food manufacturing by-products, and their nutritional composition can be altered through diet (St Hilaire et al. 2007a, Oonincx et al. 2015), making them a sustainable, environmentally-sound feed source. Determining the effects of insects in the diet on fish performance and health, nutritional content, and attractiveness to consumers is a prerequisite to wider use of insects as feed for salmonids.

Inclusion of the black soldier fly, *Hermetia illucens* (BSF), larvae and mealworm, *Tenebrio molitor*, has been assessed in salmonid diets. Replacement of 25% to 50% of the FM with BSF meal in a rainbow trout diet showed no significant effects on weight gain and feed conversion ratio but resulted in lower levels of omega-3 fatty acids in fillets (St-Hilaire et al. 2007b, Stamer et al. 2014). Sealey et al. (2011) reported satisfactory growth of rainbow trout fed a diet replacing 50% of FM with BSF reared on manure enriched with trout offal, while a diet containing BSF reared on manure only was associated with significantly slower growth compared to the commercial diet. No sensory differences between fish fed the two BSF and control diets were found. Lock et al. (2014) found 100% FM replacement by BSF meal in the diet of Atlantic salmon to have no detrimental effects on growth, histology, or sensory aspects, with the caveat that the method of insect meal preparation had a considerable impact on its usability. Replacement of 25% and 50% of FM with mealworm larva meal in a rainbow trout diet did not significantly affect growth and reduced the hepatosomatic index compared to fish fed a control diet (Gasco et al. 2014). The majority of studies focus on using processed insects as feedstuffs. Due to the approval of some insect species as "novel foods" (Anonymous 2015), the development of insect farming within the EU can be expected. This can increase the availability of live insects, which could be used as feed, especially in small local farms. Therefore, it is important to examine the possibility of using the whole insect as feed.

The house cricket, *Acheta domestica* (Gryllidae), and superworm, *Zophobas morio* (Tenebrionidae), are commonly-produced insects that can be successfully cultured on organic by-products (Fuah et al. 2015, Oonincx et al. 2015, van Broekhoven et al. 2015). The biomass produced by commercial insect cultures is permitted to be used as a valuable source of protein and fat. However, an insect-based diet may contain a number of potent bioactive compounds including antinutritional (Murefu et al. 2019) or potentially toxic compounds (Henry et al. 2015). Therefore, the replacement of a natural fish food diet might affect the activity of metabolizing enzymes in fish (Wagner et al. 2013). Cytochrome P450 (CYP) enzymes are a group of hem-containing enzymes playing a key role in the metabolism of many xenobiotics including food components (Arinç et al. 2015). Replacement of natural food of fish diet might affect the activity of metabolizing enzymes in fish. CYP1A is the most studied isoform in fish due to its important role in the metabolism of xenobiotic compounds. The measurement catalytic activity of CYP1A may provide information on the xenobiotic nature of selected insects and introduced to a fish diet.

The aim of this study was to evaluate the effect of partial to full replacement of commercial FM-based diets with live insects on growth and health parameters of rainbow trout, as well as on sensory and texture attributes and fatty acid composition in a fish muscle that can influence its nutritional value and palatability.

MATERIALS AND METHODS

Experimental fish and rearing conditions. One-hundred-forty juvenile rainbow trout, *Oncorhynchus mykiss*, weighed 264.3 ± 6.6 g (mean weight ± standard deviation) were reared in a recirculation system at the Faculty of Fisheries and Protection of Waters in Vodňany, Czech Republic. Fish were fed commercial pellets only (EFICO Enviro, 4.5 mm, Biomar) prior to the experiment. Before the experiment, 10 randomly chosen fish were measured, weighed, sacrificed, and filleted for baseline analysis of lipid content and composition. The remaining fish were separated into groups of 10, bulk-weighed, and stocked into thirteen 400-L aerated glass aquaria. The mean initial stock weight per aquarium was 2643 ± 66 g. Aquaria were filled with tap water filtered through an active carbon filter. Each aquarium was connected to an individual external filter (Eheim professionel 4+, EHEIM GmbH, Germany). Fish excrements and other sediment were drained daily at approximately 1200 h, which coincided with the replacement of ~200 L of water. Water temperature was 14.3 ± 1.2°C, oxygen content 10.1 ± 1 mg · L–1, and pH 7.2 ± 0.7. The duration of the experiment was 60 days.

Diets. Five isocaloric diets were formulated using commercial pellets and live insects. Prior to the experiment, nutrient composition of the feeds was analyzed (Table 1) by an accredited laboratory (Státní veterinární ústav Praha, Testing laboratory No. 1176) and analyzed for FA content at the Faculty of Fisheries and Protection of Waters in Vodňany, Czech Republic (Table 2). Four experimental diets were tested with three replicates. Control group (K) was fed commercial pellets (EFICO Enviro 4.5 mm, Biomar) only. For other groups, 25% of the crude energy of pellets was replaced with live adult house crickets (C), live superworm larvae (L), or a combination of 12.5% crude:gross energy each of the insect species (LC). The insect-only group (I) was fed live crickets and superworms only (50:50 crude energy). For economic reasons, this group was not replicated, therefore, they were not included in the statistical evaluation for survival, weight gain, and feed efficiency.

Table 1

The approximate composition of feed sources, used in the presently reported experiment of feeding rainbow trout, *Oncorhynchus mykiss*, with insects

Feed	Composition (as-is basis)					
	Crude protein [%]	Crude fat [%]	Carbohydrates [%]	Ash [%]	Moisture [%]	Gross energy [MJ · kg^{-1}]
Pellets	42.9	30.1	15.2	5.8	4.9	24.4
Crickets	21.7	5.6	4.1	1.9	68.8	6.1
Superworm	19.0	18.3	4.7	1.8	56.2	10.1

Crickets = *Acheta domestica*, Superworm = *Zophobas morio* larvae; the analysis was carried out by the Testing laboratory No. 1176, State Veterinary Institute Prague, Czech Republic.

Table 2

Fatty acid composition of pellets, house crickets (*Acheta domestica*), and superworm (*Zophobas morio*) larvae used in the feeding experiment of rainbow trout, *Oncorhynchus mykiss*

Fatty acid	Feed		
	Pellets	House crickets	Superworm larvae
Fat content	27.26 ± 0.01	6.68 ± 0.70	18.21 ± 2.27
14:0	1.96 ± 0.00	0.86 ± 0.05	1.06 ± 0.06
16:0	9.92 ± 0.06	24.98 ± 0.63	32.36 ± 1.76
16:1	2.22 ± 0.00	1.17 ± 0.06	0.80 ± 0.15
18:0	3.35 ± 0.01	7.50 ± 0.33	7.53 ± 0.73
18:1 *n*-9	44.63 ± 0.05	21.51 ± 0.19	33.89 ± 3.63
18:1 *n*-7	3.12 ± 0.01	0.70 ± 0.01	0.31 ± 0.04
18:2 *n*-6	15.20 ± 0.07	39.59 ± 0.53	22.53 ± 1.95
18:3 *n*-3	6.76 ± 0.03	1.31 ± 0.02	0.92 ± 0.13
20:0	0.40 ± 0.01	0.38 ± 0.00	0.17 ± 0.02
20:1 *n*-9	2.51 ± 0.01	0.44 ± 0.03	0.16 ± 0.04
20:2 *n*-6	0.49 ± 0.01	0.08 ± 0.00	0.07 ± 0.01
20:4 *n*-6	0.21 ± 0.00	0.27 ± 0.05	0.02 ± 0.03
20:4 *n*-3	1.84 ± 0.02	0.04 ± 0.00	0.03 ± 0.01
22:0	0.25 ± 0.01	0.11 ± 0.02	0.05 ± 0.03
20:5 *n*-3	2.68 ± 0.00	0.76 ± 0.01	0.05 ± 0.07
22:5 *n*-3	0.60 ± 0.02	0.03 ± 0.01	0.23 ± 0.03
22:6 *n*-3	2.88 ± 0.01	0.21 ± 0.02	0.05 ± 0.08
∑SFA	15.64 ± 0.06	33.85 ± 0.33	41.18 ± 1.98
∑MUFA	52.48 ± 0.08	23.89 ± 0.28	35.17 ± 3.78
∑PUFA	30.47 ± 0.04	42.26 ± 0.55	23.65 ± 1.92
∑*n*-3	15.70 ± 0.06	2.29 ± 0.03	1.03 ± 0.13
∑*n*-6	14.77 ± 0.03	39.94 ± 0.58	22.62 ± 1.93
∑*n*-:∑*n*-3	1.06 ± 0.01	17.44 ± 0.43	22.39 ± 3.71

Values are mean ± standard deviation (n = 3); data are expressed as percent of total fatty acids, fat content as percent weight on an as-is basis; SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

The insects were purchased fresh from a local producer (Vladimír Šefl, Bušanovice, Czech Republic) twice per week. The crickets were held at 6°C to ensure that they remained inactive, and superworms were kept in barley bran at 22°C according to producer recommendations.

After three days of acclimatization, feeding was initiated in all tanks. Pellets were fed at 1.5% of stock weight daily and at 1% after monitoring on day 26. For Groups C, L, and LC, the pellets were decreased to 75% and supplemented with the appropriate proportion of insects. In Group I, the pelleted feed was replaced with insects. The required quantity of insects was calculated based on the weight necessary to provide energy content similar to pellets:

1 g pellets = 4 g house crickets = 2.4 g superworms.

The feed adaptation phase was carried out for five days, during which Group K was fed 25% of its allocated daily ration, and other groups were fed the insect portion only. From day 8, each group received the full feed ration. Fish were fed manually four times per day, and all feed provided was consumed. Expected weight gain was calculated with respect to a feed conversion ratio (FCR) of 1:1 (based on full pellet portion weight). Fish were not fed on the monitoring and final sampling days (26, 48, and 60) or on a preceding day.

Growth monitoring, including individual weight and biometric measurements, was carried out in all aquaria on days 26 and 48. At the conclusion of the trial, all fish were individually weighed. For each aquarium, feed conversion ratio (FCR), protein efficiency ratio (PER), and gross energy (GE) utilization was calculated

$$\mathrm{FCR} = W_F \cdot W_G^{-1}$$

$$\mathrm{PER} = W_G \cdot P_i^{-1}$$

$$\text{GE utilization} = W_G \cdot \mathrm{GE}_i^{-1}$$

where W_F is feed weight (dry) [g], W_G is weight gained [g], P_i is protein intake [g] and GE_i is gross energy intake [MJ].

Ten fish per group were sacrificed, bled out, and hand-filleted. Individual weight [g] standard length [mm], and total length [mm] were determined. Condition factor (CF) was calculated for each fish

$$\mathrm{CF} = 100 \cdot (W \cdot \mathrm{TL}^3)$$

where W is total weight [g] and TL is total length [cm].

Viscera and liver were weighed for determination of viscerosomatic (VSI) and hepatosomatic (HSI) indices

$$\mathrm{VSI} = (W_v \cdot W^{-1}) \cdot 100$$

$$\mathrm{HSI} = (W_l \cdot W^{-1}) \cdot 100$$

where W_v is weight of viscera [g], W_l is weight of liver [g] and W is total weight [g]. Samples of raw, skinned fillets were used for the analysis of fatty acid (FA) composition and for sensory evaluation, while liver tissue was used for further preparation of microsomal fractions and measuring of CYP mediated reaction.

Fatty acid and lipid content analysis. Lipid extraction was performed in duplicate according to Hara and Radin (1978) and lipid content was quantified gravimetrically. For FA analyses, methylation of total lipids was conducted according to Appelqvist (1968). FA composition was analyzed by gas chromatography (Trace Ultra FID; Thermo Scientific, Milan, Italy) using a BPX-70 50 m fused silica capillary column (id 0.22 mm, 0.25 µm film thickness, SGE, USA). The peaks were identified by comparing sample retention times to those of the standard mixture GLC-68-A (Nu-Chek Prep, Elysian, MN, USA). For statistical comparison, we selected FA with a mean percentage of more than 0.2% of total FA in the least one group of fish.

Sensory analyses. The sensory quality of fillets was evaluated with respect to attributes such as aroma, taste, aftertaste, and consistency (Martinsdóttir et al. 2009). One-hundred 30-g samples (five groups, 10 fish from each, in duplicate) were prepared for a panel of 10 members of a trained jury from the Faculty of Fisheries and Protections of Waters. Tasting samples were composed of six small pieces of flesh, each from a different fish of the appropriate group (ISO 6658, 2005). Samples were taken from corresponding areas of the fish body, stored on ice for 2 h, and cooked separately in code-labeled 0.15 L glass jars for 15 min at 150°C in an electric oven. To conform to ISO 6658 (2005) and ISO 8589 (2007) criteria, no salt, oil, or spices were added. Panelists were separated from one another in individual cubicles (ISO 8589, 2007). Each panelist was provided with still water, distilled spirits, and bread to cleanse the palate. Samples were rated on a hedonic consumer scale (Martinsdóttir et al. 2009) modified according to Kříž et al. (2007). Panelists were asked to evaluate the intensity of aroma, taste, aftertaste, and consistency and to indicate a rating by assigning a point on a 100 mm unstructured abscissa (0 mm = very good quality; 100 mm = unacceptable).

Instrumental analysis of color and texture. Flesh color was assessed at three locations above the lateral line (anterior, middle, and caudal) of each fillet (n = 7 per group) using a color spectrophotometer CM-600d (Konica Minolta, Japan). Colorimetric data were represented according to the Commission Internationale de l'Eclairage (Anonymous 2004) as L^* = whiteness, a^* = the red-green axis, and b^* = the yellow-blue axis were measured directly on the fillet with each spot evaluated in duplicate. Measurements were performed within 1 h post-mortem.

Samples (n = 10 per group) for texture analysis were taken from the dorsal area of fillets between the end of the dorsal fin and the beginning of the anal fin. Hardness, defined as the maximum force detected during initial compression, was measured using a TPA-meter (TA. XTPlus, Stable Micro Systems, Godalming, Surrey, UK). A 10 mm diameter cylindrical probe (sms p/10*) was set at pretest speed of 5 mm · s^{-1} and a test speed of 2 mm · s^{-1} until the fillet was compressed to 50% of its original thickness.

Histology. During dissection, gross examination of intestines, liver, gills, and heart was performed. For histological examination, samples of liver, heart, stomach, and intestine (mid-section) were fixed in 10% natural buffered formalin, paraffin-embedded, and routinely processed as described by Bancroft and Gamble (2002). Sections (4 µm) were stained with Mayer's hematoxylin-eosin. Pathological alterations were scored from mild (1) to severe (6) and were determined as defined in Bernet et al. (1999), Steinbach et al. (2016), and Saraiva et al. (2016). Slides were examined at a magnification of 10–40× using an Olympus SZ9 microscope.

Ethoxyresorufin-*O*-deethylase activity (EROD). Resorufin, 7-ethoxyresorufin, and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma-Aldrich (Steinheim, Germany).

Microsomal fraction preparation and protein analysis: Fish hepatic microsomes were obtained by differential centrifugation. Briefly, liver (~1 g) was homogenized in three volumes of Tris-sucrose buffer (10 mM Tris–HCl, 250 mM sucrose, and 0.1 mM EDTA, pH 7.4) with subsequent centrifugation (Beckman Coulter Optima™ L-90 K) at 30 000 rpm for 15 min at 4 °C. The supernatant was further centrifuged at 100 000 g rpm for 60 min at

* *https://www.stablemicrosystems.com/Probes.html*.

4°C. As a final step, the microsomal fraction was diluted in glycerol buffer (0.1 mM EDTA, 20% glycerol, 50 mM Tris and 10 mM potassium phosphate, pH 7.4) and homogenized (UltraTurrax; Ika, Germany). All steps were carried out on ice. Microsomal fractions were immediately frozen and stored at –80°C for 7-ethoxyresorufin-*O*-deethylase (EROD) analysis. The protein levels were estimated spectrophotometrically as described by Smith et al. (1985) using bovine serum albumin as standard. The microsomes were diluted to obtain a protein concentration of 10 mg · mL–1.

The catalytic activity of CYP1A was measured as the rate of formation of resorufin from 7-ethoxyresorufin (Kennedy and Jones 1994). The incubation mixtures contained 0.5 mg microsomal protein in an incubation medium of 50 mM potassium phosphate buffer (pH 7.4) with 1.0 mM NADPH and 2 μM of 7-ethoxyresorufin. The fluorescence detector (Infinite 200 – Photometer TECAN) was used for the detection of resorufin (excitation:emission 544:590 nm). Enzyme activity was expressed as pmol resorufin · mg–1 protein · min–1 (detection limit was 1 pmol · min–1).

Statistical analysis. Sensory attributes, color and texture analyses, biometric data, and FA profile were subjected to one-way ANOVA. The differences among means were tested by post-hoc Tukey's honest significant difference test. Differences among means for instrument-based color and texture analyses were assessed by Fisher's LSD test. Data on the percentage of FA were arcsin transformed. Homogeneity of variance was tested using the Cochran-Hartley-Bartlett test. The survival of fish was compared with the Pearson and maximum likelihood $\chi 2$ test. All analysis was done using Statistica 12.0 (StatSoft CR, Prague, Czech Republic). Differences were considered significant when $P < 0.05$.

RESULTS

Growth and biometric parameters. After the feed adaptation phase, all fish in experimental groups consumed insects actively and preferentially consumed insects over pellets. No differences were found in mean fish weight between groups at monitoring days or at the end of the experiment (Fig. 1). The feeding regime was not associated with survival, total weight gain, FCR, or utilization of feed gross energy. Protein efficiency ratio (PER) in the co-fed groups was significantly lower than in the control group, with the exception of Group L (Table 3). No dietary effects were found in the mean final condition factor (CF) or VSI in fish sampled for FA analysis (n = 10 per group). Fish from Group I displayed significantly higher (P = 0.022) HSI values than did Group C. Mean final CF in Group I was significantly (P = 0.009) higher than in fish sampled at beginning of the trial (Table 4).

Fat content and fatty acid composition. The values of lipid content and FA composition are given in Table 5. Total fat content did not differ significantly between groups but was slightly higher in Group K compared to the experimental groups. Significant between-group differences were found in all selected FAs as well as in relative content of saturated FA (SFA), mono-unsaturated FA (MUFA), and poly-unsaturated FA (PUFA) (Table 5).

Palmitic acid (16:0) was the predominant SFA, and stearic acid (18:0) constituted >1% of total lipid in all groups. Their relative content was significantly lower in Group K compared to the other groups, with the highest values found in Group I. Other SFAs made up less than 1% of total FA. The quantity of SFA was significantly higher in Group I than in C, L, and LC groups and was lowest in Group K.

The level of total MUFA observed in Group K was significantly higher than in other groups, intermediate levels were seen in C, L, and LC, and lowest in Group I. A similar pattern was observed in levels of oleic acid (18:1 *n*-9) (the predominant MUFA in all groups), vaccenic acid (18:1 *n*-7), and erucic acid (22:1 *n*-11).

Linoleic acid (LA, 18:2 *n*-6) was the predominant PUFA in all groups and showed the highest relative level in Group I. Significant differences between groups were found in relative levels of docosahexaenoic acid (DHA, 22:6 *n*-3), alpha-linoleic acid (ALA, 18:3 *n*-3), eicosapentaenoic acid (EPA, 20:5, *n*-3), eicosadienoic acid (20:2, *n*-6), and other PUFAs, each of which represented <1% of total FA (Table 5). In general *n*-3 FA showed lower proportions in the insect fed groups (C, L, LC, and I), while *n*-6FA were higher in those groups, reflecting the FA composition of the diet. No significant differences in relative PUFA proportion were found between Groups K, C, L, and LC or between Groups LC and I. Group I showed significantly lower $\sum n$-3 FA proportion than observed in all other groups. In contrast, $\sum n$-6 FA content was highest in Group I, intermediate in Groups C and LC, and lowest in K and L. The $\sum n$-6:$\sum$ n-3 ratio was significantly affected by diet, with the lowest value in Group K and the highest in Group I (Table 5).

Sensory analyses. The results of the sensory evaluation showed significantly lower acceptability of Group I fillets with respect to aroma and taste in comparison with fillets from Groups K, L, and LC (Fig. 2). Fillets from Group C did not show differences from the other groups in these

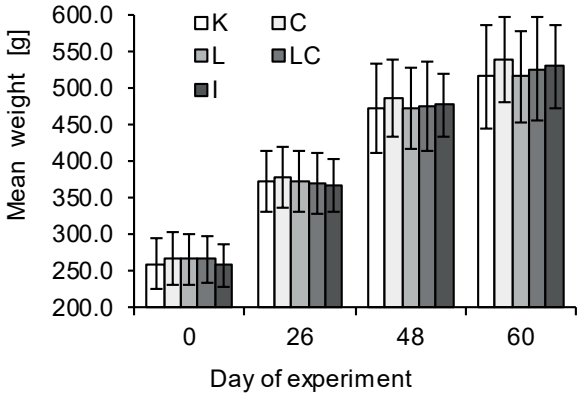

Fig. 1. Weight (mean ± standard deviation) of rainbow trout, *Oncorhynchus mykiss*, fed four different diets during a 60-day feeding trial; K = control, C = 25% live house crickets (*Acheta domestica*), L = 25% live superworm (*Zophobas morio*) larvae, LC 25% of an equal combination of insect species, I = 100% combination of insect species

Table 3

Survival, weight gain, and feed efficiency of rainbow trout, *Oncorhynchus mykiss*, groups fed different diets in a 60-day feeding trial

Dietary treatment	Survival [%]	Weight gain [g · tank^{-1}]	Feed intake [g · tank–1]			FCR	PER	GE utilization
			Pellets[1]	Crickets[1]	Superworms[1]			
K	100	2562 ± 69	2015 ± 37	—	—	0.75 ± 0.02	2.96 ± 0.06^{a}	52.1 ± 1.1
C	96.7	2676± 202	1476 ± 57	2179 ± 79	—	0.79 ± 0.03	2.42 ± 0.11^{c}	54.2 ± 2.3
L	90.0	2342 ± 83	1399 ± 55	—	1243± 46	0.80 ± 0.01	2.80 ± 0.05^{ab}	50.2 ± 0.9
LC	100	2602 ± 131	1491 ± 43	1097 ± 32	658 ± 19	0.79 ± 0.04	2.60 ± 0.14^{bc}	52.3 ± 2.8
I	100	2726	—	4023	2414	0.86	2.05	55.7

Values are mean ± standard deviation (n = 3); K = control, C = 25% live house crickets (*Acheta domestica*), L = 25% live superworm (*Zophobas morio*) larvae, LC 25% of an equal combination of insect species, I = 100% combination of insect species); value of I group (100% combination of insect species, n = 1) was not included in statistical analysis; 1weight of feed expressed on an as-is basis, FCR (feed conversion ratio) = weight of feed (dry basis) per weight gained [g], PER (protein efficiency ratio) = wet weight gain per protein intake [g], GE (gross energy) utilization = wet weight gain ([g] per GE intake [MJ]; Different superscripts letters indicate significant (P < 0.05) differences between groups at the end of the experiment according to ANOVA, post-hoc Tukey HSD test.

Table 4

The effect of four diets on selected vital parameters of rainbow trout, *Oncorhynchus mykiss*, in a 60-day feeding trial

Dietary treatment	Parameter					
	SL [mm]	TL [mm]	*W* [g]	CF	VSI [%]	HSI [%]
0	249.5 ± 12.6	279.5 ± 12.6	271.6 ± 41.2	1.74 ± 0.18	12.86 ± 0.75	1.20 ± 0.18
K	304.5 ± 9.6	339.0 ± 10.9	509.7 ± 76.1	1.79 ± 0.16	12.76 ± 1.05	1.32 ± 0.20^{a}
C	308.0 ± 12.3	344.0 ± 13.2	540.6 ± 66.2	1.84 ± 0.10	12.78 ± 0.70	1.14 ± 0.09^{a}
L	299.5 ± 15.6	335.0 ± 16.0	505.0 ± 82.5	1.87 ± 0.11	12.92 ± 1.26	1.21 ± 0.12^{a}
LC	307.5 ± 11.9	341.5 ± 12.7	534.4 ± 55.3	1.84 ± 0.11	12.75 ± 0.66	1.22 ± 0.13^{a}
I	300.6 ± 9.9	333.1 ± 8.0	530.7 ± 50.8	**1.95 ± 0.13**	13.96 ±2.11	1.35 ± 0.14^{b}

Values are mean ± standard deviation, n = 10 in each group; SL = standard length, TL = total length, W = weight, CF = condition factor, VSI = viscerosomatic index, HSI hepatosomatic index; 0 = day 0 (stocking), K = control, C = 25% live house crickets (*Acheta domestica*), L = 25% live superworm (*Zophobas morio*) larvae, LC 25% of an equal combination of insect species; I = 100% combination of insect species); different superscripts letters indicate significant (P < 0.05) differences between groups at the end of the experiment according to ANOVA, post-hoc Tukey HSD test; **bold type** indicates significant (P < 0.05) differences in CF, VSI, and HSI between day 0 and at end of experiment for each group according to ANOVA, post-hoc Tukey HSD test.

attributes. The presence of an aftertaste was significantly higher for Group I compared to other groups. No effect of diet was observed in the consistency scores.

Instrument-based color and texture analyses. The inclusion of insects in the feed formulation (Group LC and I) significantly increased L^* whiteness of fillets (P < 0.05). Redness a^* was only slightly influenced by the dietary regime, with only group LC exhibiting significantly lower redness value from Group C. There was no difference in yellowness b^* between groups (Fig. 3). Control Group K showed significantly lower hardness compared to the insect diets (P < 0.05) (Fig. 4).

Histology. There was no gross morphological alteration in the heart, liver, or intestine, but there was a large quantity of fat around the intestines in all groups. Hepatocytes were characterized by a moderate to a severe presence of vacuoles in all fish. There were no signs of pathology in the liver or histopathological aberrations in the heart, stomach, or intestine of any group.

Ethoxyresorufin-*O*-deethylase activity (EROD). No effect of diet treatment on EROD activity was detected. Slightly higher values were seen in Group L fish (5.30 ±1.46) compared to other groups (3.97 ± 1.26 for Group K; 4.04 ± 0.99 for C; 4.02 ± 1.12 for LC and 4.00 ± 0.95 for I.). All data are presented as mean ± SD, pmol · mg–1 protein · min–1 (n = 10).

DISCUSSION

Growth and biometric parameters. Results indicated that house cricket and superworm can be used as a partial or total isocaloric replacement of commercial diet for rainbow trout without negative effects on growth, survival, FCR, or gross energy utilization. The observed growth rate in Group I fish demonstrated that a combination of raw crickets and superworm larvae is nutritionally adequate for growth compared to a commercial feed of similar energy value. This is not surprising, as insects are an important component of the natural prey of salmonids (Groot 1996), including rainbow trout (Raleigh et al. 1984). Nevertheless, the majority of studies have reported total replacement of FM with insect meal to be unsuccessful, generally due to nutritional imbalances or deficiencies (Henry et al. 2015), for example in calcium (Makkar et al. 2014) and amino acids including histidine, lysine, and tryptophan (Sánchez-Muros et al. 2014). Therefore, a negative effect of nutritional imbalance in case of longer insect-based feeding than in our 60 days experiment could

Table 5

The effect of four diets on fatty acid composition of fish fillets in a 60-day feeding trial of rainbow trout, *Oncorhynchus mykiss*

Fatty acid	Stocking (Day 0)	Dietary treatment				
		K	C	L	LC	I
16:0	11.96 ± 0.51	11.79 ± 0.30^{a}	**14.40 ± 1.38**^{b}	**15.21 ± 2.48**^{b}	**14.43 ± 0.99**^{b}	**21.25 ± 0.78**^{c}
16:1	2.35 ± 0.18	2.39 ± 0.09^{a}	2.28 ± 0.17^{a}	2.20 ± 0.20^{ab}	**2.14 ± 0.19**^{b}	**2.01 ± 0.18**^{b}
18:0	3.07 ± 0.14	**2.87 ± 0.15**^{a}	**4.03 ± 0.53**^{b}	**3.80 ± 0.68**^{b}	**3.78 ± 0.35**^{b}	**6.42 ± 0.54**^{c}
18:1 *n*-9	44.06 ± 1.52	44.59 ± 0.52^{a}	**41.54 ± 1.85**^{b}	42.79 ± 1.49^{b}	**41.87 ± 1.10**^{b}	**37.09 ± 1.21**^{c}
18:1 *n*-7	3.19 ± 0.05	**3.29 ± 0.05**^{a}	**2.91 ± 0.16**^{b}	**2.73 ± 0.33**^{b}	**2.78 ± 0.13**^{b}	**1.65 ± 0.15**^{c}
18:2 *n*-6	13.87 ± 0.51	**14.50 ± 0.27**^{a}	**15.99 ± 0.59**^{b}	**14.72 ± 0.26**^{a}	**15.62 ± 0.54**^{b}	**17.73 ± 0.72**^{c}
18:3 *n*-3	4.67 ± 0.24	**5.09 ± 0.17**^{a}	**4.21 ± 0.32**^{b}	**3.95 ± 0.56**^{b}	**4.16 ± 0.26**^{b}	**2.09 ± 0.28**^{c}
20:0	0.28 ± 0.02	0.28 ± 0.02^{ab}	0.30 ± 0.02^{b}	**0.23 ± 0.03**^{c}	0.26 ± 0.02^{ac}	**0.25 ± 0.04**^{ac}
20:1 *n*-9	2.70 ± 0.33	2.63 ± 0.09^{a}	2.59 ± 0.19^{a}	**2.41 ± 0.23**^{a}	**2.44 ± 0.11**^{a}	**1.81 ± 0.21**^{b}
20:2 *n*-6	0.81 ± 0.10	**0.98 ± 0.07**^{a}	**1.19 ± 0.13**^{cd}	**1.02 ± 0.10**^{ab}	**1.14 ± 0.09**^{bc}	**1.29 ± 0.12**^{d}
20:3 *n*-3	0.29 ± 0.04	**0.40 ± 0.03**^{a}	**0.49 ± 0.16**^{a}	**0.51 ± 0.10**^{a}	**0.50 ± 0.07**^{a}	**1.13 ± 0.22**^{b}
20:4 *n*-6	0.41 ± 0.07	**0.31 ± 0.03**^{a}	0.39 ± 0.11^{a}	0.41 ± 0.08^{a}	0.40 ± 0.06^{a}	**0.97 ± 0.19**^{b}
20:4 *n*-3	0.23 ± 0.02	**0.25 ± 0.03**^{a}	0.24 ± 0.04^{a}	0.21 ± 0.03^{b}	0.24 ± 0.03^{a}	**0.09 ± 0.02**^{c}
22:0	0.20 ± 0.10	0.25 ± 0.02^{a}	0.22 ± 0.01^{b}	0.20 ± 0.01^{bc}	0.20 ± 0.01^{c}	0.17 ± 0.01^{d}
22:1	1.11 ± 0.52	0.98 ± 0.08^{a}	0.88 ± 0.07^{b}	0.80 ± 0.11^{b}	0.86 ± 0.03^{b}	**0.49 ± 0.06**^{c}
20:5 *n*-3	1.84 ± 0.36	1.90 ± 0.11^{a}	**1.43 ± 0.25**^{b}	1.53± 0.33^{b}	**1.50 ±0.24**^{b}	**0.86 ± 0.13**^{c}
24:1	0.46 ± 0.07	0.45 ± 0.06^{a}	0.42 ± 0.05^{ab}	**0.37 ± 0.05**^{b}	**0.36 ± 0.11**^{b}	**0.25 ± 0.03**^{c}
22:5 *n*-3	0.62 ± 0.36	0.58 ± 0.03^{a}	**0.48 ± 0.09**^{b}	**0.52 ± 0.11**^{ab}	**0.52 ± 0.06**^{ab}	**0.30 ± 0.06**^{c}
22:6 *n*-3	6.62 ± 0.92	6.30 ± 0.68^{a}	5.89 ± 1.46^{a}	6.26 ± 1.29^{a}	6.71 ± 0.79^{a}	**3.95 ± 0.58**^{b}
Fat content	6.52 ± 2.42	8.48 ± 1.70	6.43 ± 2.31	7.22 ± 2.20	6.96 ± 1.33	6.86 ± 1.10
∑SFA	17.40 ± 1.00	**15.31 ± 0.40**^{a}	**19.05 ± 1.84**^{b}	19.52 ± 3.12^{b}	**18.75 ± 1.32**^{b}	**28.14 ± 1.23**^{c}
∑MUFA	54.23 ± 1.55	54.33 ± 0.52^{a}	**50.63 ± 2.17**^{b}	**51.30 ± 2.12**^{b}	**51.41 ± 1.41**^{b}	**43.30 ± 1.51**^{c}
∑PUFA	28.36 ± 1.25	**32.21 ± 0.66**^{a}	**31.73 ± 2.03**^{a}	**30.67 ± 2.22**^{ab}	**32.30 ± 0.96**^{a}	29.28 ± 1.01^{b}
∑*n*-3	14.04 ± 1.24	14.53 ± 0.66^{a}	12.74 ± 1.91^{a}	12.98 ± 2.08^{a}	13.63 ± 1.05^{a}	**8.43 ± 0.90**^{b}
∑*n*-6	14.32 ± 0.51	**15.79 ± 0.25**^{a}	**17.57 ± 0.62**^{b}	**16.15 ± 0.33**^{a}	**17.16 ± 0.56**^{b}	**19.99 ± 0.88**^{c}
∑*n*-6:∑*n*-3	1.03 ± 0.11	1.09 ± 0.05^{a}	**1.41 ± 0.25**^{b}	**1.27 ± 0.21**^{ab}	**1.27 ± 0.12**^{ab}	**2.40 ± 0.28**^{c}

Values expressed as a percent of total fatty acids, fat content as weight percent on an as-is basis (mean ± standard deviation; $n = 10$); K = control, C = 25% live house crickets (*Acheta domestica*), L = 25% live superworm (*Zophobas morio*) larvae, LC 25% of an equal combination of insect species; I = 100% combination of insect species); SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids; different superscripts indicate significant ($P < 0.05$) differences between groups according to ANOVA, post-hoc Tukey HSD test; **bold type** indicates significant ($P < 0.05$) differences between values at day 0 and at end of the experiment for each group according to ANOVA, post-hoc Tukey.

not be excluded. Partial fish meal replacement with processed insect meal without negative effect on the growth of salmonids was reported Gasco et al. (2014), who successfully replaced up to 50% of fish meal with mealworm larva meal, and Stamer et al. (2014), who used BSF meal in the rainbow trout diet. Fish meal replacement with insect meal ≥50% was associated with significantly reduced growth in the majority of studies (St-Hilaire et al. 2007b, Sealey et al. 2011, Stamer et al. 2014), although total FM replacement with BSF meal without deterioration of growth parameters was reported by Lock et al. (2014) in Atlantic salmon. Potential utilization of live or raw insects in salmonid mass culture is problematic; however, a collaboration of fish and insect farming may be possible with local producers. The readiness of fish to eat inactive crickets in our study showed the potential of using frozen insects for rainbow trout without influencing palatability and/or digestibility, which can be a problem with methods of processing insect meal (Lock et al. 2014).

A major obstacle to the use of insects in commercial farms is their high cost. In the presently reported study, the cost of 1 MJ gross energy from crickets was 25-fold higher and, from superworms, 8-fold higher than of the commercial pellets. Nevertheless, local insect producers may be able to less expensively provide overproduced or dead insects to fish farms. The price of insects may be reduced by using organic by-products (Oonincx et al. 2015) including remains from fish processing (Vladimir Šefl, personal communication) for insect production.

Fatty acid composition. From a human nutrition point of view, fish are a good source of long-chain *n*-3 FA (Tacon and Metian 2013). These *n*-3 fatty acids, especially 20:5 *n*-3 (EPA) and 22:6 *n*-3 (DHA) have multiple functions in metabolism and are associated with the prevention of cardiovascular and inflammatory diseases as well as certain forms of cancer (Simopoulos 2002a, Rudkowska 2010, Calder 2014). Therefore, it is important to maintain a high content of these FA in fish. This is usually obtained by the use of fish oil as a fat source in the feed. If the fat

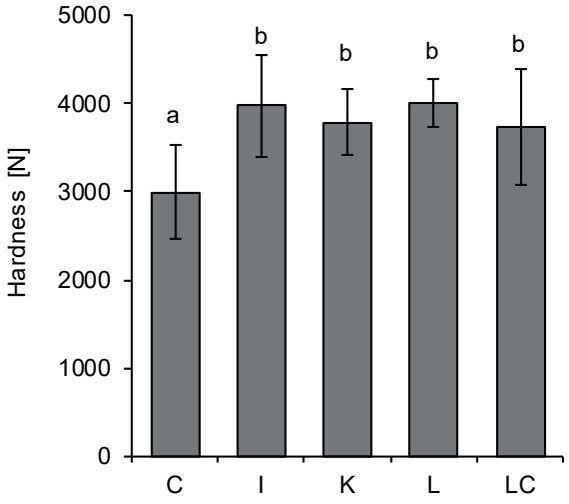

Fig. 2. Sensory attributes of rainbow trout, *Oncorhynchus mykiss*, fillets fed different diets during a 60-day feeding trial; K = control, C = 25% live house crickets (*Acheta domestica*), L = 25% live superworm (*Zophobas morio*) larvae, LC 25% of an equal combination of insect species, I = 100% combination of insect species; sensory evaluation is presented as mean (bars) ± standard deviation (whiskers); different letters indicate significant differences ($P < 0.05$) between groups according to ANOVA, post-hoc Tukey HSD test

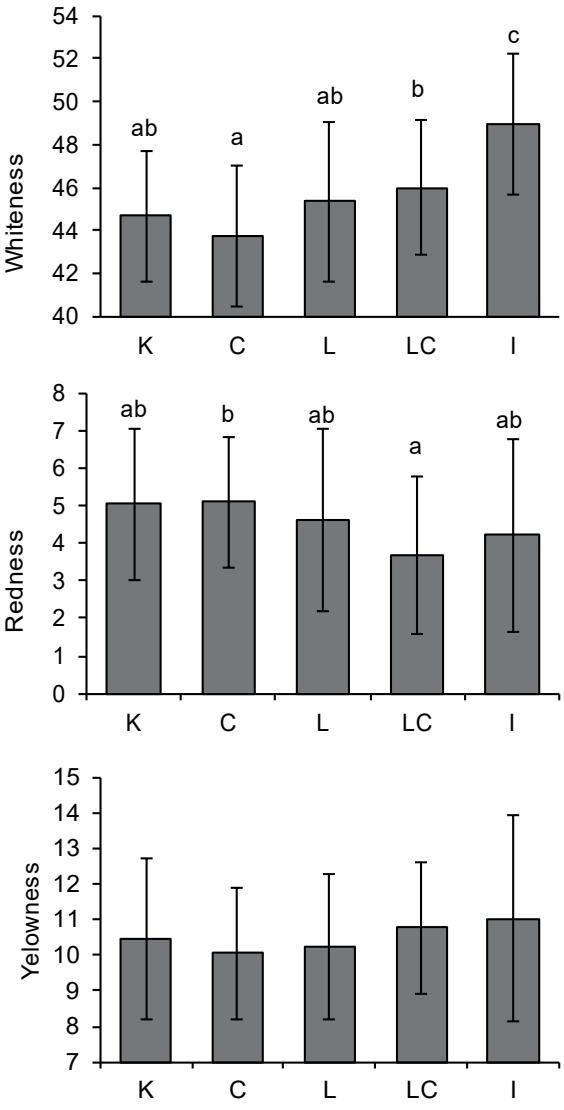

Fig. 3. Fillet color parameters of rainbow trout, *Oncorhynchus mykiss*, fed different diets during a 60-day feeding trial, represented as Whiteness, Redness and Yellowness (mean ± standard deviation); $n = 7$; K = control, C = 25% live house crickets (*Acheta domestica*), L = 25% live superworm (*Zophobas morio*) larvae, LC 25% of an equal combination of insect species, I = 100% combination of insect species; different letters indicate significant ($P < 0.05$) differences between groups according to ANOVA, Fisher's LSD test

is replaced by other sources, FA composition of the diet is generally mirrored in the fish muscle (Sargent et al. 1999, Morris 2001, Shearer 2001). In the presently reported study, an increased proportion of insects corresponded with increasing proportions of the FA dominant in the insects. This was especially notable in the significantly higher proportion of 16:0 (palmitic acid) and significantly lower proportions of EPA and DHA in the group fed insects only. However, in the groups with 25% insect replacement, the proportion of DHA was comparable to the control fish. In contrast, EPA was significantly decreased with insect replacement of 25%. The addition of crickets only, but not superworm, to the feed resulted in increased proportions of 18:2 *n*-6 (LA) and a consequent increase in the *n*-6:*n*-3 ratio. As *n*-6 and *n*-3 FA are metabolized via the same enzyme system (Palmquist 2009) but have opposing effects (Schmitz and Ecker 2008), it is important to keep the *n*-6:*n*-3 ratio as low as possible. The recommendation is 1:4 (Simopoulos 2002b). The fish with the 100% insect diet were in that range, but the ratio, as well as the content of the long-chain *n*-3 PUFA EPA and DHA, should be monitored carefully if insects are used on a bigger scale in fish feeds. Already, replacement of 25% of energy in the pellets by insects resulted in a lower content of the nutritional valuable *n*-3 FA.

The 100% insect diet resulted in EPA and DHA of approximately 45% and 63%, respectively, respectively, of that in fish fed the control diet. This indicates a decrease in the nutritional value of these fish for human nutrition and needs to be addressed. One solution to restore the level of *n*-3 FA after feeding an insect-based diet could be a so-called finishing feeding strategy, where a relatively short final feeding period with diets containing a rich blend of fishmeal and fish oil (Parés-Sierra et al. 2014). In fish fed 25% of the energy as insects, EPA was reduced to 75-80% that of controls, while DHA was comparable, demonstrating that partial replacement does not have a great effect on the fish nutritional value.

Sensory analyses and instrument-based color and texture analyses. Higher cricket content resulted in lower sensory scores for aroma, taste, and aftertaste by the majority of

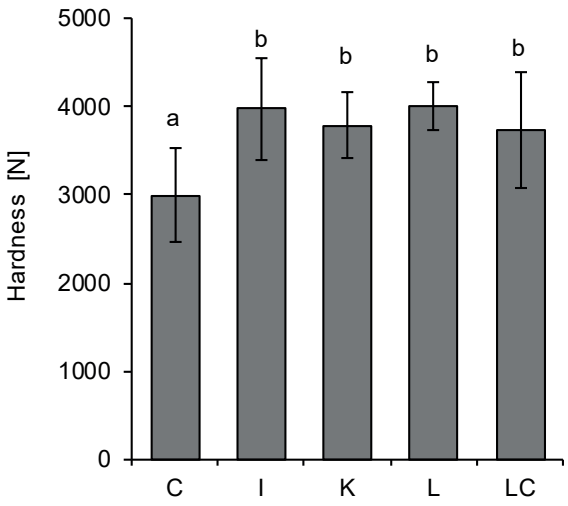

Fig. 4. Fillet hardness of rainbow trout, *Oncorhynchus mykiss*, fed different diets during a 60-day feeding trial (mean ± standard deviation); $n = 7$; K = control, C = 25% live house crickets (*Acheta domestica*), L = 25% live superworm (*Zophobas morio*) larvae, LC 25% of an equal combination of insect species, I = 100% combination of insect species; different letters indicate significant ($P < 0.05$) differences between groups according to ANOVA, Fisher's LSD test

panelists (Fig. 2). This is contradictory to studies replacing FM with BSF meal (Sealey et al. 2011) and soybean meal (D'Souza et al. 2006), in which consumers were unable to differentiate between fish fed the control and experimental diets. This may limit the widespread use of crickets, and probably other *Orthoptera* sp., for salmonid production. Despite this, two panelists evaluated taste as good (<10) and did not detect aftertaste (0) in fillets from Group I in both testing replicates. This could indicate that the distinct taste of fish fed insects may be acceptable to some consumers. Instrumental color analyses showed trout flesh to be lighter in color in groups fed the insect combination. Since color is an important trait to consumers when buying fresh fish, when replacing commercial feed with insects, it is important to use a finishing feeding strategy to obtain the desired flesh color. All insect-fed groups showed higher hardness compared to controls, probably related to the slightly higher lipid content of control fish, also observed by Hardy and Lee (2010). The firm texture of fillets is an important trait for consumers, indicating fresh fish.

Ethoxyresorufin-*O*-deethylase activity (EROD), histology, and pathology. A potential increase in EROD activity with alternative feeds has been reported in some studies (Mráz et al. 2010, Trattner et al. 2011). Similar EROD activity between groups in the presently reported study indicated that the raw insects used were unlikely to contain xenobiotic compounds. Fish showed no pathological and morphological abnormalities, thus house cricket and superworm are considered safe alternatives to commercial pellets from a fish health standpoint. However, there is a risk of insect toxicity related to rearing on biological (especially plant) waste or by-products (Sword 2001).

CONCLUSION

Unprocessed superworm larvae and house crickets are sustainable as feed for rainbow trout. Fish fed the diet containing insects at 25% and 100% of gross energy showed similar growth and feed efficiency as those fed a commercial diet with the same calories, except in PER.

Neither partial nor total replacement of commercial diet with raw insects showed a detrimental effect on fish health.

The insect-containing diet resulted in lower *n*-3 FA content of fillets. Fillet EPA and DHA of fish fed insects only was significantly reduced and may indicate the necessity of a final feeding period with an FM/FO rich diet.

Changes in sensory attributes, texture, and color of flesh from insect-fed trout, particularly those fed a high proportion of house crickets, may decrease their acceptability to consumers.

The high cost of "pet quality" insects, as well the manipulation with live insects, represents a significant limitation to the wider use of insects in commercial trout production. It may be possible to use overproduced or lower quality insects as supplementary fish feed for local producers, especially when insect and fish farming are combined within one farm.

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