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Can the inclusion of black soldier fly (*Hermetia illucens*) in diet affect the flesh quality/nutritional traits of rainbow trout (*Oncorhynchus mykiss*) after freezing and cooking?

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Abstract

Physical and chemical parameters of rainbow trout fillets were tested during 120 days of frozen storage and following cooking. The fillets were obtained from rainbow trout fed diets where dietary fishmeal was substituted with *Hermetia illucens* meal at 0, 25 and 50%

inclusion levels, corresponding to control (C), Hi25, and Hi50 diets. Variations in quality traits of fillets emerged after 30 days of frozen storage, however they remained almost unchanged for the other 90 days. Increasing levels of *H. illucens* did not affect pH, shear stress, colour, and water holding capacity of fillets. Saturated fatty acids were highly present in Hi50 samples, polyunsaturated and some monounsaturated ones were higher in C than Hi50, whereas Hi25 always assumed intermediate contents in raw and cooked samples. A medium substitution at the 25% seemed to be the most practical application of insect meal in order to not modify too much the fillets characteristics.

Keywords: Feedstuffs; Insect meal; *Hermetia illucens*; Flesh quality; Malondialdehyde.

1. Introduction

In a world where the global population is expected to grow up to 9 billion by 2050 and the fish stocks are overexploited despite the decline of catches, aquaculture industry has gained an increasingly important role in the fish supply for human consumption (Clarke and Bostock 2017). The expansion of aquaculture industry necessarily implies an increase of aquafeeds production, where high quality fishmeal (FM) has been over the years the preferred protein source, in particular for carnivorous species (Oliva-Teles et al. 2015; Moutinho et al. 2017). However, this resource is limited and cannot be produced in enough quantities to sustain the further development of aquaculture industry. Thus, the decrease of global availability and rising price of this feed ingredient have challenged industry to find more sustainable and cost-effective alternatives to FM (Clarke and Bostock 2017; Piccolo et al. 2017).

Recently, the use of insects as alternative protein source to FM has been intensely studied (van Huis et al. 2013; Makkar et al. 2014; Sánchez-Muros et al. 2014; Henry et al. 2015). Insects grow fast, reproduce easily, require much less land, energy, and water than plant and

other conventional animal protein feedstuffs. Furthermore, some insects may be raised on low-value products and their waste can be used as organic fertilizer, they have few animal welfare issues, and low hazard of transferring zoonotic infections (van Huis et al. 2013; Sánchez-Muros et al. 2014; Henry et al. 2015). Although insect production industry is recent, it has high potential and is developing fast (Rumpold and Schlüter 2013; van Huis et al. 2013). Among different species, *Hermetia illucens* larvae and prepupae have been widely studied. They contain approximately 40-44% of protein (on dry matter basis, DM) with an amino acid profile particularly rich in lysine (6-8% of the protein), and up to 40% (on DM) of fat. *H. illucens* larvae are rich in minerals such as calcium (5–8% DM) and phosphorous (0.6–1.5% DM) (St-Hilaire et al. 2007; van Huis et al. 2013; Oliva-Teles et al. 2015) and earlier studies showed that *H. illucens* can reduce harmful bacteria in the microflora of manure, acting as natural antibiotic (Makkar et al. 2014; Henry et al. 2015).

Rainbow trout (*Oncorhynchus mykiss*) is one of the leading cultured freshwater fish species in Europe and therefore has a huge economic importance in the European aquaculture (Clarke and Bostock 2017). Its role in human nutrition is mainly linked to its high biological value protein and polyunsaturated fatty acid (PUFA) content, as for many fish species. However, fillets composition may deeply vary due to both *infra vitam* and *post mortem* factors. Diets and storage are two of those. For this reason, the present study aimed to primarily evaluate the effect of dietary replacement of FM by *H. illucens* meal on quality traits of rainbow trout fillets, and secondarily, in order to understand physical and chemical modification occurred in fillets as a commercial product, the effect of frozen storage up to three months along with the effect of cooking (i.e. ready to eat) was also studied.

2. Materials and methods

2.1. Experimental diets, fish feeding and sampling

Three experimental diets were formulated to be isoproteic (crude protein: 45% DM), isolipidic (ether extract: 15% DM) and isoenergetic (22 MJ kg^{-1} gross energy). Control diet (C) was formulated including only fishmeal as protein source while the other two experimental diets presented a substitution of fishmeal with *H. illucens* larvae meal (Hi; Hermetia Deutschland GmbH & Co., Baruth/Mark, Germany) at the substitution percentages of 25% (Hi25) and 50% (Hi50). Three-hundred and sixty rainbow trout (*O. mykiss*) were housed in twelve tanks (four thanks per diet). Farming condition and chemical composition of the experimental diets were reported in previous publications (Borgogno et al. 2017; Renna et al. 2017). At the end of the feeding trial, all fish were individually weighed and 4 fish from each tank (16 fish per diet, for a total of 48 animal) were euthanized by an overdose of anaesthesia (Tricaine methane-sulfonate, MS-222; 60 mg L^{-1}; PHARMAQ Ltd., United Kingdom). The day after slaughtering, the fish were transported, in refrigerated boxes, to the Department of Agri-Food Production and Environmental Sciences (DISPAA), University of Florence (Italy).

In order to investigate the effect of frozen storage, the fish were kept at -10 °C and analysed fresh (1 day after slaughtering, T1) and after 30, 90 and 120 days of frozen storage (T30, T90 and T120). During this period, all fish were maintained in obscurity in polystyrene boxes. At each different storage time, the fish (12 in total, i.e. 4 for each treatment per time) were dissected and filleted. Right fillets were analysed as raw, while left fillets were analysed as cooked. Left fillets were weighed, vacuum packed in plastic bags and boiled (at 98-100 °C) for 5 minutes, until the core temperature arrived at 58 °C; then, the bags containing the samples were removed from the water and cooled at room temperature and the fillets, removed from the bags, were weighed again in order to calculate the cooking loss.

2.2. Physical analyses

Muscle pH was measured in triplicate on the cranial, medial and caudal positions of the epaxial region of the raw fillets. The mean value for each fish was utilized in data analysis. A Mettler Toledo DevenGo SG2™ pH-meter (Novate Milanese, Milano, Italy) equipped with an Inlab puncture electrode (Mettler-Toldedo, Ltd) was utilized.

For texture measurements, a ZwickRoell® 109 texturometer (Zwick Roell, Ulm, Germany), equipped with a 1 kN load cell and supplied by the Text Expert II® software was utilized. The Warner-Bratzler shear test was performed on a muscle sample (3×3 cm) obtained from the cranial part of the epaxial region of the fillet (one measurement for each fillet). A straight blade (width of 7 cm), perpendicular to muscle fibre direction, was utilized at a crosshead speed of 30 mm min^{-1} to 50% of total deformation as described in Iaconisi et al. (2018). Maximum shear force was determined in both raw and cooked fillets.

Colour measurements were performed in both raw and cooked fillets on both the dorsal and ventral regions, by a Spectro-color®116 colorimeter (Bell Technology Ltd., Auckland, New Zealand) equipped with Spectral qc 3.6 software. Colour was expressed as lightness (L^*), redness index (a^*) and yellowness index (b^*) according to the CIELab system (CIE 1976). From L^*, a^* and b^* values, the software automatically calculates Chroma (C*, saturation) and Hue (H*, perception) indexes.

Water Holding Capacity (WHC), performed only in the raw fillets, was determined according to Iaconisi et al. (2018). WHC was calculated as the percentage of water loss after centrifugation (5 min at 210 × g) in relation to the water content of the sample. This last value was obtained gravimetrically on 2 g of sample by weighing samples before and after 24 hours at 105 °C. Two measurements for each sample were performed.

2.3. Chemical analyses

2.3.1. Fatty acid profile

Total lipid fraction was extracted from both raw and cooked fillets (Folch et al. 1957) and subsequently gravimetrically quantified. The extracted lipids were utilized for the analysis of fatty acid (FA) profile.

The FA composition was determined by gas chromatography (GC) using a Varian GC 430 gas chromatograph (Agilent, Palo Alto, CA, USA), equipped with a flame ionization detector (FID); a Supelco Omegawax™ 320 capillary column (30 m × 0.32 mm i.d., 0.25 μm film and polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA) was utilized. The oven temperature was held at 100 °C for 2 minutes, increased to 160 °C over 4 minutes at the rate of 12 °C min^{-1}, and then increased to 220 °C over 14 min at the rate of 3 °C min^{-1} and kept at 220 °C for 25 min.. The injector and the detector temperatures were set at 220 °C and 300 °C, respectively. A quantity of 1 μL of sample in hexane was injected into the column with the carrier gas (helium) kept at a constant flow of 1.5 mL min^{-1}. The split ratio was 1:20. Chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 computing integrator software (Agilent). FAs were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA). Individual FAs were quantified using tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) as internal standard and expressed as a percentage of total FAME.

From the FA profile, fat quality indexes as atherogenicity index (AI) and thrombogenicity index (TI) (Ulbricht and Southgate 1991) and hypocholesterolemic/hypercholesterolemic FA ratio (HH) (Santos-Silva et al. 2002) were calculated as follows:

$$AI = [C12{:}0 + (C14{:}0 \times 4) + C16{:}0]/(PUFA\omega3 + PUFA\omega6 + MUFA)$$

$$TI = [(C14{:}0 + C16{:}0 + C18{:}0)]/[(MUFA \times 0.5) + (PUFA\omega6 \times 0.5) + (PUFA\omega3 \times 3) + (PUFA\omega3 / PUFA\omega6)]$$

$$HH = (C18{:}1\omega9 + C18{:}2\omega6 + C20{:}4\omega6 + C18{:}3\omega3 + C20{:}5\omega3 + C22{:}5\omega3 + C22{:}6\omega3) / (C14{:}0 + C16{:}0)$$

Furthermore, PUFAω6/PUFAω3 and PUFA/SFA ratios were calculated.

2.3.2. Lipid oxidation products

The content of conjugated dienes (CD) was measured by a colorimetric method (Srinivasan et al. 1996), using hexane as solvent. CD were quantified at 233 nm (50 Scan spectrophotometer equipped with Cary Win UV software, Varian, Palo Alto, CA, USA), using a molar extinction coefficient of 29 000 L mol^{-1} cm^{-1}. Results were expressed as mmol hydroperoxides kg^{-1} sample. The 2-thiobarbituric acid-reactive substances (TBARS) were measured by a colorimetric method (Salih et al. 1987). TBARS were extracted in 10 mL of trichloroacetic acid (TCA, 50 g L^{-1}). Five mL of the filtered extract was then added with 5 mL of thiobarbituric acid (TBA,0.02 mol L^{-1}). After 40 minutes of incubation at 97 °C, the malondialdehyde equivalents (MDA-eq) were determined spectrophotometrically at 532 nm. A calibration curve obtained by 1,1,3,3-tetraethoxypropane (TEP) at a concentration ranging from 0.8 to 8 µmol L^{-1} was utilized in order to calculate the mg MDA-eq. for kg sample.

2.4. Statistical analyses

Data obtained on raw fillets (pH, WHC, cooking loss, maximum shear force, colour, FA profile and lipid oxidation products) and on cooked samples (maximum shear force, colour and FA profile) were analysed separately. A two-way ANOVA analysis was applied with the diet (D; C, Hi25 and Hi50) and the storage time (T; T1, T30, T60 and T120) as fixed factors. Lipid oxidation products (CD, and TBARS) on raw samples were analysed only at T1 and T120.

Interaction D × T was assessed and the significance level was set at 5% (statistically significant for $P < 0.05$), and the differences were assessed using Tukey's test. Variability was expressed as Root Mean Square Error (RMSE).

Moreover, data of raw and cooked samples were also separately analysed through a principal component analysis (PCA). Both raw and cooked sample data were mean centered and scaled to a unit standard deviation before PCA analyses.

Statistical analyses were performed with R free statistical software.

3. Results and discussions

3.1. Physical analyses

Table 1 shows the results obtained from physical analyses (pH, shear stress, colour, WHC and cooking loss) of raw and cooked fillets. Regarding data of raw fillets, none of the considered parameters has been affected by the substitution of fishmeal with *H. illucens* larvae meal, thus confirming previous findings (Renna et al. 2017). Indeed, both pH value, *a** and *b** colour values were in line to those proposed during the evaluation of the suitability of a partially defatted *H. illucens* meal as ingredient for rainbow trout diet (Renna et al. 2017).

On the contrary, storage time had a significant effect on pH and texture, by significantly increasing the first and decreasing the second parameter. Specifically, pH remained unaltered along the first 30 storage days, and then slightly increased by raising its highest value at T120. Texture instead, expressed as shear force, widely decreased from T1 and T30, remained stable during the following 60 days, until T90, and finally it raised an intermediate value, among the other times, at the end of the storage period. Concerning colour, *L**, *b**, and C* values of both the considered regions resulted significantly increased by increasing the storage time. Since years, authors have been investigating the effect of storage on colour parameters studying the combination temperature-duration. An increase in *L**, *a** and *b** with long storage time was found in pigmented rainbow trout vacuum packed fillets (No and Storebakken 1991) and in Atlantic salmon (*Salmo salar*) (Regost et al. 2004), thus being in line with the data obtained in the present study.

However, it is possible to observe that colour modification differently occurred in dorsal and ventral regions. Indeed, *L**, *b**, and C* values of the dorsal region tended to remain stable during the first 30 days of storage, then gradually increased ($P < 0.05$) during the storage till assuming their highest values at T120. Instead, *L**, *b**, and C* of the ventral region already changed ($P < 0.05$) between T1 and T30 by raising their maximum values which were no more modified during the remaining storage days. Other authors have previously noticed that different part of the fish fillet might present different colour values. No and Storebakken (1991) for example, underlined that *L**, *a**, and *b** significantly differed among neck, back, and tail positions along rainbow trout fillets. Also other authors found that epaxial and hypaxial regions of trout flesh presented different *b** values (Iaconisi et al. 2017). Since colour is correlated with proximate composition, especially with lipid content, and considering the high variability in lipid distribution along fish fillets, we might attribute the different pattern in colour maintenance during storage to a different stability of chemical components of flesh (Martelli et al. 2014).

Water holding capacity of raw fillets resulted unaffected by both diet and storage, assuming a mean value around 85%. Diet did not affect cooking loss as well, while a light trend of increase was shown during the storage trial, however T1 and T120 not significantly differed.

As reported for raw samples, also quantifications of shear stress and colour on cooked fillets were not significantly modified by the diet (Table 1). Colour is widely reported to play an important role in consumers' acceptance of cooked fish (Gai et al. 2016), hence the absence of a significant effect of diet on this quality parameter should be considered as positive when evaluating *H. illucens* meal inclusion as substitute for fishmeal in rainbow trout feed.

As found for raw fillets, storage time had a significant effect on physical parameters. Shear stress increased up to T120, when its value was +37% of T1. Finally, colour evaluation of cooked fillets revealed that a* values of both dorsal and ventral regions were modified by

storage time but following an opposite pattern. Specifically, dorsal region presented an increase ($P < 0.05$) in a* value during the first 30 days and no other modifications were noted till the end of the trial. Contrarily, a* values of ventral portion decreased from T1 to T120, however this last sampling point was not significantly different from the first one.

3.2. Chemical analyses

3.2.1. Fatty acid profile

Total lipid and fatty acid contents were unaffected by the diet. Nevertheless, fatty acid profile of raw fillets was strongly affected by *H. illucens* larvae meal inclusion level in the diet, as shown in Table 2. An overall increase of saturated fatty acid (SFA) was observed while increasing the percentage of fish meal replacement with *H. illucens* meal. As a consequence, the highest SFA value was found in fillets from Hi50, mostly by C12:0 and C14:0 wide increase; Hi25 assumed intermediate values, whereas C group presented the lowest SFA value. Among this lipid category, C16:0 and C18:0 were unaltered by the diet. The opposite pattern was shown by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAω3 and total PUFA) groups. The contents of all the MUFA were significantly lower in Hi50 than in C, with the exception of C16:1ω7. PUFAω3 gradually decreased with the inclusion of *H. illucens* larvae meal in aquafeed. The highest and lowest PUFAω3 contents were found in fillets from C and Hi50 diet, respectively, whilst Hi25 once again assumed intermediate values. A dramatic half reduction of eicosapentaenoic acid (EPA, C20:5ω3) as well as the decrease of docosahexaenoic acid (DHA, C22:6ω3) lead the overall PUFAω3 reduction. On the contrary, PUFAω6 fraction was constant between fillets from trout fed C and Hi50 diet. Due to this deeply modification, PUFA/SFA ratio was found below 1 only in Hi50 group, whereas a ratio of 1.71 and 1.12 was calculated for C and Hi25, respectively. Fish fatty acid profile usually reflects the one of the administered diet. In this sense, the

progressive decrease of PUFAω3, together with the increase of SFA, seems to be attributable to two factors, both related to the presence of *H. illucens* larvae meal in feed. First of all, it is important to consider that *H. illucens* meal utilized in the present trial was partially defatted, and it had around 17% of fat content. Hence, in order to maintain the diet isoenergetic, the increased presence of insect larvae meal implied the contemporary reduction of fish oil, the main source of PUFAω3 in aquafeed (Borgogno et al. 2017; Renna et al. 2017). Secondarily, *H. illucens* fat is characterized by high level of SFA, which might amount at 71.80% and 54.59% of total fatty acid in prepupae and larvae meal, respectively, irrespective the growing substrates (Borgogno et al. 2017, Renna et al. 2017). A recent study has suggested that *H. illucens* is able to both synthesize C12:0 from nutrients present in the substrate, such as carbohydrates (starch and sugars), and metabolize to C12:0 a large proportion of fatty acid (Oonincx et al. 2015) as form of biological storage. However, in light of the fact that a substitution of 50% of fish meal with *H. illucens* meal deeply reduced PUFAω3 fraction, a defatted phase should be considered while producing this insect meal for aquafeeds.

Storage time seemed to have a little effect on fatty acid profile of raw fillets, thus confirming the suitability of frozen storage for lipid preservation. SFA fraction, especially C12:0 and C14:0, slight but significantly increased during the first 30 days of storage, and they remained unchanged until T120. Thirty days of frozen storage seemed to be critical for C22:5ω3, which was significantly lower at T30, T90 and T120 than at T1. No other significant variation emerged from the statistical analysis.

The fatty acid profile of the rainbow trout cooked fillets (Table 3) was very similar to the profile of the raw ones, in terms of the percentages of each fatty acid and of trends. All the reported fatty acids were affected by the diet, except C18:0, C16:1ω7, and C18:2ω6. As found in raw fillets, SFA significantly increased in cooked fillets with the increasing of *H. illucens* meal in diet, whereas all the other fatty acid fractions decreased ($P < 0.05$). Due to these

modifications, all the calculated nutritional indexes were negatively affected by rising insect meal level in diet. The worsening of lipid quality with insect meal introduction in aquafeed was also confirmed by the gradual but significant decrease of ω3/ω6, PUFA/SFA, and HH ratios as well as by the AI and TI values increase in fillets from trout fed Hi50. However, the only recommendation about PUFAs intake in relation with their role in human health, is the amount of EPA + DHA per day, suggested in 500 mg (Kris-Etherton et al. 2009). While looking at present results, EPA and DHA reduction in rainbow trout cooked fillets, as a consequence of growing levels of *H. illucens* in fish diet, implied that consumers should increase the number of portions (around 100 g as suggested by US Food and Drug Administration) consumed every week. Specifically, in order to introduce the suggested 3.5 g of EPA + DHA per week, people should eat around 196 g (equivalent to 2 scarce portions), 254 g (equivalent to 2.5 portions), and 311 g (equivalent to 3 portions) of trout fed with C, Hi25, and Hi50 diets, respectively.

The effect of storage time on fatty acid profile of cooked fillets was less extended than that of the diet. Indeed, only few fatty acids resulted affected by storage. Significant increases of C12:0 and C14:0 lead the overall increase ($P < 0.05$) in total SFA fraction during the storage. On the contrary, a decrease of PUFAω3 was shown at T30 ($P < 0.01$), with a single decrease of around 8% and 12% of content for C22:5ω3 and C22:6ω3, respectively. It has to be underlined that all of these changes occurred during the first 30 days of storage, hence T1 data resulted significantly different from T30, T90, and T120, with no further modification among these last three sampling times.

3.2.2. Lipid oxidation products

Lipid oxidation was evaluated only in raw fillets by determining both primary (conjugate dienes, CD) and secondary (TBARS, as MDA-eq) oxidation products. No statistical

significance for the main factors and their interaction was found for CD quantification. Instead, interaction of treatments D × T was found significant by the statistical analysis for MDA-eq quantification (Figure 1). Figure 1 shows that fillets from C dietary group were the most susceptible to be oxidized, indeed, they contained the highest level of MDA-eq at T120, equal to 2.60 mg MDA-eq kg^{-1}. This fact might be due to the highest PUFAs content in C fillets, which is the main lipid fraction to be damaged by oxidative phenomena, as confirmed by fatty acid profile patterns during storage. However, a recent paper has observed that dietary defatted *H. illucens* larvae meal improved the antioxidant status of Jian carp (*Cyprinus carpio* var. Jian) by showing a high catalase activity (CAT) in animal serum (Li et al. 2017). Furthermore, chitin, naturally contained in insects exoskeleton, seems to have antioxidant properties attributed to *in vitro* and *in vivo* free radical-scavenging activities, as reported by Ngo and Kim (2014), which might explain the lowest TBARS content of Hi samples at the end of the storage period.

3.3. Principal component analysis

Principal component analyses on raw and cooked fillets are reported as biplot (score plot and loading plot) in Figures 2 and 3, respectively. Samples were grouped for type of diet in both plots, revealing that the PC 1 could be related to the substitution of fishmeal with Hi meal. Indeed, more fishmeal was replaced with Hi meal more the diet samples were plotted distant from the C diet samples. As revealed from fatty acid analysis, fillets were grouped mostly in relation to fatty acid profile; SFA were highly present in Hi50 samples, as well as PUFA and in part MUFA were higher in C samples than in Hi25 and Hi50 ones.

PC 2 was not representative for the storage time, as samples were mixed between time of analysis on the y axis, even if a trend to plot the T1 in the upper squares of the plots were revealed in both raw and cooked PCAs. This finding might be expected as storage time

showed to significantly affect the parameters investigated only in few cases, with major differences between T1 fillet characteristics and the characteristics of fillets analysed at the other times. Results of cooked samples followed the trends of the raw ones. The same distribution of the loadings could be highlighted in both the plots, with quite the same position of the plotted analyses (i.e. for the same type of determination the eigenvectors of the raw and the cooked PCA were comparable).

4. Conclusion

Substitution of fishmeal with *Hermetia illucens* meal in rainbow trout diet mainly induced modifications in fatty acid composition of raw and cooked fillets. Variations in quality/nutritional traits of fillets emerged after 30 days of frozen storage, with minor modification during the rest of the storage period. The worsening in nutritional characteristics of cooked fillets due to insect meal inclusion increased the fillets portion needed to carry the weekly suggested intake of EPA and DHA. A medium substitution at the 25% of fishmeal with this new ingredient for aquafeeds seemed to be the most practical application in order to not substantially modify the fillets characteristics.

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Table 1. Physical analyses (pH, shear stress, colour, water holding capacity, and cooking loss) of raw and cooked fillets from rainbow trout fed three experimental diets: Control (C) with fish meal as the main protein source, Hi25, and Hi50 with the 25% and 50% of fish meal substitution by *Hermetia illucens*, respectively. Fillets from the three groups where stored 120 days at –10 °C.

		Diet (D)			Time (T)				*P*-value		RMSE
		C	Hi25	Hi50	T1	T30	T90	T120	D	T	
Raw fillet											
pH		6.41	6.43	6.42	6.37^{z}	6.40^{yz}	6.44^{xy}	6.47^{x}	0.521	<0.001	0.050
Shear stress, N		12.79	14.59	14.68	19.42^{x}	10.34^{z}	11.12^{z}	15.21^{y}	0.060	<0.001	2.445
Colour											
Dorsal	*L**	50.38	50.63	50.33	45.95^{z}	47.36^{z}	51.86^{y}	56.61^{x}	0.946	<0.001	2.661
	*a**	-0.21	-0.35	0.14	-0.14	-0.13	0.07	-0.36	0.435	0.820	1.091
	*b**	5.59	5.40	6.23	3.67^{y}	4.64^{y}	7.38^{x}	7.27^{x}	0.188	<0.001	1.315
	C*	5.69	5.50	6.67	4.22^{y}	4.79^{y}	7.43^{x}	7.37^{x}	0.051	<0.001	1.388
	H*	94.16	97.22	94.63	100.31	95.00	90.92	95.11	0.764	0.3585	12.651
Ventral	*L**	50.94	51.33	49.63	47.99^{z}	49.37^{yz}	51.29^{xy}	53.90^{x}	0.159	<0.001	2.557
	*a**	0.78	1.20	0.92	0.24	1.73	1.49	0.42	0.684	0.253	1.391
	*b**	6.35	7.07	6.96	4.03^{y}	6.95^{x}	8.41^{x}	7.78^{x}	0.268	<0.001	1.333
	C*	6.60	7.42	7.29	4.27^{y}	7.35^{x}	8.76^{x}	8.04^{x}	0.240	<0.001	1.441
	H*	83.76	83.56	87.77	89.52^{x}	79.21^{y}	82.24^{xy}	89.15^{x}	0.330	0.015	8.873
WHC, %		84.72	85.16	84.25	86.96	83.47	84.49	83.92	0.715	0.054	3.176
Cooking loss, %		7.90	10.22	10.02	7.92^{xy}	12.55^{x}	6.01^{y}	11.05^{xy}	0.109	<0.001	3.355
Cooked fillet											
Shear stress, N		13.46	14.75	11.73	9.89^{y}	12.66^{xy}	13.67^{xy}	17.05^{x}	0.363	0.045	5.946
Colour											
Dorsal	L*	77.48	76.08	74.66	0.75	78.63	74.04	76.34	0.226	0.105	4.542
	a*	-0.25	-0.33	-0.25	-0.88^{y}	-0.01^{x}	-0.15^{xy}	-0.06^{x}	0.920	0.010	0.678
	b*	11.28	11.05	12.29	11.93	12.26	10.54	11.41	0.187	0.188	2.000
	C*	11.32	11.09	12.36	12.03	12.29	10.59	11.44	0.175	0.180	1.988
	H*	92.11	92.72	91.97	95.56^{x}	90.74^{y}	91.93^{xy}	90.84^{y}	0.836	0.010	3.759
Ventral	L*	74.07	72.56	70.28	70.67^{xy}	76.40^{x}	66.97^{y}	75.18^{x}	0.141	<0.001	5.306
	a*	0.36	0.63	0.74	0.91^{xy}	1.02^{x}	0.40^{xy}	-0.02^{y}	0.475	0.028	0.896
	b*	12.44	12.22	12.9	13.02	13.68	11.24	12.13	0.714	0.083	2.370
	C*	12.48	12.31	12.97	13.15	13.76	11.28	12.15	0.727	0.078	2.409
	H*	88.92	88.13	87.44	87.17^{xy}	86.19^{y}	88.74^{xy}	90.55^{x}	0.526	0.032	3.648

RMSE, root mean square error.

x, y, z: Different letters indicate significantly different values ($P < 0.05$) within Time (T).

The interaction D × T was not significant ($P < 0.05$).

Table 2. Total lipids (g kg^{-1} fillet), total fatty acids (g kg^{-1} fillet), and fatty acid profile (% of total fatty acids) of raw fillets from rainbow trout fed three experimental diets: Control (C) with fish meal as the main protein source, Hi25, and Hi50 with the 25% and 50% of fish meal substitution by *Hermetia illucens*, respectively. Fillets from the three groups were stored 120 days at –10 °C.

	Diet (D)			Time (T)				*P*-value		RMSE
	C	Hi25	Hi50	T1	T30	T90	T120	D	T	
Total lipids	88.0	96.2	98.0	116.3^{x}	89.9^{y}	86.9^{y}	83.0^{y}	0.183	<0.001	1.59
Total fatty acids	70.5	76.8	78.4	92.8^{x}	72.1^{y}	69.6^{y}	66.5^{y}	0.196	<0.001	1.27
C12:0	0.60^{c}	6.33^{b}	12.08^{a}	5.79^{y}	6.45^{x}	6.44^{x}	6.67^{x}	<0.001	0.004	0.569
C14:0	3.23^{c}	4.29^{b}	5.35^{a}	4.14^{y}	4.32^{x}	4.30^{x}	4.39^{x}	<0.001	0.004	0.159
C16:0	15.23	15.65	15.61	15.14	15.63	15.69	15.52	0.085	0.100	0.574
C18:0	3.62	3.72	3.64	3.68	3.69	3.63	3.64	0.242	0.739	0.174
SFA	23.49^{c}	30.71^{b}	37.30^{a}	29.50^{y}	30.83^{x}	30.75^{x}	30.93^{x}	<0.001	0.008	1.085
C16:1ω7	5.43	5.56	5.52	5.29	5.61	5.55	5.57	0.585	0.170	0.372
C18:1ω7	2.81^{a}	2.53^{b}	2.30^{c}	2.55	2.53	2.57	2.54	<0.001	0.372	0.068
C18:1ω9	22.63^{a}	22.08^{ab}	21.56^{b}	22.16	22.14	22.2	21.87	0.001	0.650	0.703
C20:1ω9	2.33^{a}	2.26^{a}	1.90^{b}	2.22	2.20	2.19	2.05	0.008	0.694	0.391
C22:1ω11	1.34^{a}	1.07^{b}	0.73^{c}	1.07	1.04	1.01	1.06	<0.001	0.604	0.113
MUFA	36.17^{a}	34.91^{b}	33.32^{c}	34.67	34.91	34.93	34.69	<0.001	0.863	0.955
C18:3ω3	1.86^{a}	1.61^{b}	1.53^{b}	1.73	1.65	1.66	1.63	<0.001	0.402	0.146
C20:5ω3	4.12^{a}	3.16^{b}	2.18^{c}	3.27	3.14	3.07	3.22	<0.001	0.070	0.183
C22:5ω3	2.40^{a}	1.74^{b}	1.19^{c}	1.91^{x}	1.70^{y}	1.75^{y}	1.75^{y}	<0.001	0.003	0.137
C22:6ω3	18.58^{a}	15.96^{b}	12.72^{c}	16.15	15.59	15.6	15.66	<0.001	0.219	0.749
PUFAω3	29.12^{a}	24.13^{b}	18.87^{c}	24.7	23.71	23.78	23.98	<0.001	0.307	0.966
C18:2ω6	7.73	7.24	7.83	8.00	7.50	7.54	7.36	0.076	0.210	0.760
PUFAω6	10.11^{a}	9.31^{b}	9.80^{ab}	10.20	9.64	9.66	9.47	0.040	0.201	0.864
PUFA	40.13^{a}	34.58^{b}	29.52^{c}	35.83	34.26	34.51	34.38	<0.001	0.127	1.778

RMSE, root mean square error.

a, b, c: Different letters indicate significantly different values ($P < 0.05$) within Diet (D).

x, y, z: Different letters indicate significantly different values ($P < 0.05$) within Time (T).

The fatty acids C13:0, C15:0, C17:0, C20:0, C22:0, C24:0, C14:1ω5, C16:1ω9, C17:1, C20:1ω7, C20:1ω11, C22:1ω9, C22:1ω7, C18:4ω3, C20:3ω3, C20:4ω3, C21:5ω3, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, C22:2ω6, C22:4ω6, C22:5ω6 were detected but not listed in the table because below 1.5%. All the mentioned fatty acids have been utilized for calculating the sums of the lipid fractions.

The interaction D × T was not significant ($P < 0.05$).

Table 3. Total lipids (g kg^{-1} fillet), total fatty acids (g kg^{-1} fillet), and fatty acid profile (% of total fatty acids) of cooked fillets from rainbow trout fed three experimental diets: Control (C) with fish meal as the main protein source, Hi25, and Hi50 with the 25% and 50% of fish meal substitution by *Hermetia illucens*, respectively. Fillets from the three groups were stored 120 days at –10 °C.

	Diet (D)			Time (T)				*P*-value		RMSE
	C	Hi25	Hi50	T1	T30	T90	T120	D	T	
Total lipids	78.4	81.3	77.3	84.3	74.0	80.4	77.2	0.766	0.449	1.603
Total fatty acids	6.27	6.51	6.17	6.75	5.92	6.42	6.17	0.755	0.442	1.282
C12:0	1.17^{c}	6.00^{b}	11.68^{a}	5.64^{y}	6.95^{x}	6.07^{xy}	6.48^{xy}	<0.001	0.038	1.103
C14:0	3.23^{c}	4.18^{b}	5.08^{a}	3.77^{y}	4.32^{x}	4.23^{x}	4.35^{x}	0.019	<0.001	0.478
C16:0	15.22^{b}	15.67^{a}	15.73^{a}	15.17	15.63	15.68	15.67	0.010	0.143	0.487
C18:0	3.61	3.74	3.67	3.68	3.71	3.63	3.68	0.100	0.688	0.161
SFA	24.03^{c}	30.30^{b}	36.66^{a}	28.82^{y}	31.33^{x}	30.30^{x}	30.89^{x}	<0.001	0.003	1.597
C16:1ω7	5.20	5.39	5.44	5.06	5.39	5.48	5.42	0.173	0.146	0.378
C18:1ω7	2.75^{a}	2.49^{b}	2.27^{c}	2.49	2.45	2.54	2.53	<0.001	0.353	0.068
C18:1ω9	21.86^{a}	21.61^{ab}	21.14^{b}	21.43	21.49	21.83	21.39	0.016	0.388	0.677
C20:1ω9	2.43^{a}	2.22^{a}	1.65^{b}	2.17	2.15	2.18	1.91	<0.001	0.230	0.364
C22:1ω11	1.31^{a}	1.07^{b}	0.69^{c}	1.04	1.00	1.00	1.06	<0.001	0.746	0.147
MUFA	35.00^{a}	34.17^{a}	32.45^{b}	33.54	33.86	34.43	33.67	<0.001	0.240	1.123
C18:3ω3	1.81^{a}	1.57^{b}	1.50^{b}	1.69	1.60	1.62	1.59	<0.001	0.307	0.136
C20:5ω3	4.21^{a}	3.23^{b}	2.29^{c}	3.37	3.16	3.14	3.31	<0.001	0.213	0.189
C22:5ω3	2.39^{a}	1.80^{b}	1.23^{c}	1.98^{x}	1.70^{y}	1.80^{y}	1.76^{y}	<0.001	0.001	0.159
C22:6ω3	19.58^{a}	17.18^{b}	14.19^{c}	18.05^{x}	16.47^{y}	16.56^{y}	16.86^{y}	<0.001	0.005	1.118
PUFAω3	30.03^{a}	25.43^{b}	20.43^{c}	26.66^{x}	24.55^{y}	24.80^{y}	25.19^{y}	<0.001	0.002	1.349
C18:2ω6	7.50	7.03	7.63	7.76	7.25	7.38	7.16	0.052	0.188	0.696
PUFAω6	9.90	9.19	9.74	10.12	9.39	9.59	9.35	0.053	0.098	0.820
PUFA	40.97^{a}	35.53^{b}	30.88^{c}	37.64^{x}	34.81^{y}	35.27^{y}	35.44^{y}	<0.001	0.010	2.084
ω3/ω6	3.04^{a}	2.78^{b}	2.10^{c}	2.66	2.64	2.58	2.68	<0.001	0.393	0.146
PUFA/SFA	1.72^{a}	1.18^{b}	0.85^{c}	1.37^{x}	1.16^{y}	1.23^{y}	1.23^{y}	<0.001	0.010	0.142
AI	0.39^{c}	0.56^{b}	0.76^{a}	0.51^{y}	0.60^{x}	0.57^{x}	0.60^{x}	<0.001	0.003	0.061
TI	0.19^{c}	0.23^{b}	0.29^{a}	0.22^{y}	0.25^{x}	0.24^{x}	0.24^{x}	<0.001	0.002	0.020
HH	3.16^{a}	2.69^{b}	2.38^{c}	2.96^{x}	2.65^{y}	2.69^{y}	2.66^{y}	<0.001	0.004	0.222

RMSE, root mean square error.

a, b, c: Different letters indicate significantly different values ($P < 0.05$) within Diet (D).

x, y, z: Different letters indicate significantly different values ($P < 0.05$) within Time (T).

The fatty acids C13:0, C15:0, C17:0, C20:0, C22:0, C24:0, C14:1ω5, C16:1ω9, C17:1, C20:1ω7, C20:1ω11, C22:1ω9, C22:1ω7, C18:4ω3, C20:3ω3, C20:4ω3, C21:5ω3, C18:3ω6, C20:2ω6, C20:3ω6,C20:4ω6, C22:2ω6,

C22:4ω6, C22:5ω6 were detected but not listed in the table because below 1.5%. All the mentioned fatty acids have been utilized for calculating the sums of the lipid fractions.

The interaction D × T was not significant ($P < 0.05$).

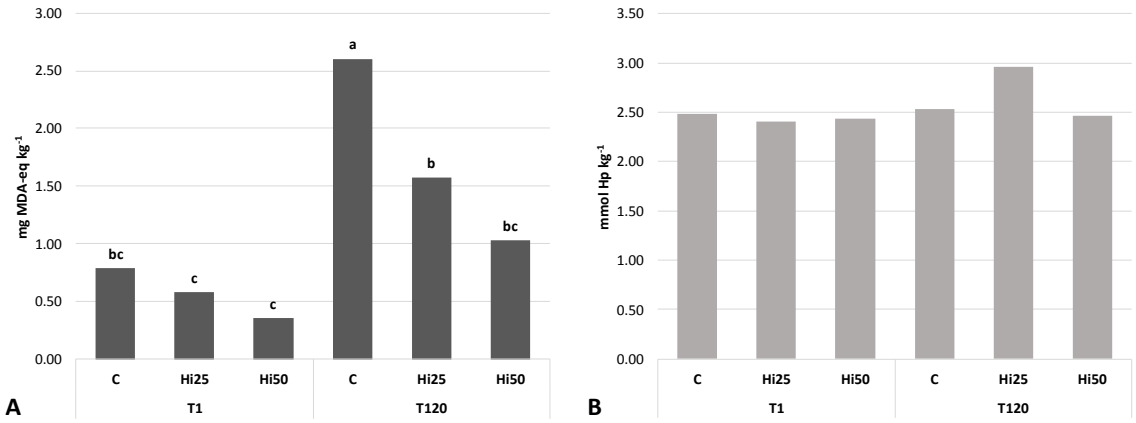
Captions of Figures

Figure 1. TBARS and CD contents at T1 and T120 (mg MDA-eq kg^{-1}; mmol Hp kg^{-1} fillets) of raw fillets from rainbow trout fed three experimental diets: Control (C) with fish meal as the main protein source, Hi25, and Hi50 with the 25% and 50% of fish meal substitution by *Hermetia illucens*, respectively.

Figure 2. Biplot of principal component (PC) analysis of raw fillets.

Figure 3. Biplot of principal component (PC) analysis of cooked fillets.

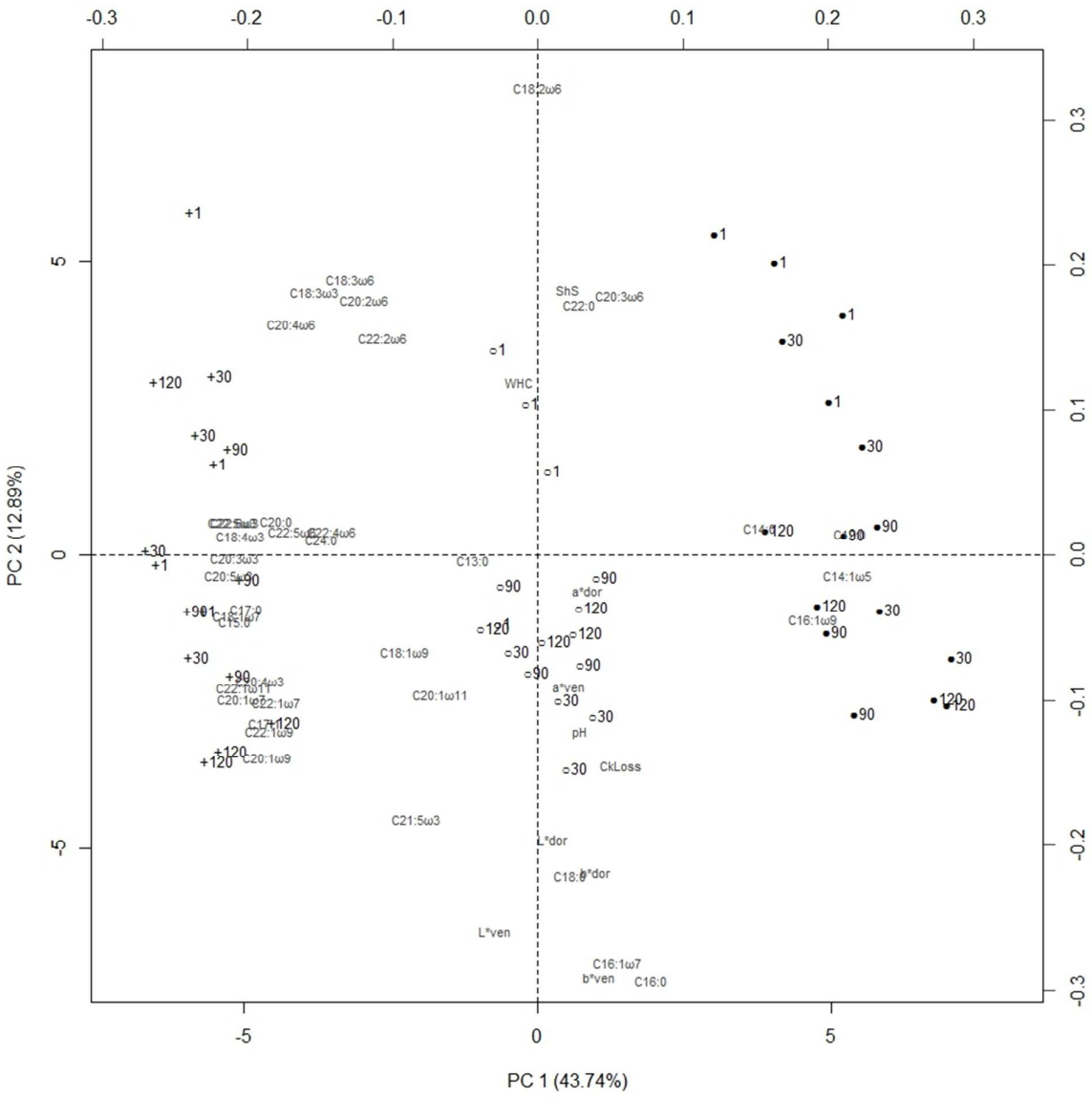
Figure 1.



A. TBARS - thiobarbituric acid-reactive substances; B. CD - conjugated dienes.

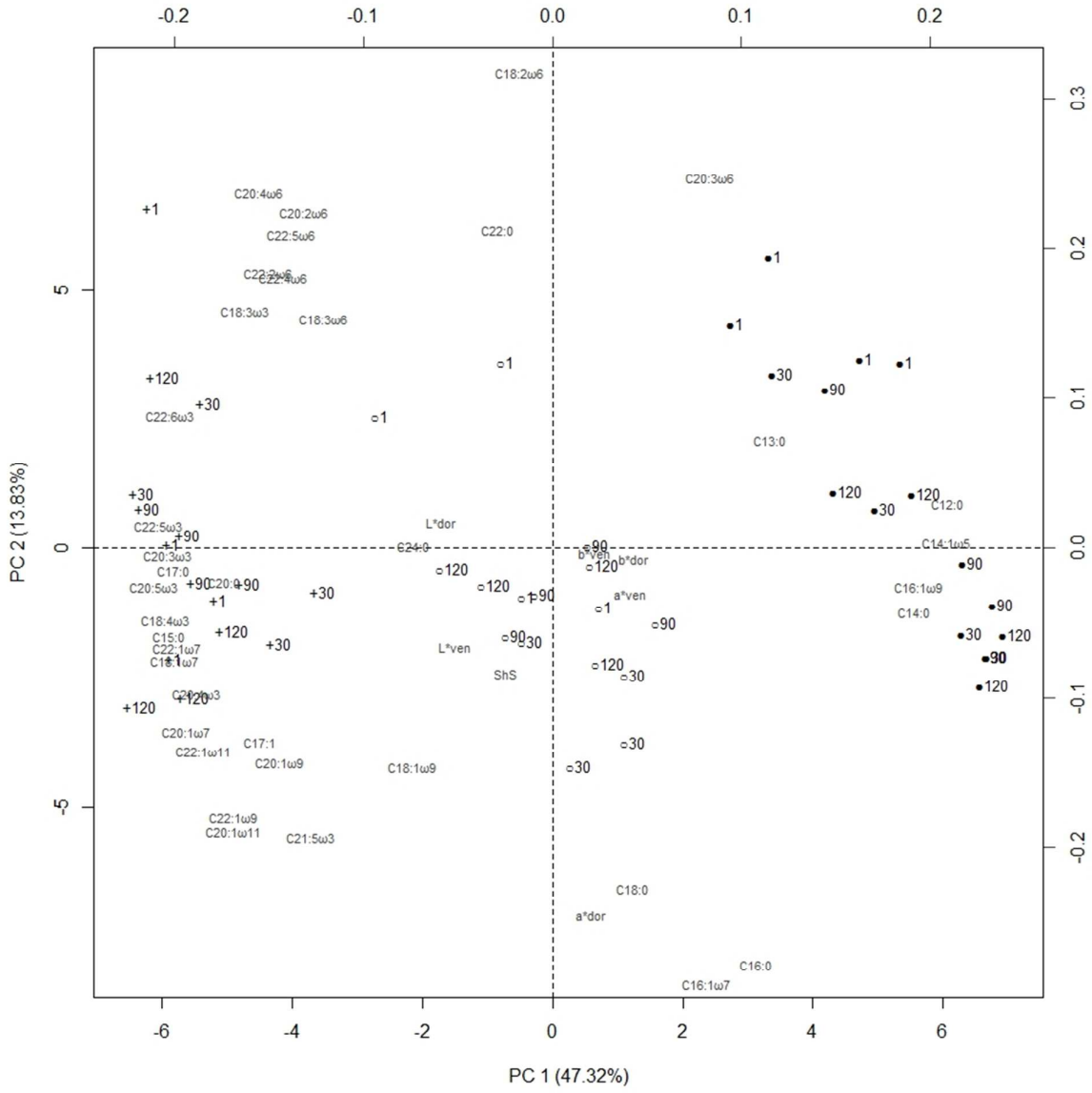
a, b, c: Different letters indicate significantly different values ($P < 0.05$).

Figure 2.



+: Control (C) diet; ○: 25% of fish meal substitution by *Hermetia illucens* meal (Hi25); ●: 50% of fish meal substitution by *Hermetia illucens* meal (Hi50).

Figure 3.



+: Control (C) diet; ○: 25% of fish meal substitution by *Hermetia illucens* meal (Hi25); ●: 50% of fish meal substitution by *Hermetia illucens* meal (Hi50).