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1 Organoleptic qualities of rainbow trout fed with new ingredients
2 added to a plant-based diet

3 Short title: New feed ingredients and trout fillet quality

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23

24 **Abstract**

25 Background: The variety of new ingredients used to replace traditional marine raw materials in
26 fish feed requires to study their possible impact on products quality. Rainbow trout were fed
27 four different experimental diets: a 100% plant-based diet (V1), supplemented or not with
28 alternative ingredients, insect and yeast (V2), and processed animal products (PA), and
29 compared to fish fed a control (Co) commercial-like diet, including some marine ingredients.
30 Organoleptic qualities of raw, cooked, and smoked fillets were evaluated by both instrumental
31 and sensory measurements, at pan-size and large trout stages.

32 Results: Instrumental assessments of quality showed that the tested diets had little or no impact
33 on fillet pH, dry matter content or texture parameters. The main effect of the tested diets was
34 measured on fillet color. Fillet from fish fed the Co diet were the most colorful, whether raw,
35 cooked or smoked. Fish fed the PA diet had raw, cooked, and smoked fillet with the lowest a*
36 and b* values. Sensory analysis also showed a difference in fillet color both for cooked fillets
37 at pan-size and for cooked and smoked fillets of large trout. Some other differences between
38 the diets were noted by the sensory panel, particularly on the smoked fillets from the large trout.

39 Conclusion: The inclusion of new ingredients such as insect or yeast had no major impact on
40 organoleptic quality, but the incorporation of processed animal products considerably affected
41 fillet color, even after cooking or smoking, and, to a lesser extent, the perceived flavor.

42 **Key-words:** salmonids; quality, diet, replacement, fillet color, raw and processed quality

43

44 Introduction

45 The sustainability of the fish farming industry requires the replacement of traditional marine
46 raw materials, fish meal and oil, by new ingredients like vegetable raw materials (1, 2). For
47 carnivorous fish, a high replacement rate with plant-based diets can reduce fish growth and
48 even impact some quality traits such as technological quality, reducing for example carcass
49 yield via a higher peri-visceral fat deposition (3). Nutritional quality is also often affected, with
50 a lower flesh poly-unsaturated fatty acids (PUFA) content, miming those of diet ingredients,
51 and in some case a lower protein content (4). Sensory qualities are less affected by the
52 substitution of fish ingredients by vegetable ones in particular if fish growth is not affected (1,
53 5-9). Nevertheless, many studies report a perceptible impact on fillet odor, flavor or color, but
54 also sometimes on texture parameters (4, 5, 9-14).

55 To overcome these disadvantages, two strategies were explored: 1) tailoring the plant-based
56 feed by mixing plant sources and/or introducing new ingredients (11), and 2) selective breeding
57 to get fish better adapted to use these new feeds (15).

58 Among the new ingredients, various insects species was tested (raw or defatted meal or
59 hydrolysates), or algae, particularly microalgae rich in PUFA which can restore the nutritional
60 quality (16, 17). Many studies, mostly in rainbow trout, report that the inclusion of insect meal
61 in diet does not alter the sensory qualities of the products (18-20). However, other studies report
62 effects on color (21, 22), flavor (21, 23), or texture (21, 23), especially when the substitution
63 rate is high. Moreover, the effects observed can differ depending on fish species (seabass,
64 seabream or trout), so the inclusion of insect meal should be observed species by species (24).

65 Yeast is a novel high-quality protein source, and has been shown to have a positive effect on
66 intestinal health in fish fed plant-based feeds, particularly with soya meal (25, 26). In rainbow
67 trout, a mix of microorganisms, including yeast, was shown not to affect fillet sensory quality
68 (27), but some yeasts contain pigments that can affect the color of fillets (28). Processed animal

69 products (PAP) in fish feed enables to use by-products from the production of terrestrial animals
70 and provides high-quality nutrients for fish, particularly for carnivorous species. As for other
71 raw materials mentioned above, the substitution of marine ingredients by PAP can affect fish
72 growth (26), muscle fatty acids profile (29) and sensory qualities (11, 30). However, a number
73 of studies have shown that there are no major drawbacks to its use, at least with a limited
74 substitution rate (29, 31). It could nevertheless be pointed out that the introduction of blood
75 meal can lead to carotenoid pigment degradation in feed resulting in lower fillet color score
76 (31). With regard to the replacement of fish oil with fat of terrestrial origin, it has been shown
77 that the high rate (75%) substitution of fish oil by chicken fat affected the muscle content of
78 some volatile compounds, without any difference being detected by a sensory panel (10).

79 The other route to more sustainable aquaculture that limits the use of marine ingredients is to
80 have fish better adapted to these new feeds. Indeed, genetic selection was shown to efficiently
81 improve rainbow trout survival and growth (15, 32). We chose to test the effects of diets on this
82 specific model in order to benefit from fish with the best growth potential using alternative
83 feeds.

84 In addition, given the lack of data on the sensory qualities on market size and processed
85 products, we decided to assess the consequences of experimental diets, at two commercial
86 stages, for raw, cooked and smoked fillets. This approach makes it possible to evaluate the real
87 consequences for the product consumed, and to see whether characterization of the raw product
88 will predict the quality of the processed product.

89 The aim of this study was therefore to assess the impact of adding new feed ingredients (insect,
90 yeast and PAP) to a 100% plant-based diet on the organoleptic qualities of raw, cooked and
91 smoked rainbow trout fillet.

92 **Materials and methods**

93 **Fish, diets and rearing experiment**

94 Eggs from females PEIMA's experimental "Autumnal" strain were fertilized with sperm from
95 males of the 4th generation of selective breeding for ability to survive and grow on a full plant-
96 based diet (32).

97 Fish were distributed among 12 circular 2 m diameter 1.8 m³ tanks (n= 350 fish per tank, 70g
98 mean weight at the start of the trial), representing each of the four diets in triplicates. They were
99 reared in a flow through system at natural photoperiod, constant oxygen (7.5 to 9.5 mg/L) and
100 ambient temperature (6°C to 19°C). Fish were fed automatically to satiation twice daily and the
101 unfed pellets were collected and recorded to adjust feed distribution.

102 Fish were first all fed the same commercial diet during seven months before the start of
103 experimentation. Experimental diets were then distributed up to commercial pan-size (13
104 weeks) and large trout stages (45 weeks) (Figure1). The four experimental diets were: a
105 commercial-like diet (**CO**), a plant-based diet (**V1**) considered as a low-cost diet, the same
106 plant-based diet supplemented with 5% insect hydrolysate extract and 5% yeast wall extract
107 (**V2**), or the previous V2 supplemented plant-based diet added with 5% processed animal
108 proteins (**PA**) (formulation and proximate composition of diets in Table S1). These diets were
109 formulated and produced by Le Gouessant (Lamballe-Armor, France) to be iso-proteic, iso-
110 lipidic and iso-energetic.

111 **Fish slaughter and sampling**

112 Sampling was carried out after 13 (pan-size) and 45 (large trout) weeks of feeding on the
113 experimental diets. The fish were fasted for 48 h before slaughter.

114 For fish traits measurements, 10 fish per tank (30 per diet) were harvested as fast as possible
115 with a handling net, anesthetized with tricaine MS-22 (0.05 g/L) in a separate tank, stunned by
116 a sharp blow on the head and bled by gill arch section. Fish characteristics were assessed as

117 previously described (33), and data are reported in supplemental Tables S2 and S3. For half of
118 the fish, fillets were vacuum-packed within 1 hour after fish death. For both pan-size and large
119 trout stages, the right fillet was stored on ice until further analysis as raw fillet at 2 days *post-*
120 *mortem* (dpm). For the left fillet, it was either packed and stored in ice for measurement after
121 cooking (pan-size stage) or packed and stored in a cold-room (2°C) until salting and smoking
122 at 2 dpm (large trout stage) for measurement as smoked fillet.

123 Fish for sensory evaluation were caught as fast as possible with a fishing net, stunned by a blow
124 on the head and bled by gill arch section in water. They were weighted (data in Table S2),
125 gutted, and filleted. At pan-size, the fillets of five fish were individually vacuum packed in
126 marked plastic bag and stored in ice for two days before rapid freezing at -60°C with liquid CO₂
127 in a cryogenic chilling cell (Messer, France) and storing at -80°C for three weeks until sensory
128 tests. For large trout, fillets from ten fish were also vacuum packed. The ten right fillets were
129 stored in ice for two days before freezing as described above, whereas the ten left fillets were
130 stored two days in a 2°C cold room before smoking. The smoked fillets were then frozen at 7
131 dpm as described above.

132 **Fillet smoking**

133 After two days of storage, **fillet was salted and** smoked at PEIMA fish processing facility. Fillet
134 was weighed and hand-salted proportionally to the fillet weight (7%) with "**Sel de Guérande**"
135 **salt (86% sodium chloride)** for 4 h on grids. Fillet was thereafter rinsed with tap water to remove
136 excess salt, drained, and then cold-smoked for 5 h at 23°C with green beech wood in an air-
137 conditioned and horizontally-ventilated smoking cabinet equipped with a GF 200 automatic
138 smoke generator (Arcos® CTF 100 SH). Fillet was weighed before and after the salting and
139 smoking procedure to estimate the smoking yield of fillet. Smoked fillets were deboned, then
140 vacuum-packed and cold-stored (0–4 °C) until instrumentally or sensory quality assessments.

141 Instrumental measurements of quality

142 Fillet were measured for raw quality parameters at 2 dpm, cooked quality parameters at 4 dpm
143 after cooking at 2 dpm (pan-size stage), or smoked quality parameters at 7 dpm after
144 salting/smoking at 2 dpm as previously described (large trout stage). All the measurements were
145 done at room temperature. Raw fillet color was assessed as previously described (33), with the
146 mean value of two (pan-size) or three (large trout) measurements (Figure 1). Ultimate pH, dry
147 matter content and mechanical strength of fillet (Figure 1) were measured as previously
148 described (33).

149 At pan-size, at 2 dpm, whereas one fillet was measured as raw fillet, the second fillet was
150 cooked as previously described (33) and cooking yield was calculated as fillet weight after
151 cooking / weight before cooking. Physical measurements of the quality parameters for the
152 cooked fillets were assessed in the same way as for raw fillets.

153 Instrumental quality parameters of the smoked fillet of large trout were measured as described
154 above for the raw fillet.

155 Sensory measurements of quality

156 Three conventional profiling tests were conducted according to ISO 13299 (34) standard, two
157 on cooked fillets (at pan-size stage and large trout stages) and one on smoked fillet (large trout).
158 The descriptors used were chosen from previous studies (33, 35) supplemented by a flash
159 profile descriptor generation session (36) using the study cooked products. Twenty-two
160 panelists belonging to the IFREMER staff experienced in seafood evaluation were invited to
161 score the samples. The day before the sensory test, samples were thawed overnight at 4°C. For
162 both cooked and smoked fillets evaluation, panelists received a sample of the same dorsal part
163 of the fillet. Fillet portions of 50-60 g were cooked in a glass bowl covered with a cap. The four
164 diet groups, assigned 3-digit numbers, were cooked simultaneously in a microwave oven for
165 1.5 minutes at 600 watts, randomized for the order of presentation and served to the panelists.

166 For smoked fillet, panelists received four pieces 1cm wide, cut from the dorsal part of the fillet.
167 Sessions were performed in individual partitioned booths controlled for temperature (20°C) and
168 light (day light, $t = 6500^\circ\text{K}$) (37). Data were collected with a computerized system (Fizz,
169 Biosystèmes, Dijon, France). Panelists rated the sensory attributes on a continuous scale, from
170 low intensity (0) to high intensity (10).

171 **Statistical analysis**

172 All of the results are expressed as the mean \pm standard deviation. The number of fish measured
173 is specified in each table. A one-way ANOVA analysis was used to test the effect of diets on
174 instrumentally measured quality parameters. The significant level was set at $p < 0.05$. The
175 significant differences between the mean values of each diet were determined using the
176 Newman-Keuls test. All of these analyses were performed using Statistica for Windows
177 (version 5.1) software.

178 A two-ways ANOVA analysis, with feed and panellists as independent factors, was performed
179 on sensory data with Fizz 2.46b software (FIZZ system, Biosystèmes, Dijon, France). In cases
180 where significant differences occurred, the means were compared by the Duncan test at the 0.05
181 level of probability.

182 **Results**

183 **Instrumental measurements of quality**

184 For **raw** fillet from **pan-size** trout, diet affected fillet **color**. If no effect was measured on fillet
185 lightness L^* , fillet redness a^* was higher for Co fed fish, intermediate for V1 and V2 fed fish,
186 and the lowest for PA fed fish (Figures 2 & 3). Fillet from PA fed fish also showed lower
187 yellowness b^* value than the three other groups (Table 1). **Mechanical resistance** of fillet was
188 slightly affected with a lower value of maximal force measured for fillet from fish fed V1,
189 compared to those from the three other diets, but no difference was measured on the weighted
190 parameter (Table 1). The comparison of the means of muscle **dry matter content** between the

191 four groups showed no significant difference (Table 1). No effect of either diet was measured
192 on muscle ultimate **pH** (Table 1).

193 The effect of diet on **raw fillet color** parameters was confirmed with **large trout**. The
194 comparison of fillet from fish fed the four diets showed a lower lightness for fillet from Co
195 group, lower values of a* and b* for fillet from PA group, compared to the three other diets,
196 and a higher redness of fillet from Co group (Table 1 and Figures 2 & 3). A slight effect of diet
197 was observed on specific resistance with a higher value for fillet from fish fed Co compared to
198 those from V1 and V2 diets, the PA group giving intermediate values not different from the
199 other groups (Table 1). No effect of diet was measured on muscle **ultimate pH** and no
200 difference in muscle **dry matter content** was measured between the groups for large trout
201 (Table 1).

202 At **pan-size**, higher **cooking yield** was measured for fillet from fish fed Co diet compared to
203 those from fish fed V2 diet ($p < 0.05$), whereas intermediate values were obtained for V1 and
204 PA diets groups (Table 2). The analysis of **cooked fillet** showed that diet affected color and dry
205 matter content, but not pH or mechanical resistance. Cooked fillet redness was the lowest for
206 the PA group and the highest for Co group, V1 and V2 groups giving intermediate values
207 (Figure 2). Cooked fillet from fish fed V2 diet had higher value of yellowness than those fed
208 PA diet, whereas Co and V1 groups gave intermediate values. The dry matter content of cooked
209 fillet from V2 group was higher than that of V1 and PA groups, whereas Co group gave
210 intermediate value.

211 In **large trout**, no significant effect of feed was measured on salting/smoking yield (Table 2).
212 A significant effect of diet was measured on smoked fillet lightness and redness. Higher
213 lightness was measured for smoked fillet from the PA group, compared to Co and V1 groups
214 ($p < 0.001$). Smoked fillet from the PA group also had a lower value of redness than all three
215 other diet groups. Moreover, smoked fillet from the Co group gave higher value of redness than

216 those from V2 group, fillet from V1 group giving intermediate values (Figures 2 & 3). No
217 difference in mechanical resistance was measured between diets.

218 **Sensory quality**

219 Sensory evaluation of **cooked fillet** from **pan-size** fish fed the four diets showed that only few
220 criteria distinguish the products (Table S4). Only the PA group seemed to show some
221 differences with the other three groups. For this group, fillet color was significantly less orange
222 (Figure 2), the texture in the mouth was moister and the overall flavor less intense. The earthy
223 taste was less pronounced in the Co group than in the other groups (Figure 4).

224 The sensory analysis of **large trout cooked fillet** showed that only color perception was
225 significantly affected by diet ($p < 0.001$). Fillet from the Co group were the most colorful, fillet
226 from PA group were the least, and fillet from V1 and V2 groups were intermediate (Table S5
227 & Figure 2). A few more tendencies (p -value around 0.10) were observed as a less strong overall
228 flavor for the PA group as well as a slightly more metallic and "blood/meat" taste for the V2
229 and PA diets (Figure 4). For this last criterion, we could note that panelists had no particular
230 training, and, that the observed differences were mainly due to the scoring of six panelists.
231 However, the comments after tasting indicate a specificity in the PA group, which was not
232 always appreciated and difficult to describe.

233 **Smoked fillets from large trout** were all globally perceived as very smoked, with a mean score
234 around 5 for odor and flavor. Comparing the four diets, the main differences observed
235 concerned the PA group, the difference in color, previously identified between the PA group
236 and the others, was confirmed in the smoked product (Figures 2 & 4). The only odor descriptor
237 that differentiates the products is the smell of butter, significantly more intense in the Co group
238 (Table S6 and Figure 4). Concerning texture perception, compared with the other groups, the
239 PA group was among those the least firm and with more melt-in-the-mouth. This group was
240 also perceived as less smoky and less salty than the other three groups.

241 **Discussion**242 **Plant-based diet had limited but significant impact on sensory quality**

243 The impact of replacing traditional marine raw materials with plant ingredients in fish feed on
244 the flesh organoleptic qualities has been widely studied for many years. The conclusions of
245 these studies are not unanimous, and this can be due to various impacting factors as the choice
246 of substituting either fish meal, or fish oil or both, the rate of substitution, the duration of
247 substituted feed distribution and the nature of the plants chosen as substitute (1, 2, 4). Overall,
248 we can conclude that replacement by plant ingredients does not have a major impact on sensory
249 qualities, but minor effects are observed in almost all the studies, and may concern all
250 dimensions of sensory quality, i.e., color, flavor and texture. These effects are all the more
251 marked when the fish growth is affected by the substitution, but an impact is often reported
252 even when fish have similar body weight (5, 10-12). Our data are broadly in line with previous
253 works.

254 Fish fed the V1 full plant-based diet were indeed smaller than those fed with Co diet, but with
255 no major impact on organoleptic quality, at least for texture and flavor. The slightly lower
256 instrumentally firmness we measured for fish fed a plant-based diet was already observed in
257 sea bass (14) or sea bream (7). But an opposite result, with higher fillet hardness for fish fed
258 plant-based diet has also be observed (5), as was no effect (9), suggesting that this impact is not
259 a general trend of plant-based diet on fillet quality. Moreover, no difference between Co et V1
260 groups was measured in sensory texture parameters, which is consistent with previous studies
261 (9, 14).

262 Cooked fillet from fish fed plant-based diets often exhibit fewer fishy sensory perception than
263 those fed fish meal diet (5, 6, 9). However, some other works do not report any impact of the
264 replacement on sensory flavor parameters (8, 10). We did not observe significant difference in
265 odor or flavor in cooked fillets from fish fed Co and V1 diets, whether at pan-size or large trout

266 stage. This may be due to our experimental design, which also included other diets, such as the
267 PA diet, which had a greater incidence on the odor and flavor perception by the sensory
268 panelists, which may have masked a more subtle difference between the Co and V1 diets. A
269 significant lower perception of butter odor was nonetheless measured for smoked fillet from
270 fish fed V1 diet compared to those fed Co diet. This unexpected effect of a plant-based diet
271 merits to be further explored, as almost no data are available on the effect of the substitution of
272 marine feedstuffs on organoleptic quality of smoked products.

273 The main effect we observed, between Co and V1 diets, concerned fillet color. Indeed, fish fed
274 V1 diet exhibited a higher raw fillet lightness (L^*) and a lower redness (a^*) for both raw and
275 cooked fillet in instrumental color assessment. A lower cooked fillet orange intensity was also
276 perceived by the sensory panel. Such an effect of plant-based diet was already observed in
277 rainbow trout (5). In salmonid, the main determinant of fillet color is the amount of pigment
278 deposited in muscle. Here, all the diets were equally supplemented with astaxanthin, but fish
279 fed V1 diet were smaller, so we can assume they ingested less feed, and so less pigment, which
280 could explain a lower fillet pigmentation. Furthermore, replacing marine ingredients by plant-
281 based ones greatly impact the fatty acid composition of muscle, decreasing the PUFA content
282 (11), and the type and quantity of PUFA was suspected to affect uptake and deposition of
283 carotenoids pigments in salmon (38). Such an effect may also explain the difference in fillet
284 color we observed.

285 **Adding new ingredients in plant-based diet did not affect sensory quality**

286 Previous studies on the incorporation of insect feedstuff in fish feed show that this new
287 ingredient can affect sensory qualities or not, depending on insect species and quantity
288 incorporated in feed (19, 23). The effect of yeast incorporation is however less much
289 documented, even though a few studies have assessed the impact on product sensory quality
290 (27, 28). In the present study, when comparing groups of fish fed V2 vs V1 diet, we can

291 conclude that the incorporation of insect hydrolysate and yeast extract proteins had quite no
292 impact on product quality. No difference was measured on the raw product. The dry matter
293 content of cooked fillet was higher for V2 group at pan-size, but that can be explained by a
294 slightly, even if not significant, lower cooking yield and higher dry matter content for this
295 group. No difference was measured with sensory assessment of cooked product, both at pan-
296 size and large trout stages. Only a slightly more pronounced melting texture was only measured
297 in the smoke of fish fed V2 compared to those fed V1. The lack of effect of adding insect and
298 yeast ingredients in plant-based diet is probably due to the low rate of incorporation of these
299 ingredients, with only 5% of insect hydrolysate and 5% yeast extract proteins added in V2 diet,
300 compared to V1 diet. The effects, reported in the literature, of substituting fishmeal with insect
301 meal on organoleptic qualities indeed appear most often for high substitution rates (23).

302 **PA diet greatly affected sensory quality**

303 Of the diets tested in the present study, the one that most affected sensory qualities was PA diet.
304 The main effect observed was on fillet color, as PA diet led to a less colored fillet both for raw,
305 cooked and smoked fillet, and this was measured both with instrumental assessment and by the
306 sensory panel. If PA diet had no other impact on other measured physical parameters, a
307 significant impact on cooked and smoked sensory texture and flavor was also measured. The
308 PAP incorporated in PA diet were blood meal and poultry meal. The main effect on fillet color
309 we observed can be attributed to the degradation of feed astaxanthin catalyzed by iron present
310 in blood meal, as previously measured in a trial on salmon fed porcine blood meal (31).

311 The other observed effects of PA diet, especially on flavor criteria, appear more complex to
312 explain. Indeed, a specific flavor to the PA group was detected during pan-size evaluation,
313 which led us to add the "blood/meat" flavor criterion for large stage evaluation, which gave
314 slightly higher scores ($p < 0.10$) for the PA group. Such a marked impact of diet included PAP
315 on fillet flavor has not been reported in previous studies. However, a less intense fish aroma

316 was noted in pan-size rainbow trout fed a diet in which 100% fishmeal was substituted by
317 poultry by-products (11), and a lower acceptance of fish fed PAP diet was also mentioned for
318 pan-size rainbow trout in another study (30). Other studies report no effect in incorporating
319 PAP in fish feed, or an improvement in sensory quality (10, 31). This discrepancy may be due
320 to the nature of the PAPs used as fish meal or oil substitution in fish feed, and considering the
321 sensory attributes affected in our study, we can presume that it is mainly the presence of blood
322 meal that contributes to the alteration of the sensory perception we observed. A specific flavor
323 of the PA group was also measured with smoked fillet which were perceived with a less smoked
324 and salty flavor. As this is the first study dealing with the impact of diet including PAP on the
325 sensory qualities of smoked products, it is difficult to place this result in the context of previous
326 work. Whatever, these effects are all the more notable given that, in our study, only 5% PAP
327 was added in the PA diet.

328 Conclusion

329 The main effect of ingredients tested were on fillet color, especially for the diet included PAP
330 which led to less pigmented raw, cooked and smoked fillets. Sensory analysis nevertheless
331 revealed more subtle effects of the PAP including diet on the flavor of cooked and smoked
332 products. Most of results were similar at pan-size stage, after only 3 months of experimental
333 feeding, and at large trout stage, after almost 1 year of experimental diets distribution. However,
334 the detrimental effect of the PAP containing diet, noticeable from the pan-size stage, was greater
335 in large trout after long-term feeding with experimental diet, and affected sensory attributes of
336 smoked fillets.

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344 [Ethical statement](#)

345 The fish involved in the present study were produced and reared in the INRAE-PEIMA facilities
346 (PEIMA, INRAE, Fish Farming systems Experimental Facility, DOI:
347 10.15454/1.5572329612068406E12, Sizun, France). This structure is authorized for animal
348 experimentation under French regulation D29-277-02. The experiments were carried out from
349 July 2018 to June 2019 following the Guidelines of the National Legislation on Animal Care
350 of the French Ministry of Research (Decree N°2001-464, May 29, 2001). For this experiment,
351 project authorization was not required. Experiments were conducted under the official license
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353

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Figures

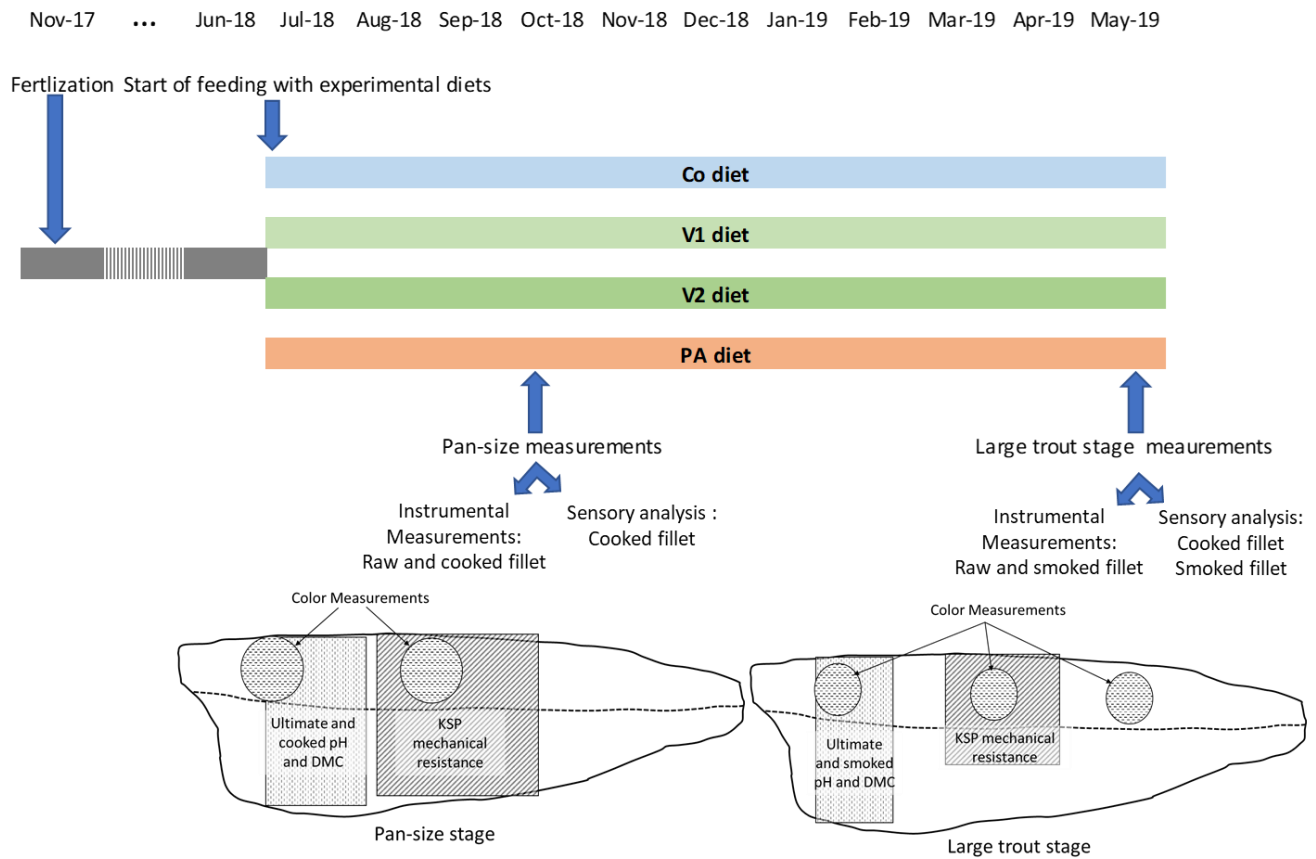


Figure 1: Schematic representation of the experimental design and measurements carried out on right raw fillet, cooked or smoked left fillets; for details see text. KSP: Kramer Shear Press, DMC: Dry Matter Content.

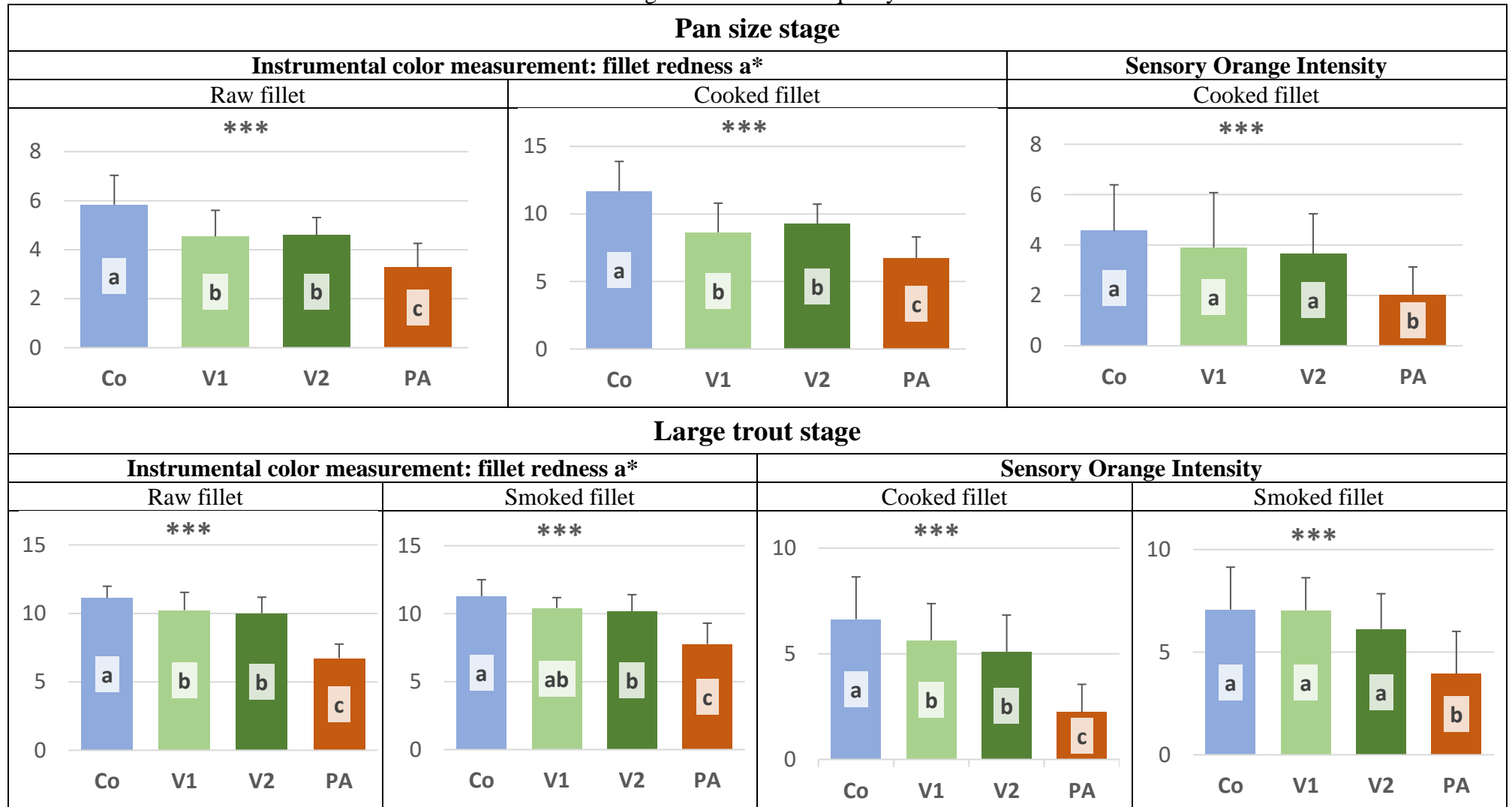


Figure 2: Effect of the four diets, Co, V1, V2, and PA on pan-size and large trout raw, cooked and smoked fillets color evaluated either with instrumental Minolta measurement or sensory assessment; ***: p<0.001.

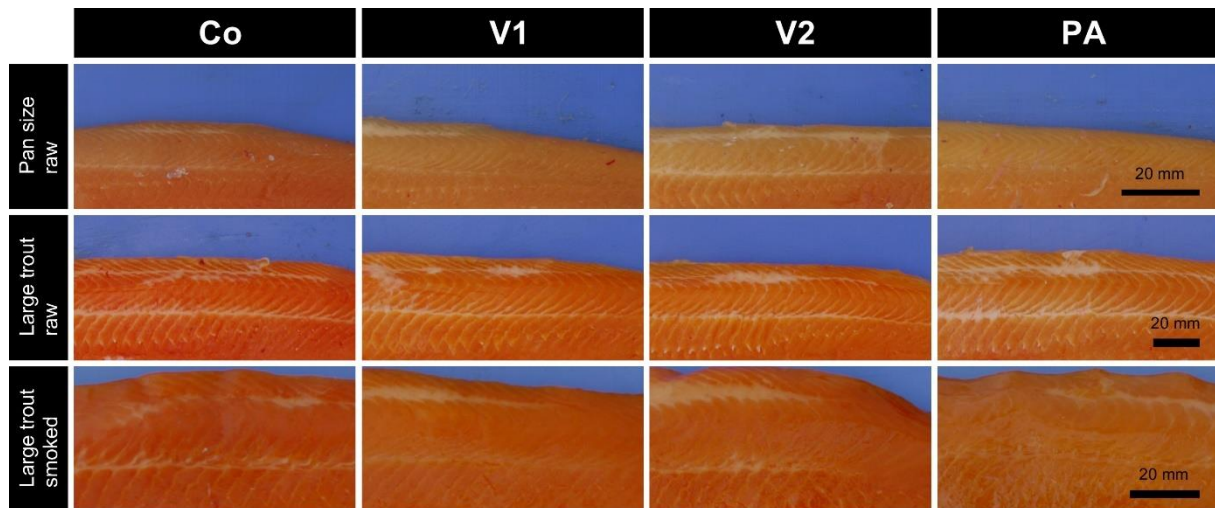


Figure 3: Illustration of the effects of the four diets, Co, V1, V2, PA, on raw and smoked fillets appearance from pan-size (raw) and large (raw and smoked) trout. The photos chosen are those of fillet whose redness a^* value corresponds to the mean of the group.

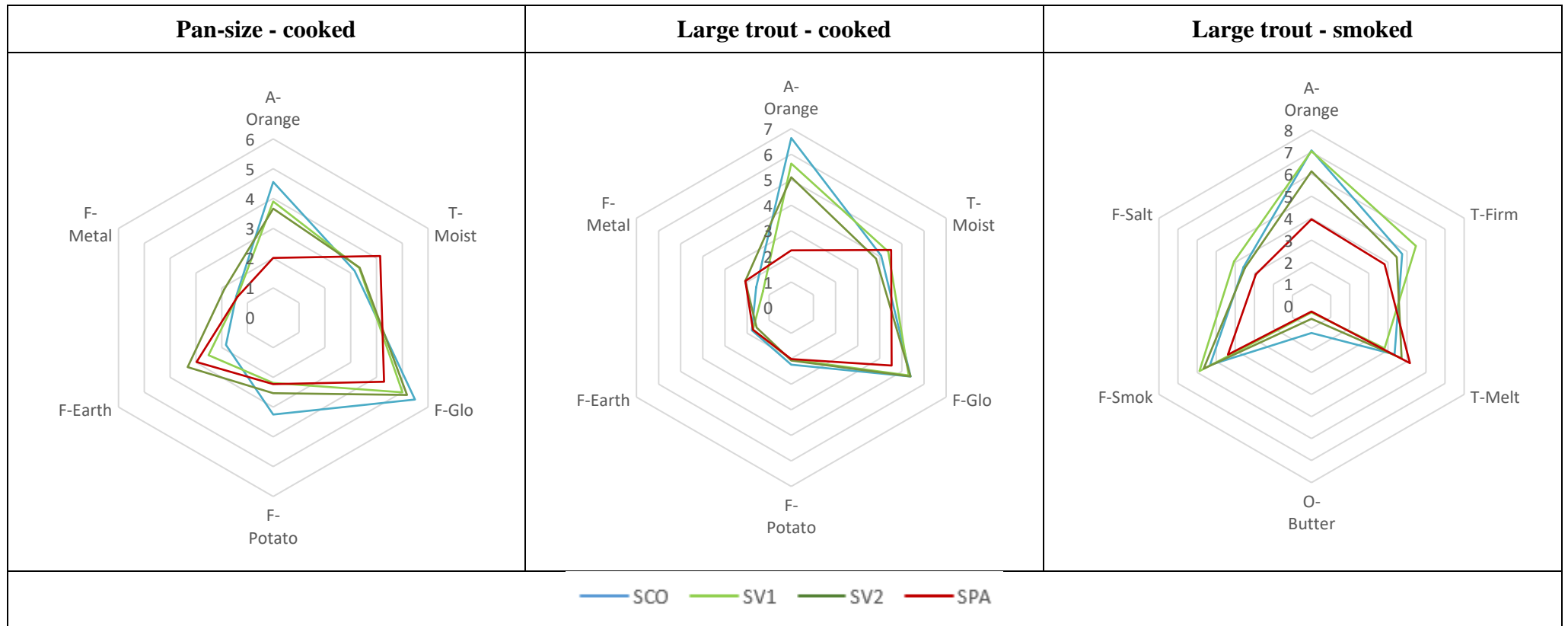


Figure 4: Sensory parameters impacted by diets for cooked fillet at pan-size and large trout stages, and for large trout smoked fillet; Mean scores of 20 panellists. A: Appearance; T: Texture; O: Odor; F: Flavor. The complete data of sensory analysis are purchased in supplementary tables S5, S6, and S7.

Tables

Table 1: Effect of diet on raw fillet characteristics, mean \pm standard deviation, n=15.

Diet:	Co	V1	V2	PA	Feed effect p value:
Pan-Size stage					
pHu	6.41 \pm 0.05	6.44 \pm 0.04	6.42 \pm 0.06	6.41 \pm 0.04	0.17
L*	39.7 \pm 2.3	40.5 \pm 1.2	40.7 \pm 1.6	40.2 \pm 1.9	0.44
a*	5.8 \pm 1.2a	4.5 \pm 1.1b	4.6 \pm 0.7b	3.3 \pm 1.0c	<0.001
b*	13.0 \pm 1.5a	13.2 \pm 2.2a	13.5 \pm 1.4a	11.1 \pm 1.8b	0.002
DMC (%)	27.4 \pm 1.3a	26.4 \pm 1.3a	27.6 \pm 1.2a	26.6 \pm 1.4a	0.037
Fmax (N)	578 \pm 75a	515 \pm 63b	529 \pm 85ab	581 \pm 51ab	0.020
Spe Rstce (N/g)	16.6 \pm 1.6	17.8 \pm 2.1	17.4 \pm 3.1	18.3 \pm 2.1	0.26
Large Trout stage					
pHu	6.32 \pm 0.08	6.31 \pm 0.04	6.29 \pm 0.05	6.31 \pm 0.07	0.60
L*	41.7 \pm 1.5b	43.6 \pm 2.4a	43.7 \pm 1.6a	44.1 \pm 1.7a	0.003
a*	11.1 \pm 0.9a	10.2 \pm 1.3b	10.0 \pm 1.2b	6.7 \pm 1.0c	<0.001
b*	19.9 \pm 1.3a	20.3 \pm 2.1a	20.1 \pm 2.0a	17.3 \pm 1.7b	<0.001
DMC (%)	32.2 \pm 1.6	32.8 \pm 1.6	31.9 \pm 1.7	31.2 \pm 1.4	0.13
Fmax (N)	719 \pm 128	634 \pm 75	650 \pm 92	659 \pm 82	0.095
Spe Rstce (N/g)	8.7 \pm 1.5a	7.5 \pm 0.8b	7.8 \pm 0.9b	7.8 \pm 1.1ab	0.040

pHu: ultimate pH (measured 48h *post-mortem*); DMC: Dry Matter Content; L*: Lightness; a*: redness; b*: yellowness; Fmax: maximal force; Spe. Rstce: Kramer shear press specific resistance = Fmax/sample weight. Means with different letters in the same row are significantly different (p<0.05). Bold number and characters indicate significant difference at p value < 0.05.

Table 2: Effect of diet on cooking and smoking yield, and cooked (pan-size) and smoked (large trout) fillet characteristics, mean \pm standard deviation, n=15.

Diet:	Co	V1	V2	PA	Feed effect p value:
Cooked fillet - Pan-Size stage					
Cooking Yield (%)	90.3 \pm 1.2a	89.1 \pm 1.5ab	88.7 \pm 1.4b	89.5 \pm 1.8ab	0.028
pHc	6.62 \pm 0.03	6.65 \pm 0.04	6.61 \pm 0.06	6.62 \pm 0.06	0.25
L*	71.7 \pm 2.1	71.2 \pm 2.9	71.5 \pm 2.6	72.9 \pm 3.0	0.31
a*	11.7 \pm 2.2a	8.6 \pm 2.2b	9.3 \pm 1.4b	6.7 \pm 1.6c	<0.001
b*	27.3 \pm 2.5ab	27.2 \pm 3.1ab	28.9 \pm 1.9a	25.8 \pm 2.3b	0.018
DMC (%)	28.9 \pm 1.5ab	28.4 \pm 1.8b	29.9 \pm 1.4a	28.1 \pm 0.9b	0.006
Fmax (N)	713 \pm 99	605 \pm 100	653 \pm 144	698 \pm 160	0.11
Spe Rstce (N/g)	24.5 \pm 2.9	23.7 \pm 3.8	25.1 \pm 3.2	25.7 \pm 3.7	0.42
Smoked fillet - Large Trout stage					
Smoking Yield (%)	84.6 \pm 1.7	85.5 \pm 1.5	85.6 \pm 1.4	85.1 \pm 1.9	0.33
pHs	6.15 \pm 0.05	6.13 \pm 0.03	6.13 \pm 0.04	6.13 \pm 0.03	0.53
L*	39.9 \pm 1.0b	41.0 \pm 1.5b	40.8 \pm 1.3ab	42.0 \pm 1.5a	0.001
a*	11.3 \pm 1.2a	10.4 \pm 0.8ab	10.2 \pm 1.2b	7.8 \pm 1.5c	<0.001
b*	21.6 \pm 1.7	21.8 \pm 1.3	21.5 \pm 1.4	21.7 \pm 2.2	0.95
DMC (%)	37.2 \pm 1.8	38.0 \pm 1.8	37.7 \pm 1.5	36.7 \pm 1.4	0.15
Fmax (N)	535 \pm 143	506 \pm 75	510 \pm 92	500 \pm 108	0.81
Spe Rstce (N/g)	7.2 \pm 2.2	6.9 \pm 1.1	6.9 \pm 1.1	6.5 \pm 1.1	0.70

Cooking yield = cooked fillet weight / raw fillet weight; pHc: cooked fillet pH; DMC: Dry Matter Content; L*: Lightness; a*: redness; b*: yellowness; Fmax: maximal force; Spe. Resistance: Kramer shear press specific resistance = Fmax / sample weight; Smoking yield = smoked fillet weight / raw fillet weight; pHs: smoked fillet pH; Means with different letters in the same row are significantly different (p<0.05). Bold number and characters indicate significant difference at p value < 0.05.

Supplemental Tables

Table S1: Formulation and proximate composition of experimental diets: CO commercial-like diet, V1 a plant-based diet, V2 the V1 plant-based diet supplemented with 5% insect hydrolysate extract and 5% yeast wall extract, PA the V2 supplemented plant-based diet added with 5% processed animal proteins. Two feed granulometries were distributed, from starting the experiment to pan-size stage (PS) trout, and from pan-size to large trout stage (LT).

Ingredients (g/100g)	CO		V1		V2		PA	
	PS	LT	PS	LT	PS	LT	PS	LT
Fish meal	25.6	23,5						
Fish oil	6	7						
DHA-rich algae meal			5.4	6.4	5.4	6.4	5.4	6.4
Insect hydrolysate					5	5	5	5
Yeast extract proteins					5	5	5	
Processed animal proteins ¹							5	5
Vegetal oils ²	13	16.3	16.6	20	15.8	19.4	15.7	19.2
Plant proteins ³	52.5	50	72.5	68	63.6	59.1	59	54.7
Additives ⁴	2.9	3.3	5.5	5.5	5.2	5.1	5.9	4.7
Astaxanthin 10%	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Proximate composition								
Dry Matter (DM in %)	93.0	95.7	92.1	94.7	91.9	94.1	92.2	94.4
Proteins (% DM)	39.6	41.7	40	41.8	39.2	40.5	39.5	40.2
Lipids (% DM)	22.3	27.6	21.2	28.8	22.2	29.6	22.5	29.5
Ash (% DM)	7.5	6.7	4.5	4.4	4.8	4.4	4.7	4.9

1: Processed animal proteins: blood meal / poultry meal 2/3

2: Vegetable oils: rapeseed oil, linseed oil

3: Plant proteins: CO wheat gluten, corn gluten, soy concentrate, soybean meal, rapeseed meal, peeled fava bean and wheat; V1: corn gluten, pea protein, soybean meal, guar meal, peeled faba bean, wheat; V2 & PA: a fraction of wheat gluten, corn gluten, pea protein, soybean meal, guar meal and wheat as well as a fraction of rapeseed oil were substituted by insect hydrolysate & yeast extract & processed animal protein fraction

4: including monocalcium phosphate, phytase, amino acids (Met, Thr, Lys), vitamin and mineral premix, antioxidant, anti-fungus, and rapeseed lecithin (only in CO diet)

Table S2: Body weight of the fish sampled and measured at pan-size and large trout for instrumental and sensory quality evaluations, mean \pm standard deviation,

Diet:	Co	V1	V2	PA	Feed effect p value:
Pan-Size stage					
Quality instrumental measurements at slaughter					
n=30	345 \pm 46a	283 \pm 32c	303 \pm 49bc	322 \pm 50ab	<0.001
Including Quality instrumental measurements of raw fillet at 2 dpm and cooked fillet					
n=15	340 \pm 35a	283 \pm 38b	295 \pm 48b	318 \pm 47ab	0.003
Sensory evaluation – Cooked fillet					
n=5	384 \pm 60	343 \pm 50	354 \pm 43	355 \pm 35	0.58
Large Trout stage					
Quality instrumental measurements at slaughter					
n=30	1807 \pm 329a	1646 \pm 254b	1619 \pm 254b	1644 \pm 248ab	0.034
Including Quality instrumental measurements of raw fillet at 2 dpm and smoked fillet					
n=15	1716 \pm 295	1609 \pm 218	1602 \pm 200	1635 \pm 174	0.50
Sensory evaluation – Cooked and smoked fillet					
n=10	2336 \pm 211a	1904 \pm 288b	1880 \pm 329b	1885 \pm 144b	<0.001

Table S3: Effect of diet on trout condition factor, adiposity, and technological qualities, mean \pm standard deviation, n=30 for raw cutting yields, n=15 for dressed skinned fillet yields, and cooked/smoked fillet yield.

Diet:	Co	V1	V2	PA	Feed effect p value:
Pan-Size stage					
CF	2.04 \pm 0.13a	1.90 \pm 0.14b	2.02 \pm 0.18a	1.98 \pm 0.14a	0.003
Fat-meter[®] value (%)	5.5 \pm 1.0a	4.4 \pm 0.9b	5.6 \pm 1.0a	5.2 \pm 0.7a	<0.001
Carcass Yield (%)	88.9 \pm 1.1b	89.6 \pm 0.8a	88.9 \pm 0.8b	88.6 \pm 1.2b	0.005
VSI (%)	9.8 \pm 1.1a	9.2 \pm 0.9b	9.7 \pm 0.8a	9.9 \pm 1.0a	0.03
HSI (%)	1.00 \pm 0.15	0.90 \pm 0.15	0.98 \pm 0.15	0.95 \pm 0.14	0.08
GSI (%)	0.052 \pm 0.024	0.060 \pm 0.036	0.065 \pm 0.026	0.065 \pm 0.021	0.23
Head (% body weight)	13.3 \pm 0.6b	13.7 \pm 0.7a	13.4 \pm 0.6ab	13.1 \pm 0.7b	0.003
Raw fillet yield (%)	63.1 \pm 2.8	62.9 \pm 2.1	63.3 \pm 2.1	63.5 \pm 2.1	0.76
Dressed skinned fillet yield (%)	47.6 \pm 2.7	46.8 \pm 2.3	47.2 \pm 1.9	47.0 \pm 2.7	0.79
Cooked Fillet Yield (%)	43.0 \pm 2.7	41.7 \pm 2.3	41.9 \pm 2.2	42.0 \pm 2.3	0.42
Large Trout stage					
CF	2.37 \pm 0.18	2.30 \pm 0.16	2.41 \pm 0.24	2.41 \pm 0.22	0.11
Fat-meter[®] value (%)	8.50 \pm 1.99	9.28 \pm 1.99	9.55 \pm 1.92	8.75 \pm 1.83	0.14
Carcass Yield (%)	87.1 \pm 1.5	87.8 \pm 1.4	87.5 \pm 1.8	86.9 \pm 1.9	0.17
VSI (%)	11.5 \pm 1.5	10.9 \pm 1.3	11.2 \pm 1.7	11.9 \pm 1.8	0.096
HSI (%)	1.11 \pm 0.13a	1.02 \pm 0.12b	1.06 \pm 0.15ab	0.99 \pm 0.08b	<0.01
GSI (%)	0.18 \pm 0.09	0.16 \pm 0.07	0.15 \pm 0.09	0.16 \pm 0.10	0.53
Head (% bodyweight)	11.8 \pm 0.8	11.7 \pm 0.9	11.6 \pm 0.8	11.3 \pm 0.6	0.057
Raw fillet yield (%)	66.0 \pm 2.0	66.2 \pm 2.4	66.6 \pm 2.5	66.5 \pm 2.5	0.70
Dressed skinned fillet yield (%)	49.5 \pm 1.7	49.0 \pm 2.2	50.4 \pm 2.3	49.5 \pm 2.7	0.37
Smoked Fillet Yield (%)	41.9 \pm 2.0	41.9 \pm 2.4	43.2 \pm 2.3	42.1 \pm 2.8	0.39

CF: condition factor $K = \text{body weight} / \text{standard length}^3$; VSI: Viscero-Somatic Index = viscera weight/ body weight; HIS: Hepato-Somatic Index = liver weight/ body weight; GSI: Gonado-Somatic Index = gonad weight/ body weight; Cooked fillet yield = cooked fillet weight/ fish weight; Smoked fillet yield = smoked fillet weight/ fish weight. Means with different letters in the same row are significantly different ($p < 0.05$). Bold number and characters indicate significant difference at p value < 0.05 .

Table S4: Pan size trout - Effect of diet on sensory characteristics of cooked fillet. Mean scores of 20 panellists.

	Sensory descriptors	Co	V1	V2	PA	Feed effect ¹ p value
ODOR	Global intensity	5,30	4,70	4,92	4,70	0.46
	Fat fish	3,68	3,67	3,20	3,19	0.63
	Earth	2,97	2,14	2,38	2,17	0.54
	Cooked potato	2,38	2,18	2,02	1,69	0.32
	Milky	2,04	2,48	1,69	1,80	0.17
	Sour	0,56	0,56	0,86	0,54	0.72
APPEARANCE	Orange color	4,55 a	3,90 a	3,66 a	2,01 b	<0,001
TEXTURE	Firmness	4,33	3,85	4,53	3,69	0.20
	Fibrous texture	4,27	3,27	4,15	3,81	0.11
	Moisture	3,15 b	3,33 b	3,35 b	4,14 a	0,048
	Pasty texture	2,63	2,11	2,65	1,93	0.22
	Sticky texture	2,95	2,60	3,19	2,65	0.60
FLAVOR	Global intensity	5,50 a	5,01 a	5,18 a	4,29 b	0,003
	Fat fish	4,42	4,24	4,08	3,75	0.37
	Cooked potato	3,25	2,19	2,54	2,23	0.07
	Earth	1,83 b	2,51 ab	3,32 a	2,98 a	0,031
	Acid taste	0,68	0,87	1,04	0,57	0.18
	Bitter taste	0,81	0,62	0,92	0,68	0.48
	Metallic taste	1,44	1,40	1,90	1,39	0.60

¹Significant level for feed effect in the two factors (panellists, feed) analysis of variance. Bold numbers and characters indicate significant difference at p value < 0.05. Means with different letters in the same raw are significantly different (p < 0.05).

Table S5: Large trout - Effect of diet on sensory characteristics of cooked fillet. Mean scores of 22 panellists.

	Sensory descriptors	Co	V1	V2	PA	Feed effect ¹ p value
ODOR	Global intensity	5,05	4,91	5,04	4,60	0.79
	Fat fish	3,94	3,62	3,82	3,81	0.90
	Earth	1,98	1,86	1,06	1,71	0.27
	Cooked potato	1,98	2,70	2,13	2,16	0.30
	Milky	1,53	1,38	1,75	1,37	0.69
	Sour	0,16	0,59	0,56	0,33	0.21
APPEARANCE	Orange color	6,64 a	5,64 b	5,10 b	2,24 c	<0,001
TEXTURE	Firmness	4,38	4,19	4,58	3,98	0.54
	Fibrous texture	4,92	4,30	4,99	4,97	0.18
	Moisture	4,06	4,38	3,83	4,51	0.57
	Pasty texture	2,73	2,89	2,92	2,76	0.96
	Sticky texture	3,68	3,42	3,99	3,94	0.64
FLAVOR	Global intensity	5,40	5,31	5,39	4,54	0.11
	Fat fish	4,60	3,95	4,31	4,05	0.37
	Cooked potato	2,24	2,03	2,07	2,01	0.92
	Earth	1,78	1,73	1,56	1,74	0.88
	Acid taste	0,35	0,72	0,64	0,48	0.17
	Bitter taste	0,48	0,50	0,55	0,45	0.97
	Metallic taste	1,59	1,28	2,08	2,08	0.10
	Blood / meat	0,61	0,60	0,96	1,21	0.10

¹Significant level for feed effect in the two factors (panellists, feed) analysis of variance. Bold numbers and characters indicate significant difference at p value < 0.05 or a tendency at p ≤ 0.10. Means with different letters in the same raw are significantly different (p < 0.05).

Table S6: Large smoked trout - Effect of diet on sensory characteristics of smoked fillet. Mean scores of 14 panellists.

	Sensory descriptors	Co	V1	V2	PA	feed effect ¹ p value
ODOR	Smoke intensity	4,97	5,34	5,73	4,79	0.42
	Cold ashes	3,26	3,34	3,22	2,79	0.85
	Fat Fish	2,68	2,04	2,01	2,49	0.16
	Butter	1,21 a	0,29 b	0,57 ab	0,24 b	0,0477
	Earth	0,07	0,06	0,05	0,07	0.21
	Acid / pungent	0,40	0,44	0,44	0,36	0.95
APPEARANCE	Orange color	7,08 a	7,04 a	6,13 a	3,95 b	<0,001
	Firmness	4,75 ab	5,47 a	4,46 ab	3,83 b	0,0218
TEXTURE	Melting texture	4,36 ab	3,82 b	4,72 a	5,16 a	0,0178
	Fatty texture	2,84	2,61	3,09	3,36	0.41
FLAVEUR	Smoke intensity	5,32 ab	5,88 a	5,66 a	4,39 b	0,0456
	Cold ashes	3,06	3,41	3,22	2,64	0.61
	Salted taste	3,56 ab	4,07 a	3,49 ab	2,91 b	0,0424
	Fat fish	3,30	3,61	3,27	3,57	0.61
	Acid taste	0,52	0,26	0,49	0,39	0.24
	Earth	0,17	0,14	0,21	0,17	0.66
	Metallic	0,36	0,29	0,56	0,70	0.36

¹Significant level for feed effect in the two factors (panellists, feed) analysis of variance. Bold numbers and characters indicate significant difference at p value < 0.05. Means with different letters in the same raw are significantly different (p < 0.05).