

Rainbow trout prefer diets rich in omega-3 long chain polyunsaturated fatty acids DHA and EPA

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29 ABSTRACT

30 The control of feed intake in fish in aquaculture requires the development of new techniques 31 to improve diet composition, feed conversion efficiency and growth. The aim must be 32 sustainability and an effective use of resources. The effect of replacing traditional aqua-feed 33 ingredients (fishmeal and fish oil) by a 100% plant-based diet is known to drastically 34 decrease fish performance (survival and growth). The present study examined the feed 35 preference of rainbow trout Oncorhynchus mykiss for three diets containing distinct levels of 36 omega-3 long chain polyunsaturated fatty acids (ω-3 LCPUFA): eicosapentaenoic acid 37 (EPA) and docosahexaenoic acid (DHA) (0% for low, 5% for medium and 20% for high, total fatty acid content). Feed preference values for each group (low v. medium ω -3 diets, 38 39 medium v. high ω -3 diets and low v. high ω -3 diets) were observed using two self-feeders 40 positioned at opposite sides of the tank. The hypothesis was that the decrease of fish growth 41 and survival rate of fish fed with 100% plant-based diet could be explained by the absence of 42 ω -3 LCPUFA relating to decrease of food intake. This could explain the tasting role of ω -3 43 LCPUFA in the feeding behavior of rainbow trout (which reflects the motivation to consume 44 feed). The results showed that rainbow trout could discriminate between the diets containing 45 different level of ω -3 LCPUFA even if unable to differentiate between level of 5% (no 46 preference observed in low v. medium ω -3 diets). Overall they had a preference for diet high 47 in ω -3 LCPUFA: 59.5% preference for high ω -3 diet in high v. low ω -3 diets, and 75.6% 48 preference for high ω -3 diet in medium v. high ω -3 diets respectively. This preference was 49 repeated after 21 days and for a further 21 days when the feeds were exchanged between the 50 two self- feeders in each tank: 63.3% preference for high ω -3 diet in high v. low ω -3 diets, 51 and 69,5% preference for high ω -3 diet in medium v. high ω -3 diets respectively. The tests 52 also indicated a difference in the extent of food waste of each of the three diets revealed by 53 uneaten pellets after feed demands. During two periods of test, high ω -3 diet was the most

54	appreciated, the least wasted and the most eaten (all choice groups) whereas the most uneaten
55	feed remained the least appreciated diet in three choices diets (low ω -3 diet in low v. medium
56	ω -3 diets, medium in medium v. high ω -3 diets and low in low v. high ω -3). In conclusion,
57	this study highlighted the influence of ω -3 LCPUFA in the feeding behavior of juvenile
58	rainbow trout, levels of ω -3 LCPUFA drove dietary choices in the fish.
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62	KEYWORDS:
63	rainbow trout; ω -3 long chain polyunsaturated fatty acid; feed preference; diet selection; self-
64	feeding.
65	

66 1. INTRODUCTION

67

Omega-3 long chain polyunsaturated fatty acids (ω -3 LCPUFA), such as eicosapentaenoic 68 69 acid (EPA) and docosahexaenoic acid (DHA), are essential components for all living organism [1]. For more than three decades, ω -3 LCPUFA have been known for their 70 71 importance in development (primarily brain, eyes and nerves) and numerous beneficial 72 effects and functions in humans including lower risk of cancer, cardiovascular disease, skin 73 disorders, anxiety and stress, obesity, inflammation, rheumatoid arthritis, osteoporosis, 74 asthma and allergies [2]. The main dietary source of ω -3 LCPUFA for humans is seafood 75 but, paradoxically, farmed fish is also reliant on marine fisheries for fishmeal and fish oil, traditionally major ingredients of aquafeeds. Aquaculture production has increased almost 76 77 12-fold [3] over the last three decades and this rapid growth has resulted in an increased demand for aqua-feed. In addition, in order to reduce the reliance of aquaculture on wild fish 78 79 resources, while ensuring the sustainability of salmonid aquaculture, the traditional marine 80 ingredients of aquafeeds must be replaced by renewable, eco-friendly and less costly 81 alternative resources. In this way, previous studies revealed that the replacement of marine 82 ingredients and in particular the substitution of fish oil and fish meal with vegetable 83 ingredients, led to reduced fish performance in rainbow trout (Oncorhynchus mykiss) fed 84 with a completely plant-based diet compared to fish fed with a commercial diet [4]. 85 Furthermore, extensive use of plant ingredients like vegetable oils has several disadvantages, an unfavorable modification of the fatty-acid composition of farmed fish [5,6] particularly of 86 87 ω -3 PUFA [4,7–10]. Indeed, while none of the vegetable oils contain ω -3 LCPUFA, such as 88 DHA and EPA, they are rich in C18 ω -3 (alpha linolenic acid) and ω -6 PUFA (mainly 89 18:2ω-6: linoleic acid) [11].

90 To date, studies that have investigated the impact of the substitution of fishmeal and 91 fish oil with alternative dietary products (*e.g.* plants, insects, yeast and algae) have focused

92 their scope on fish performance, metabolism and functional genomics ([4,12–16]). In farmed 93 fish, qualitative (food type) and quantitative (consumption) feeding is essential for adequate 94 growth, survival and reproduction. Hence, understanding feeding behavior in farmed fish 95 will provide extensive information about how ingredients are perceived by fishes to enhance 96 growth and feed utilization. Increased feed conversion efficiency and reduced nutrient losses, 97 are major objectives in intensive aquaculture. It is therefore important to understand feeding 98 behavior of farmed fish and to find appropriate alternative ingredients to promote growth and 99 health performance.

100 Among alternative ingredients, those containing no ω -3 LCPUFA are known to 101 impact feeding of farmed fish. In early stages (larvae) inadequate levels of dietary ω -3 102 LCPUFA resulted in reduced swimming activities, feeding rhythms (delayed the response to 103 a visual stimulus) [17] and behavioral development (schooling), and increased abnormal 104 behavior (longer escape latency and defect in spatial retention) [18,19]. ω -3 LCPUFA are 105 also particularly important for marine fish larval growth and survival [20,21], and essential in 106 the regulation and resistance to different stress response in gilthead seabream (Sparus 107 aurata) [22–24], normal behavior and vigilance in yellowtail (Seriola quinqueradiata) and 108 gilthead seabream [25,26], sexual maturation and reproduction in rainbow trout [27,28] and 109 development of brain function in larval Japanese flounder (Paralichthys olivaceus) [29-31]. 110 In farmed fish, except for a study about the effect on the origin of the fat source on feed 111 selection in fish [32], the specific role of lipids and particularly ω -3 LCPUFA on feeding 112 behavior (preference, food intake and uneaten food) has not yet been investigated. This 113 information is crucial to understand if the decrease of survival rate and growth performance 114 of fish fed with plant-based diets could be explained by the modification of feeding behavior 115 due to the absence of ω -3 LCPUFA. These ω -3 LCPUFA could play an active role of tasting 116 in feeding behavior (palatability).

In this study, the objective was to investigate the feed preference of rainbow trout for three different dietary levels of ω -3 LCPUFA (DHA and EPA): low 0%, medium 5% and high 20% total fatty acid content in the diet. The hypothesis was that the absence of ω -3 LCPUFA in plant-based diet would lead to a decrease of food intake of farmed fish due to the lack of the palatability (which reflects motivation to consume feed) for this diet.

122 For this purpose, the feed preference of the fish for each diet was measured during 123 two periods of 3 weeks by means of self-feeders and their preference was tested by offering 124 the choice between two of the three diets containing low, medium or high dietary levels of ω -125 3 LCPUFA. The first test period was followed by the second period when the diets were 126 exchanged between feeders in order to observe whether the fish had learnt their preferences. 127 Finally, we analyzed fish growth performance, feed intake variables (total consumed and 128 uneaten food) and feed preferences (daily and cumulative in absolute and relative terms 129 during the test and exchange periods).

130

131 2. MATERIALS AND METHODS

132 2.1. Ethics statement

133 The experiment was conducted following the Guidelines of the National Legislation 134 on Animal Care of the French Ministry of Research (Decree No 2013-118, 1 February 2013) 135 and in accordance with EU legal frameworks relating to the protection of animals used for 136 scientific purposes (*i.e.* Directive 2010/63/EU). The scientists in charge of the experiments 137 received training and personal authorization. The experiment was conducted at the INRA 138 NuMeA facilities (permit number A64.495.1 delivered by French veterinary services), and 139 approved by the ethical committee (C2EA-73) of INRA "Comité d'éthique Aquitain poissons 140 oiseaux" (N° agreement INRA 17829, 9 May, 2019).

142 2.2. Experimental diets

Diets were manufactured at the INRA experimental facilities at Donzacq (permit number A40-228.1, Landes, France) using a twinscrew extruder (Clextral). Pellets were produced with 3 mm diameter and 3 mm length.

146 Composition and proximate analysis of the three diets are given in Table 1. All diets 147 were formulated based on the same feed ingredient composition, differing only in their oil 148 derivation (Table 1). A commercial diet (of marine and vegetable derivation) served as a 149 reference diet. In order to avoid exceeding anti-nutrient threshold levels, we used a blend of 150 wheat gluten, extruded peas and whole wheat, corn gluten meal, rapeseed meal and white 151 lupin as protein sources (c. 44.47% of total diet). Synthetic L-lysine, L-methionine, 152 dicalciumphosphate and soy-lecithin were added to all diets to correct the deficiency in 153 essential amino acids, phosphorous and phospholipids. A mineral and a vitamin premix was added to each diet. Diets were isoenergetic (c. 24.8 kJg⁻¹ of dry diet) and were formulated to 154 155 cover the nutrient requirements of rainbow trout [11]. In order to maintain a constant ratio 156 between groups of fatty acids (saturated, monounsaturated, ω -3 PUFA, ω -6 PUFA and ω -9 157 PUFA), the three experimental diets differed by the level of DHA and EPA to the benefit of 158 alpha-linolenic acid (ALA), the ω -3 PUFA precursor of these two fatty acids. To do this, the 159 dietary content of EPA and DHA was increased by adding Omegavie® marine oils DHA and 160 EPA (Polaris functional lipids, Quimper, France). The three experimental diets contained 161 23.66% crude lipids (\pm 0.05% of total diet) with 0.25% (devoid of DHA) of ω -3 DHA/EPA 162 (% of total fatty acids) for low, 4.92% for medium and 19.59% for high ω -3 diets. The fatty 163 acid composition of the diets is shown in Table 2.

164 The nutrient composition of diets was analyzed by drying the samples to constant 165 weight at 105°C for 24 h. Crude protein content was determined by the Kjeldahl method after 166 acid digestion and estimated by multiplying nitrogen by 6.25. Crude lipids were quantified

167 by petroleum diethyl ether extraction using the Soxhlet method. Gross energy was 168 determined in an adiabatic bomb calorimeter (IKA, Heitersheim Gribheimer, Germany). 169 Starch content was evaluated by an enzymatic method (Megazyme). Ash content was 170 determined by combustion in muffle furnace (550°C for 8 h). Total lipid was extracted and 171 measured gravimetrically according to the Folch method [33] using dichloromethane instead 172 of chloroform. Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of 173 total lipids using boron trifluoride (BF3) in methanol (14%) according to Shantha and 174 Ackman [34] and analyzed in a Varian 3900 gas chromatograph (Varian, les Ulis, France).

175

176 2.3. Rearing and self-feeders

177 The feeding trial was conducted in a recirculating rearing system at the INRA 178 facilities of Saint-Pee-sur-Nivelle. Rainbow trout used in this experiment originated from the 179 same parental stock (INRA Fish Farm of Lees-Athas, Permit number A64.104.1, vallée 180 d'Aspe, France).

Before starting the experiment, fish (mean *c*. 40 g) were reared to the experimental conditions (tank and self-feeder) and fed a commercial feed (Neostart1, Le Gouessant aquaculture, Lamballe, France) for 3 weeks (by hand for the first week and by self-feeders for the second 2 weeks).

After the acclimation period, juveniles (57.00 \pm 0.55 g) (Table 3) were randomly distributed among 11 fiberglass tanks (total of 200 rainbow trout; 16 to 17 fish per tank). Throughout the trial, dead fish (if any) were removed daily and weighed. Tanks of 70 1 volume were used and water flow was set to ensure an oxygen concentration >90% saturation. The tanks were individually aerated, and re-circulated water was thermostatically maintained at 17.2 \pm 0.2 °C (flow rate, 3 1 min⁻¹). Dissolved oxygen, pH (7.5) and water temperature were continuously monitored *via* probes. NH₄⁺ (0.0-0.1 mg l⁻¹), NO₂⁻ (0.0250.100 mgl⁻¹) (Microquant test kit for NH₄ and NO₂; Merck KGaA, Darmstadt, Germany)
were measured daily.

194 Each tank was equipped with two self-feeding devices (Imetronic®, Pessac, France) 195 positioned at opposite sides of the tank. Each device was composed of a trigger placed 1 cm 196 above the water surface to avoid unintentional trigger activations an interface that 197 conditioned the signal from the trigger and a feeder that delivered a predetermined amount of 198 feed (c. 45 pellets, 0.5 g) after each trigger actuation with a delay of 1s between two feed 199 rewards (in order to avoid multiple activation per demand due to the sensitivity of the 200 actuation). The entire system was connected to a computer that compiled and recorded 201 demand and distribution between feeders. Control software recorded the origin of signals and 202 controlled the feeders [35]. Each tank had a sediment trap for the daily recovery of feces and 203 uneaten pellets. Feed demands were rewarded during two periods of 2.5 h (06:00 - 08:30 and 204 18:00 - 20:30 hours), corresponding to the maximal feeding periods in unrestricted 205 conditions [36,37]. Fish were conditioned to feed during these feeding periods by means of 206 an extra 40W light. The artificial photoperiod switched on at 05:30 and off at 21:30 hours, 207 including an artificial dawn and dusk period of 30 min each (15.5 h light: 8.5 h dark).

208

209 *2.4. Procedure*

At the end of the acclimation period, feeder activity within each tank was compared to analyze feeder preferences, independently of the diet. The preferred feeder was assigned as the one receiving > 50% of total feed demands over the last 5 days of the acclimatation period (d-5- d_0). The initial preference (P_{ini} , %) was calculated as 100 x number of feed distribution in the preferred feeder d-5- d_0 / total number of feed distributions during d-5- d_0 . Groups with P_{ini} > 85% or those that did not acclimatize to the self-feeding device were excluded from the trial.

The experimental set-up has been published [32]. After the acclimation period, a 3 weeks test phase was performed. Each tank distributed two different diets through two selffeeders, offering the choice between the low, medium or high ω -3 diets. Four tanks were used for low and medium ω -3 diets and medium and high ω -3 diets and 3 tanks for low and high ω -3 diets (Table 3 and Supporting information 1). Following the test phase diets were exchanged between feeders for a further 3 weeks. The aim was to observe the behavior of the fish to discriminate between the diets.

224

225 2.5. Variables and analysis

226 Juvenile trout were counted and weighed as a group at the beginning and at the end of 227 each phase. No intermediate weighing was done to avoid stress and loss in appetite due to 228 handling. The number of demands and their distribution among the self-feeders and the feed 229 remaining in the feeders and the amount of uneaten feed were recorded daily. This was done 230 by emptying the decantation tube connected at the outlet of each tank into a sieve and 231 weighing and counting the settled feed pellets (different shades of green between pellets 232 allowed the two diets in each tank to be discriminated). Variables related to growth were 233 final body weight (FBW), body weight gain (FBW minus initial body weight, IBW), daily 234 growth coefficient (DGC, 100 x (FBW0.33-IBW0.33)/days, % per day) and feed efficiency 235 (FBW/food intake, FI). Variables related to FI were corrected for the amount of uneaten feed 236 and expressed in relative terms (% of body weight, BW, per day). Daily digestive energy 237 intake (DEI) was obtained by multiplying FI by the digestible energy (DE content) of the diet 238 (estimated as 23.07 kJg⁻¹). The variables related to feed preference (FP), the daily or 239 cumulative preferences (% total feed distribution) were calculated as absolute changes in the 240 feed demands for one diet as 100 x number of feed distributions for the feeder / total number 241 of feed distributions for the tank. As already published [32,38], to enable feeding behavior

242 and ability of fish to acclimatize to changes during the exchange period aside from the effects 243 of the training during the test period, the changes in relative preference were calculated 244 relative to the cumulative feed preference (P) at the end of the test period: 100 x (P/daily 245 cumulative feed preference during the exchange period) with daily cumulative feed 246 preference = 100 x number of feed demands in the feeder for each day / numbers total feed 247 demands in the tank for each day. The final relative cumulative feed preference varied 248 between 100% at the start of the exchange phase or in the case where no changes in feeder 249 demands occurred and 0% in the case of complete avoidance of the previously preferred 250 feeder (Figure 4C). The relative feed preference was also analyzed during the exchange 251 period as the relative cumulative feed preference (Supporting information 2). The proportion 252 of uneaten feed (UF) was expressed as percentage of total feed distributed during the two 253 periods (Figure 5). Feed intake, feed preference and uneaten feed were analyzed over the 254 total test period and for the exchange period after feed change.

255

256 2.6. Statistical analysis

257 The effect of the dietary choice selection was tested by one-way ANOVA. To evaluate 258 the effect of the diet on time course changes in daily and cumulative feed preferences for each 259 diet (Figures 1, 2, 3 and 4 and Supporting information 2), values were analyzed using 260 frequency analysis (one-sample *t*-tests). The total food consumed and total uneaten pellets 261 (Figure 5), values were analysed by *t*-tests. All data are expressed as mean \pm S.E. A *P*-value 262 of 0.05 or less was indicated as statistically significant. Statistical analyses were performed 263 using R software (v3.5.2)/R Commander package. Analyses were carried out on 264 untransformed data since criteria for normality and homogeneity of variances were fulfilled 265 (Shapiro-Wilk's and Levene's test respectively).

267 **3. RESULTS**

268 *3.1. Fish performance*

Growth and fish performance are presented in Table 4. For all tanks and groups, fish growth doubled after 6 weeks feeding (test and exchange periods), and very low mortalities were recorded. This represents a DGC of 2.65 ± 0.06 % (for all tanks) of their initial body weight and a daily feed intake > 1.2 % (BW/day). The values found for BWG, DGC, FI, FE and DEI during 6 weeks at 17 °C were unaffected by the three experimental diets (Table 4).

275 *3.2. Feed preferences*

276 3.2.1 Feed preference between low and medium EPA/DHA ω-3 diets

277 The daily and cumulative feed preferences are presented in Figure 1A and summarized 278 in Figure 4A for the test period and in Figure 1B and 4B for the exchange period. During the 279 test period, daily and cumulative feed preferences showed that fish had no feed preference 280 between the two diets (Figure 1A). The final cumulative feed preference revealed no feed 281 preference for fish between low and medium ω -3 diet (Figure 4A) with 45.8% v. 54.2% for 282 low and medium ω -3 diet, respectively. During the exchange period, this absence of 283 preference for one diet remained unchanged (Figure 1B). The final cumulative feed 284 preference showed no preference for one specific diet with 54.1% of total feed demands for 285 low ω -3 diet compared to 45.9% for medium ω -3 diet (Figure 4B). The relative cumulative 286 feed preference showed no preference for fish during the exchange period (Supporting 287 information 2A).

288

289 3.2.2 Feed preference between medium and high EPA/DHA ω -3 diets

290 During the 3 weeks test period, daily (Figure 2A; upper) and cumulative (Figure 2A; 291 lower) feed preferences for medium ω -3 diet *v*. high ω -3 diet revealed that fish had an early

292 preference for the diet with the highest level of DHA/EPA (*c*. 20%) with a final cumulative 293 feed preference of 75.62% (P<0.05) at the end of the test period (Figure 4A). Moreover, all 294 tanks had a final cumulative feed preference for high ω -3 diet between 56 and 88%.

During the exchange period (Figure 2B), fish kept their preference for the self-feeder position now containing the medium ω -3 diet. However, the first day after the diet exchange (day 1 exchange period), the cumulative feed preference was 62.05% for the medium ω -3 diet and 37.95% for the high ω -3 diet compared to 54.66% for the medium ω -3 diet and 45.33% for the high ω -3 diet for the final cumulative feed preference (Figure 4B).

This feed preference in favor of high ω -3 diet also observed (*P*=0.053) by the final relative feed preference (Figure 4C) revealed that fish had changed their relative feed preference during the exchange period (Supporting information 2B) increasing on the first day until the end of the exchange period (69.5% of relative cumulative feed preference for the high ω -3 diet *v*. 30.5% for the medium ω -3 diet).

This early relative change of preference was explained by two tanks which had changed their absolute rate preference the first day after exchange and until the end of this period.

308

309 3.2.3 Feed preference between low and high EPA/DHA ω -3 diets

For the group of fish fed with diets containing higher gaps of ω -3 LCPUFA level (*c*. 311 0% of DHA/EPA for low ω -3 diet *v*. *c*. 20% of DHA/EPA for the high ω -3 diet), the test 312 period indicated that fish had a feed preference for the high ω -3 diet (*P*<0.05 for day 14 and 313 15; Figure 3A). The cumulative feed preference showed that on each day (except for day 1) 314 fish favored the high ω -3 diet, even though this was not significant for the final cumulative 315 feed preference (59.55% for the high ω -3 diet *v*. 40.45% for the low ω -3 diet, *P*=0.059; 316 Figure 3A). It is important to note that all groups in this ω -3 diet selection (3 tanks) had a 317 final cumulative feed preference for the high ω -3 diet.

During the first 9 days of exchange period (Figure 3B), fish kept their preference for the self-feeder position now containing low ω -3 diet. However, during the last 11 days of the exchange period, results were different with 10 days of feed preferences in favor of the high ω -3 diet *v*. the low ω -3 diet (*P*<0.05; days 10, 11, 17, 18 and 20). The cumulative feed preference revealed that feed preference change (*P*>0.05) in favor of the high ω -3 diet on day 9 and 10 until an absolute change over on day 15 until the end of the experiment (Figure 4B; 53.95% of preference for high ω -3 diet *v*. 46.05% for low ω -3 diet; *P*>0.05).

325 Two of the three tanks changed their absolute rate preference during the exchange 326 period (particularly on the first day after exchange) in favor of the high ω -3 diet with final 327 cumulative feed preferences of 56.59, 57.83 and 47.40%. This absolute change of feed 328 preference was also confirmed by the relative feed preference (Figure 4C), which revealed a 329 feed preference of 63.3% for the high ω -3 diet v. 36.7% for the low ω -3 diet (P<0.05). The 330 relative cumulative feed preference also indicated that fish had changed their relative 331 preferences on the 10 days (P < 0.05 for the last 4 days) until the end of the exchange period 332 (Supporting information 2C).

333

334 *3.3. Feed consumption*

335 The total consumed and uneaten feeds are presented in Figures 5A and 5B for the test period336 and Figures 5C and 5D for the exchange period.

At the end of the test period, the proportion of total feed consumed (corrected for the amount of uneaten feed) and uneaten for all groups were in concordance to the final feed preference. Indeed, fish had consumed 79% more of high ω -3 diet during the high *v*. medium ω -3 diets test (495.9 g v. 127.5 g; *P*<0.05) and 61.6% more of the high ω -3 diet during the

341 high v. low ω -3 diets (384 g v. 239.1 g) (Figure 5A). In parallel, the proportion of the uneaten 342 feed for all ω -3 diets selection during the test period was in concordance with the final 343 cumulative feed preference even if the waste had decreased over time. For uneaten feed 344 during the test period (Figure 5B), fish spat out more of the less appreciated diet with 3.93% of uneaten feed (total feed distribution) for the low ω -3 diet during the low v. medium ω -3 345 346 diets test (four times more than the medium ω -3 diet), 18.4% for the medium ω -3 diet during 347 the medium v. high ω -3 diets test (four and a half times more than the high ω -3 diet; P<0.05) 348 and 2.5% for the low ω -3 diet during the low v. high ω -3 diets test (ten times more than high 349 ω -3 diet).

350 During the exchange period, fish consumed more of the low ω -3 diet during the low v. 351 medium ω -3 diets test (63%; 363.2 v. 207.8 g; P<0.05), 58% % of the medium ω -3 diet 352 during the medium v. high ω -3 diets test (294 v. 210 g) and 52% of the high ω -3 diet during 353 the low v. high ω -3 diets test (307.8 v. 284.2 g) (Figure 4A). Compared to the test period, 354 during the exchange period (Figure 4D), the overall increase of uneaten feed in each diet 355 (even for the medium ω -3 diet during the medium v. high ω -3 diets) revealed that fish seemed 356 to understand that there was an exchange of diet in the self-feeders. Moreover, the most 357 uneaten feed remained the least appreciated diet (even for the medium ω -3 diet during the 358 medium v. high ω -3 diets).

359 Overall, fish ate (corrected for the amount of uneaten feed) 3939 g of low, 3708 g of 360 medium and 5032 g of high ω -3 diet over the experiment (test and exchange periods).

361 4. DISCUSSION

362 In the present study, we revealed that juvenile trout preferred diet containing the 363 higher-level of ω -3 LCPUFA which was also the least wasted and the most eaten (all choice 364 groups) whereas the most uneaten feed remained the least appreciated diet in three choices 365 diets (low ω -3 diet in low v. medium ω -3 diets, medium in medium v. high ω -3 diets and low 366 in low v. high ω -3). For growth variables (BWG, DGC, FI, FE and DEI), values were 367 consistent for rainbow trout reared during 7 weeks at 17 °C and during a short time 368 experimental period, and were unaffected by the three experimental diets (Table 4). During 369 the second period (when diets were exchanged between feeders in each tank), the cumulative 370 feed preference revealed that fish could acclimatize to the change with a preference for the 371 higher diet in ω -3 for low and high ω -3 diet group between day 9 and 10 until an absolute 372 (cumulative feed preference) change on the day 15 until the end of the experiment (Figure 373 4B). Furthermore, after the first day of the exchange, rainbow trout had the ability to 374 acclimatize to change (2 tanks for medium ω -3 diet in low and medium ω -3 diets, 2 tanks for 375 high ω -3 diet in medium and high ω -3 diets and 1 tank for high diet ω -3 in low and high ω -3 376 diets) that allowed the change of their preferred food (absolute feed preference) in the other self feeder (less selected in first period). Also, when fish were fed with the high level of ω -3 377 378 LCPUFA (low ω -3 diet compared to high ω -3 diet), they changed their absolute cumulative 379 feed preference in favor of the high ω -3 diet from the 17th day.

In the present study, we hypothesized that the absence of ω -3 LCPUFA in plant-based diet would lead to a decrease of food intake of farmed fish due to the lack of the palatability (which reflects motivation to consume feed) for this diet. Here, we observed that a class of essential lipids for the development of farmed fish, ω -3 LCPUFA, drives the feeding behavior of juvenile rainbow trout. Presently, nutrition composition of aquafeed is one of the major criticisms of aquaculture. Current aquafeed is not sustainable mainly because it is composed 386 of ingredients originating from marine resources (such as fish oil and fish meal). However, 387 availability of this traditional aquafeed ingredient has not increased with demand and today 388 readily available alternative sources of proteins and lipids are required. In this current context 389 where aquaculture reliance on marine products must be reduced, several studies have 390 observed that the use of plant based-diets presents disadvantages, such as a reduction in feed-391 intake [12,13], growth performance [4,14] or a combination of lower feed intake and feed 392 efficiency. This decrease of growth performance by plant based diets is believed to be mainly 393 related to the absence of marine products, fish meal and particularly fish oil [15,16]. The 394 physiological explanation of this remains remain poorly documented. In addition, extensive 395 use of new sustainable ingredient presents disadvantages, particularly of some nutrients such 396 as ω -3 LCPUFA (DHA and EPA) suggesting an important negative alteration of feeding 397 behavior (motivation to consume feed) leading to drastically reduced growth performance and 398 survival. In this way, it seems clear that understanding the basic physiological mechanisms 399 regulating feed intake of farmed fish will allow to enhance feed conversion efficiency and 400 reduce nutrient losses, key elements in fish farming. Moreover, theses knowledge might lead 401 not only to specific adjustments in fish rearing conditions (e.g. temperature and photoperiod) and feeding strategies (e.g. time of feeding, frequency and stock density), but also to find 402 403 appropriate alternative ingredients to sustainably promote growth aquaculture while 404 improving fish growth and survival rate.

This study revealed that ω -3 LC-PUFA (DHA and EPA) drive the feeding behavior of rainbow trout. These new observations explained the results of the study that nutrient composition of aquafeed influence dietary choices in the fish. The presence of ω -3 LCPUFA, DHA and EPA in the diet influences the feeding behavior of rainbow trout lead to feed preference, a greater consumption and decrease wasted for diet containing the higher-level of ω -3 LCPUFA. Feeding behavior consists food ingestion following foraging related to hunger 411 (which reflect motivation to consume feed). In all animals, feeding and nutrient intake are 412 major regulators for growth and reproduction [39]. In addition, in aquaculture, feeding 413 strategy is essential in rearing farmed fish to allow them to survive, grow, and reproduce in an 414 environment with factors that might disrupt their feed intake. Furthermore, in farmed fish, 415 variations in several extrinsic factors, whether they are abiotic (*e.g.* temperature, oxygen level 416 and water quality) or biotic (e.g. competition and stock density) have been shown to induce 417 changes in feeding behavior [40]. Several studies have revealed that farmed fish appear to be 418 able to directly detect small differences in the levels of several micronutrients such as zinc 419 [41], vitamin C [42], individual amino acids [43] and classes of lipids. Rainbow trout were 420 found to have the capacity to discriminate the difference between a standard fish oil diet and a 421 diet containing high levels of ALA (linseed oil), linoleic acid (sunflower oil), or oleic acid 422 (rapeseed oil) [32]. In our study, we have gone further by showing that trout could 423 discriminate between defined fatty acids. Interestingly fish were not be able to discriminate 424 ω -3 LCPUFA levels < 5% (low v. medium ω -3 diets, Figure 1A); they did not have any 425 preference. In the comparison of medium v. high ω -3 diets (Figure 2A), fish discriminated 426 between the diets and showed a feed preference for the high ω -3 diet. This observation could 427 be explained because of the levels of EPA and DHA between low and medium ω -3 diets (gap 428 of 5%), which were less than those as between medium and high ω -3 diets (gap of 15%).

429 At the molecular level assumptions can be made based on studies on mammals. 430 Feeding behavior and, by extension, feed intake levels are regulated in the central nervous 431 system, where the neural circuitry integrate incoming sensorial information to orchestrate an 432 integrated feeding response. In this neural circuitry, the importance of monoamine 433 neurotransmitters (dopamine, norepinephrine, serotonin and epinephrine), opioids and 434 endocannabinoids in the regulation of many behavioural and physiological processes, 435 including feeding, cannot exclude a link between the nutritional state by ω -3 LCPUFA in this 436 trial and this non-homeostatic mechanism. This hedonic feeding based on previous 437 experience, is driven by sensory perception or pleasure and is independent of energy 438 requirements [44]. Furthermore, brain reward circuitry involved in the regulation of food 439 intake is activated by taste and consumption of palatable food [45], two essential criteria met 440 by dietary lipids like ω -3 LCPUFA. Considering that sensorial information could be mostly 441 attributable to palatability and taste sensitivity of diet products [46], studies have observed the 442 importance of this sensitivity. Moreover, due to its textural and olfactory properties, it has 443 been well accepted that dietary lipids modulate feeding behavior. Twenty years ago, an 444 additional gustatory component for the detection of lipids was proposed [47] and later well 445 documented in animals [48-50] including humans [51,52]. These studies accumulated 446 evidence supporting a taste component (savory sensation as a taste modality) for dietary fat 447 [53]. Another relevant response by which fatty acids exert their effect is the fatty acid sensing 448 system. The mammalian central nervous system, via the hypothalamus, is able to detect 449 changes in the level of LCPUFA through mechanisms contributing to energy homeostasis 450 [54,55] and control of feeding behavior. It was observed that neither saturated fatty acids like 451 palmitate (C16:0) nor the presence of two (such as in linoleic acid) or three (such as in DHA) 452 double bonds activated fatty acid sensing systems [55]. Also, nutrients such as as dietary 453 lipids are major regulators influencing feeding and growth in fish [39]. Studies revealed that 454 fatty acid in the diet of farmed fish impacted their feeding behavior [56-58] involving a 455 neuropeptide pathway (via change in the expression of anorexigenic and orexigenic 456 neuropeptides) [56,58–61]. At the molecular level, several mechanisms have been proposed to 457 explain the beneficial behavioral effects of ω -3 LCPUFA. These mechanisms included 458 modulation of membrane biophysical properties (increase number of receptors), [62] 459 regulation of neurotransmitter release [63], augmentation of cerebral blood flow in response 460 to stimulation (modulation of neuronal excitability) [64], synthesis of biologically active

461 oxygenated derivatives [65], or increased receptor-channel complex novel sites [66]. 462 Furthermore, ω -3 LCPUFA could enhance synaptogenesis [67] and synaptic plasticity [68], 463 facilitate serotonergic transmission [69], reduce inflammation [70] as well as normalize 464 choligernic and glutamergic systems [71] in order to reduce distress behaviors in mammals.

465 All these findings could explain the physiological pathways involved in the feed 466 preference of rainbow trout observed in our study for diets higher in ω -3 LCPUFA. Further 467 studies will be necessary to correlate the feed preference for diets higher in ω -3 LCPUFA and 468 the implications on the central control of food intake particularly the fatty acid sensing 469 pathways and brain reward system (hedonic feeding) in farmed fish. In addition, to maintain 470 sustainable aquaculture and resources, it will be interesting to assess the importance of the 471 intake of ω -3 LCPUFA in plant-based diets by a new sustainable food source for aquaculture 472 (algae) to restore growth and survival rate in rainbow trout fed with 100% plant based diet 473 totally devoid of ω -3 LCPUFA.

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687 **FIGURE LEGENDS**

- **Figure 1.** (A) Representative daily (upper) and cumulative (lower) feed preference for low and medium ω -3 diets during the test period (3 weeks). (B) Representative daily (upper) and cumulative (lower) feed preference for low and medium ω -3 diets during the exchange period (diets were swapped between feeders in each tank) (3 weeks). An asterisk indicates a significant difference between the two diets as determined by a *t*-test (*P* < 0.05). Results are expressed in % of distribution as mean ± S.E. (*n* = 4 tanks).
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Figure 2. (A) Representative daily (upper) and cumulative (lower) feed preference for medium and high ω -3 diets during the test period (3 weeks). (B) Representative daily (upper) and cumulative (lower) feed preference for medium and high ω -3 diets during the exchange period (diets were swapped between feeders in each tank) (3 weeks). An asterisk indicates a significant difference between the two diets as determined by a *t*-test (*P* < 0.05). Results are expressed in % of distribution as mean ± S.E. (*n* = 4 tanks).

Figure 3. (A) Representative daily (upper) and cumulative (lower) feed preference for low and high ω -3 diets during the test period (3 weeks). (B) Representative daily (upper) and cumulative (lower) feed preference for low and high ω -3 diets during the exchange period (diets were swapped between feeders in each tank) (3 weeks). An asterisk indicates a significant difference between the two diets as determined by a *t*-test (*P* < 0.05). Results are expressed in % of distribution as mean ± S.E. (*n* = 3 tanks).

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710 Figure 4. (A) Representative final cumulative feed preference after the test period in 711 all ω -3 diet selections. (B) Representative final cumulative feed preference after the exchange 712 period (diets were swapped between feeders in each tank) in all ω -3 diet selections. (C) 713 Representative final relative feed preference after the exchange period in all ω -3 diet 714 selections. An asterisk indicates a significant difference between the two diets as determined 715 by a *t*-test (P < 0.05). Results are expressed in % of distribution as mean \pm S.E. (n = 4 tanks 716 for low and medium ω -3 diets and medium and high ω -3 diets, and n= 3 tanks for low and 717 high ω -3 diets).

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719 **Figure 5.** (A) Representative total feed consumed in the test period in all ω -3 diet 720 selections. (B) Representative uneaten pellets after the test period in all ω -3 diet selections. 721 (C) Representative total feed consumed in the exchange period (diets were swapped between 722 feeders in each tank) in all ω -3 diet selections. (D) Representative uneaten pellets after the 723 exchange period in all ω -3 diet selections. An asterisk indicates a significant difference between the two diets as determined by a *t*-test ($P \le 0.05$). Results are expressed in g for total 724 725 feed (A and B) and % (of total feed distribution) for uneaten feed as mean \pm S.E. (n = 4 tanks 726 for low and medium ω -3 diets and medium and high ω -3 diets, and n= 3 tanks for low and 727 high ω -3 diets).

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Supporting information 1. Experimental set-up of self-feeder experiment.

After a 3-weeks acclimation period to the experimental conditions (tanks and self-feeders) juvenile trout were weighed and randomly distributed among 11 fiberglass tanks (16 to 17 fish per tank). Each tank was equipped with two demand feeders A and B (Imetronic®, Pessac, France) positioned at opposite sides of the tank. A 3 weeks test period was performed. Each tank distributed either two different diets, offering the choice between the 135 low, medium or high ω -3 diets (three groups: L ω 3 + M ω 3; M ω 3 + H ω 3; L ω 3 + H ω 3) with 136 different combinations (two replicate groups per diet with different self-positioning). 137 Following the test phase, a swapped phase was performed. The choice diets were swapped 138 between the two feeders for another 3 weeks until the end of the trial.

Supporting information 2. (A) Representative relative cumulative feed preference for low and medium ω -3 diets. (B) Representative relative cumulative feed preference for medium and high ω -3 diets. (C) Representative relative cumulative feed preference for (low and high ω -3 diets. An asterisk indicates a significant difference between the two diets as determined by a *t*-test (P < 0.05). Results are expressed in % of distribution as mean \pm S.E. (n= 4 tanks for low and medium ω -3 diets and medium and high ω -3 diets, and n= 3 tanks for low and high ω -3 diets).

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768 **Table 1.** Ingredients and composition of the experimental diets.

Ingredient (%)	DIET			
	LOW ω-3	MEDIUM ω-3	HIGH ω-3	
Fish meal	5.0	5.1	5.2	
Extruded whole wheat	16.0	16.0	16.0	
Corn gluten	8.0	8.0	8.0	
Wheat gluten	18.0	18.0	18.0	
Peas meal	3.0	3.0	3.0	
Extruded Peas	7.1	7.1	7.1	
White lupin seed meal	5.0	5.0	5.0	
Fish hydrolysate (CPSP®)	5.0	5.0	5.0	
Rapeseed meal	8.2	8.2	8.2	
Soy lecithin	2.0	2.0	2.0	
L-Lysine	0.4	0.4	0.4	
L-methionine	0.3	0.3	0.3	
CaHPO₄.2H₂O	0.3	0.3	0.3	
Mineral premix ^a	1.5	1.5	1.5	
Vitamin premix ^b	1.5	1.5	1.5	
Attractant mix ^c	1.5	1.5	1.5	
Fish oil	1,0	1.0	1.0	
Sunflower oil	5.0	5.0	4.0	
Rapeseed oil	5.2	5.2	3.1	
Linseed oil	6.0	3.0	1.0	
Omegavie® DHA marine oil (min 70%) ^d	0.0	1.6	4.3	
Omegavie® EPA marine oil (min 70%) ^e	0.0	1.3	3.7	
Composition (% of dry matter)				
Dry matter (in % of diet)	96.9	97.1	97.2	
Crude protein	44.7	44.0	44.8	
Crude lipid	23.6	23.7	23.7	
Starch	13.9	13.6	13.6	
Ash	4.8	4.8	4.7	
Energy (kJg-1 DM)	24.8	25.0	24.7	

^aMineral premix: (g or mg kg⁻¹ diet): calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60% Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulphate (36% Zn), 0.4 g; copper sulphate (25% Cu), 0.3 g; manganese sulphate (33% Mn), 0.3 g; dibasic calcium phosphate (20% Ca, 18% P), 5 g; cobalt sulphate, 2 mg; sodium selenite (30% Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g
(UPAE, INRA).
^bVitamin premix : (IU or mg kg⁻¹ diet): DL-a tocopherol acetate, 60 IU; sodium menadione

bisulphate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin,
30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2000 mg (UPAE, INRA).

- ^cAttractant mix: glucosamine, 0.5 g; taurine, 0.3 g; betaine, 0.3 g; glycine, 0.2 g; alanine, 0.2 g.
- 780 ^dOmegavie® DHA oil (min 70%): concentrated marine oil produced mainly from anchovy and
- sardine oil. The crude oil is first refined, then purified and concentrated. This oil contains marine DHA
- 782 Omega 3 fatty acids under Triglycerides form. From Polaris, Quimper, France.
- 783 ^eOmegavie® EPA oil (min 75%): concentrated marine oil produced mainly from anchovy and sardine
- oil. The crude oil is first refined, then purified and concentrated. This oil contains marine EPA Omega
- 785 3 fatty acids under Triglycerides form. From Polaris, Quimper, France.
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Table 2. Selected fatty acid composition (% of total fatty acids).

	Diet				
	LOW ω-3	MEDIUM ω-3	HIGH ω-3		
C14:0	0.93	1.16	1.23		
C15:0	0.12	0.15	0.12		
C16:0	12.53	13.77	12.54		
C18:0	2.64	2.51	2.55		
C20:0	0.15	0.09	0.16		
Sum saturates	16.38	17.69	16.6		
C16:1 ω-7	1.04	1.18	1.22		
C18:1 ω-9	32.7	32.84	26.87		
C20:1 ω-9	0.49	0.55	0.91		
C22:1 ω-9	0	0	0.27		
Sum monoenes	32.24	34.57	29.77		
C18:2 ω-6	30.74	30.71	27.15		
C18:3 ω-6	0	0	0		
C20:2 ω-6	0	0	0		
C20: <i>3 ω-6</i>	0	0	0		
C20:4 ω-6	0.09	0.24	0.89		
Sum n-6 PUFA	30.84	30.96	28.03		
C18:3 ω-3	18.02	11.64	5.95		
C18:4 ω-3	0.11	0.11	0.28		
C20:3 ω-3	0	0	0		
C20:4 ω-3	0	0	0.32		
C20:5 ω-3	0.256	3.39	11.6		
C22:5 ω-3	0	0	0		
C22:6 ω-3	0	1.53	7.66		
Sum ω-3 PUFA	18.39	16.67	25,81		
Sum ω -3 LC-PUFA	0.25	4.92	19.59		
ω-3 LC-PUFA / ω-6	0.01	0.16	0.7		

 Table 2. Selected fatty acid composition (% total fatty acids)

Table 3. The experimental set-up and initial biomass specifications of the different groupsused in the trials.

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Test		Validation	Number of groups	Initial biomass	Initial number	Initial body weight
D1-D21	Inversion	D21 - D42	N	g per group	per group	g per individual
LOW - MEDIUM		MEDIUM - LOW	4	929.25 ± 13.28	16.25 ± 0.25	57.19 ± 0.438
HIGH - MEDIUM		MEDIUM - HIGH	4	916.19 ± 30.96	16.25 ± 0.25	56.30 ± 1.21
LOW - HIGH		HIGH - LOW	3	894.08 ± 7.75	16.67 ± 0.33	53.70 ± 1.51
			One-way ANOVA (P-values)	0.28	0.77	0.56

796**Table 4.** Mean \pm S.E. values of growth, feed intake, feed efficiency and digestible energy797intake over the whole experimental period.

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Table 4. Mean values (± SEM) of growth, feed intake, feed efficiency and digestible energy intake over the whole experimental period

		Dietary treatment			One-way ANOVA (<i>P</i> -values)	
		LOW-MEDIUM	MEDIUM-HIGH	LOW-HIGH	Effect diet	
	Final body weight (g per ind)	122.87 ± 6.22	126.82 ± 4.47	126.85 ± 4.44	0.83	
	Body weight gain (g per ind)	65.75 ± 6.44	68.65 ± 4.63	69.75 ± 5.06	0.86	
	Daily growth coefficient (% per day)	2.59 ± 0.20	2.66 ± 0.15	2.72 ± 0.17	0.47	
	Daily feed intake (% BW/day)	1.24 ± 0.04	1.44 ± 0.13	1.48 ± 0.07	0.6	
	Feed efficiency (BWG/FI)	1.03 ± 0.08	0.98 ± 0.11	1.00 ± 0.08	0.89	
	Daily digestive energy intake (kJ kg-1 BW)	399.76 ± 31.66	441.85 ± 45.44	419.74 ± 35.53	0.62	
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Supporting information 1

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