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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,
38 total fatty acid content). Feed preference values for each group (low v. medium ω -3 diets,
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.

59

60

61

62 **KEYWORDS:**

63 rainbow trout; ω -3 long chain polyunsaturated fatty acid; feed preference; diet selection; self-
64 feeding.

65

66 1. INTRODUCTION

67

68 Omega-3 long chain polyunsaturated fatty acids (ω -3 LCPUFA), such as eicosapentaenoic
69 acid (EPA) and docosahexaenoic acid (DHA), are essential components for all living
70 organism [1]. For more than three decades, ω -3 LCPUFA have been known for their
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,
76 traditionally major ingredients of aquafeeds. Aquaculture production has increased almost
77 12-fold [3] over the last three decades and this rapid growth has resulted in an increased
78 demand for aqua-feed. In addition, in order to reduce the reliance of aquaculture on wild fish
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,
86 an unfavorable modification of the fatty-acid composition of farmed fish [5,6] particularly of
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 18:2 ω -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).

117 In this study, the objective was to investigate the feed preference of rainbow trout for
118 three different dietary levels of ω -3 LCPUFA (DHA and EPA): low 0%, medium 5% and
119 high 20% total fatty acid content in the diet. The hypothesis was that the absence of ω -3
120 LCPUFA in plant-based diet would lead to a decrease of food intake of farmed fish due to
121 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).

141

142 2.2. *Experimental diets*

143 Diets were manufactured at the INRA experimental facilities at Donzacq (permit
144 number A40-228.1, Landes, France) using a twinscrew extruder (Cleextral). Pellets were
145 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
154 added to each diet. Diets were isoenergetic (*c.* 24.8 kJg⁻¹ of dry diet) and were formulated to
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).

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

185 After the acclimation period, juveniles (57.00 ± 0.55 g) (Table 3) were randomly
186 distributed among 11 fiberglass tanks (total of 200 rainbow trout; 16 to 17 fish per tank).
187 Throughout the trial, dead fish (if any) were removed daily and weighed. Tanks of 70 l
188 volume were used and water flow was set to ensure an oxygen concentration >90%
189 saturation. The tanks were individually aerated, and re-circulated water was thermostatically
190 maintained at 17.2 ± 0.2 °C (flow rate, 3 l min⁻¹). Dissolved oxygen, pH (7.5) and water
191 temperature were continuously monitored *via* probes. NH₄⁺ (0.0-0.1 mg l⁻¹), NO₂⁻ (0.025-

192 0.100 mg l^{-1}) (Microquant test kit for NH $_4$ and NO $_2$; Merck KGaA, Darmstadt, Germany)
193 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

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

217 The experimental set-up has been published [32]. After the acclimation period, a 3
218 weeks test phase was performed. Each tank distributed two different diets through two self-
219 feeders, offering the choice between the low, medium or high ω -3 diets. Four tanks were
220 used for low and medium ω -3 diets and medium and high ω -3 diets and 3 tanks for low and
221 high ω -3 diets (Table 3 and Supporting information 1). Following the test phase diets were
222 exchanged between feeders for a further 3 weeks. The aim was to observe the behavior of the
223 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, $100 \times (\text{FBW}^{0.33} - \text{IBW}^{0.33}) / \text{days}$, % 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 $100 \times \text{number of feed distributions for the feeder} / \text{total number}$
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: $100 \times (P/\text{daily}$
245 $\text{cumulative feed preference during the exchange period})$ with daily cumulative feed
246 $\text{preference} = 100 \times \text{number of feed demands in the feeder for each day} / \text{numbers total feed}$
247 $\text{demands in the tank for each day}$. 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).

266

267 3. RESULTS

268 3.1. Fish performance

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

274

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%.

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

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

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

308

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

310 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.

318 During the first 9 days of exchange period (Figure 3B), fish kept their preference for
319 the self-feeder position now containing low ω -3 diet. However, during the last 11 days of the
320 exchange period, results were different with 10 days of feed preferences in favor of the high
321 ω -3 diet v. the low ω -3 diet ($P<0.05$; days 10, 11, 17, 18 and 20). The cumulative feed
322 preference revealed that feed preference change ($P>0.05$) in favor of the high ω -3 diet on day
323 9 and 10 until an absolute change over on day 15 until the end of the experiment (Figure 4B;
324 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 period
336 and Figures 5C and 5D for the exchange period.

337 At the end of the test period, the proportion of total feed consumed (corrected for the
338 amount of uneaten feed) and uneaten for all groups were in concordance to the final feed
339 preference. Indeed, fish had consumed 79% more of high ω -3 diet during the high v. medium
340 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%
345 of uneaten feed (total feed distribution) for the low ω -3 diet during the low v. medium ω -3
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
377 self feeder (less selected in first period). Also, when fish were fed with the high level of ω -3
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.

380 In the present study, we hypothesized that the absence of ω -3 LCPUFA in plant-based
381 diet would lead to a decrease of food intake of farmed fish due to the lack of the palatability
382 (which reflects motivation to consume feed) for this diet. Here, we observed that a class of
383 essential lipids for the development of farmed fish, ω -3 LCPUFA, drives the feeding behavior
384 of juvenile rainbow trout. Presently, nutrition composition of aquafeed is one of the major
385 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)
402 and feeding strategies (*e.g.* time of feeding, frequency and stock density), but also to find
403 appropriate alternative ingredients to sustainably promote growth aquaculture while
404 improving fish growth and survival rate.

405 This study revealed that ω -3 LC-PUFA (DHA and EPA) drive the feeding behavior of
406 rainbow trout. These new observations explained the results of the study that nutrient
407 composition of aquafeed influence dietary choices in the fish. The presence of ω -3 LCPUFA,
408 DHA and EPA in the diet influences the feeding behavior of rainbow trout lead to feed
409 preference, a greater consumption and decrease wasted for diet containing the higher-level of
410 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 cholinergic 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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683 684 685 686 687 **FIGURE LEGENDS**

688
689 **Figure 1.** (A) Representative daily (upper) and cumulative (lower) feed preference for
 690 low and medium ω -3 diets during the test period (3 weeks). (B) Representative daily (upper)
 691 and cumulative (lower) feed preference for low and medium ω -3 diets during the exchange
 692 period (diets were swapped between feeders in each tank) (3 weeks). An asterisk indicates a
 693 significant difference between the two diets as determined by a *t*-test ($P < 0.05$). Results are
 694 expressed in % of distribution as mean \pm S.E. ($n = 4$ tanks).

695
696 **Figure 2.** (A) Representative daily (upper) and cumulative (lower) feed preference for
 697 medium and high ω -3 diets during the test period (3 weeks). (B) Representative daily (upper)
 698 and cumulative (lower) feed preference for medium and high ω -3 diets during the exchange
 699 period (diets were swapped between feeders in each tank) (3 weeks). An asterisk indicates a
 700 significant difference between the two diets as determined by a *t*-test ($P < 0.05$). Results are
 701 expressed in % of distribution as mean \pm S.E. ($n = 4$ tanks).

702

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

709

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).

718

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
724 between the two diets as determined by a *t*-test ($P < 0.05$). Results are expressed in g for total
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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729 **Supporting information 1.** Experimental set-up of self-feeder experiment.

730 After a 3-weeks acclimation period to the experimental conditions (tanks and self-feeders)
731 juvenile trout were weighed and randomly distributed among 11 fiberglass tanks (16 to 17
732 fish per tank). Each tank was equipped with two demand feeders A and B (Imetronic®,
733 Pessac, France) positioned at opposite sides of the tank. A 3 weeks test period was
734 performed. Each tank distributed either two different diets, offering the choice between the

735 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
736 different combinations (two replicate groups per diet with different self-positioning).
737 Following the test phase, a swapped phase was performed. The choice diets were swapped
738 between the two feeders for another 3 weeks until the end of the trial.

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740 **Supporting information 2.** (A) Representative relative cumulative feed preference for
741 low and medium ω -3 diets. (B) Representative relative cumulative feed preference for
742 medium and high ω -3 diets. (C) Representative relative cumulative feed preference for (low
743 and high ω -3 diets. An asterisk indicates a significant difference between the two diets as
744 determined by a *t*-test ($P < 0.05$). Results are expressed in % of distribution as mean \pm S.E. (n
745 = 4 tanks for low and medium ω -3 diets and medium and high ω -3 diets, and $n= 3$ tanks for
746 low and high ω -3 diets).

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768 **Table 1.** Ingredients and composition of the experimental diets.**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
CaHPO ₄ ·2H ₂ O	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 (kJ/g-1 DM)	24.8	25.0	24.7

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770 ^aMineral premix: (g or mg kg⁻¹ diet): calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60%
771 Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulphate (36% Zn), 0.4 g;
772 copper sulphate (25% Cu), 0.3 g; manganese sulphate (33% Mn), 0.3 g; dibasic calcium phosphate
773 (20% Ca, 18% P), 5 g; cobalt sulphate, 2 mg; sodium selenite (30% Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g
774 (UPAE, INRA).

775 ^bVitamin premix : (IU or mg kg⁻¹ diet): DL- α tocopherol acetate, 60 IU; sodium menadione
776 bisulphate, 5 mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin,
777 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000
778 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2000 mg (UPAE, INRA).

779 ^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
781 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
784 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.

786

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

Table 2. Selected fatty acid composition (% 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
C20:3 ω -6	0	0	0
C20:4 ω -6	0.09	0.24	0.89
Sum <i>n</i> -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

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790 **Table 3.** The experimental set-up and initial biomass specifications of the different groups
791 used in the trials.

792

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

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

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

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Table 4. Mean values (\pm 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 \pm 6.22	126.82 \pm 4.47	126.85 \pm 4.44	0.83
Body weight gain (g per ind)	65.75 \pm 6.44	68.65 \pm 4.63	69.75 \pm 5.06	0.86
Daily growth coefficient (% per day)	2.59 \pm 0.20	2.66 \pm 0.15	2.72 \pm 0.17	0.47
Daily feed intake (% BW/day)	1.24 \pm 0.04	1.44 \pm 0.13	1.48 \pm 0.07	0.6
Feed efficiency (BWG/FI)	1.03 \pm 0.08	0.98 \pm 0.11	1.00 \pm 0.08	0.89
Daily digestive energy intake (kJ kg ⁻¹ BW)	399.76 \pm 31.66	441.85 \pm 45.44	419.74 \pm 35.53	0.62

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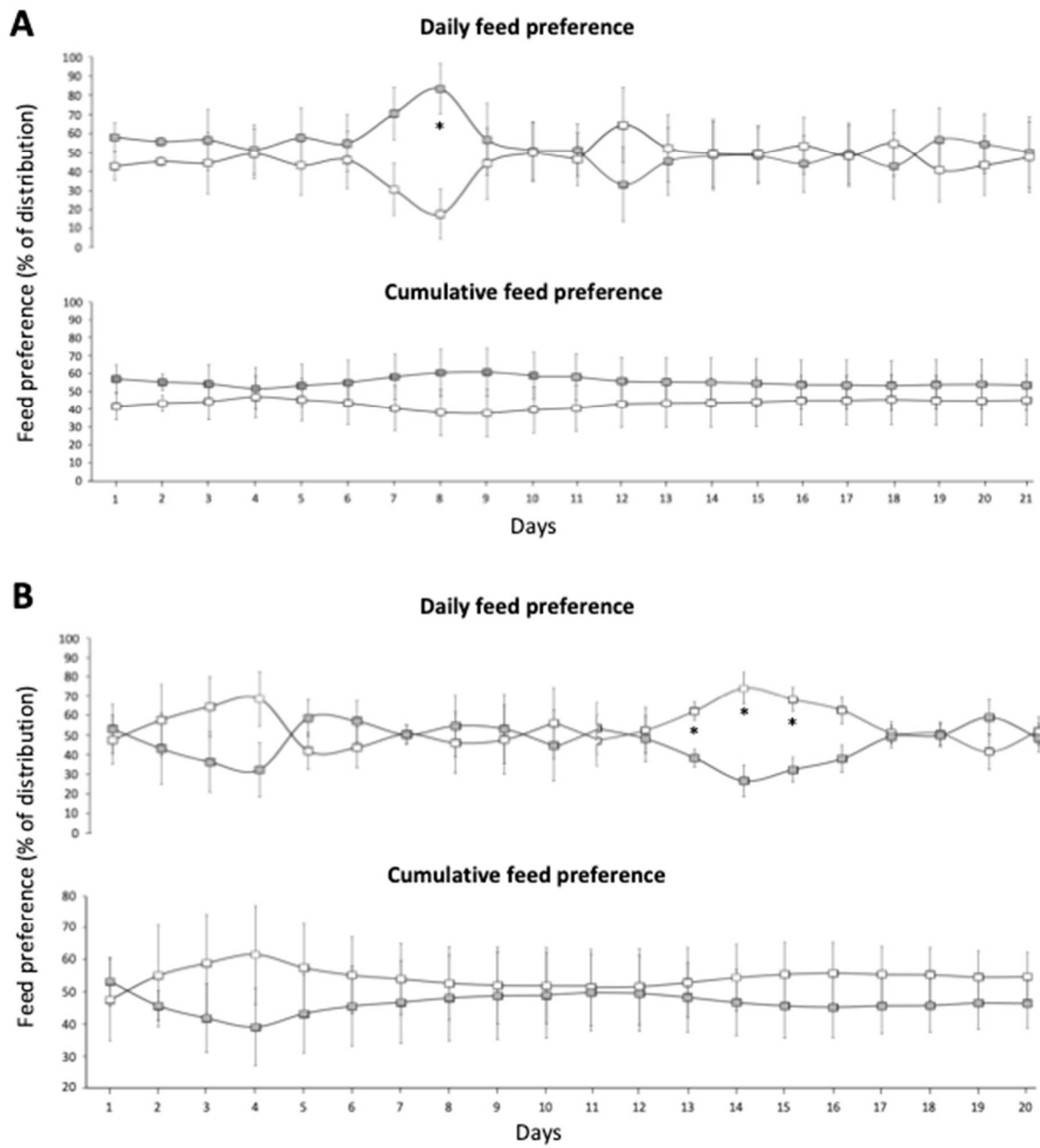
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Figure 1

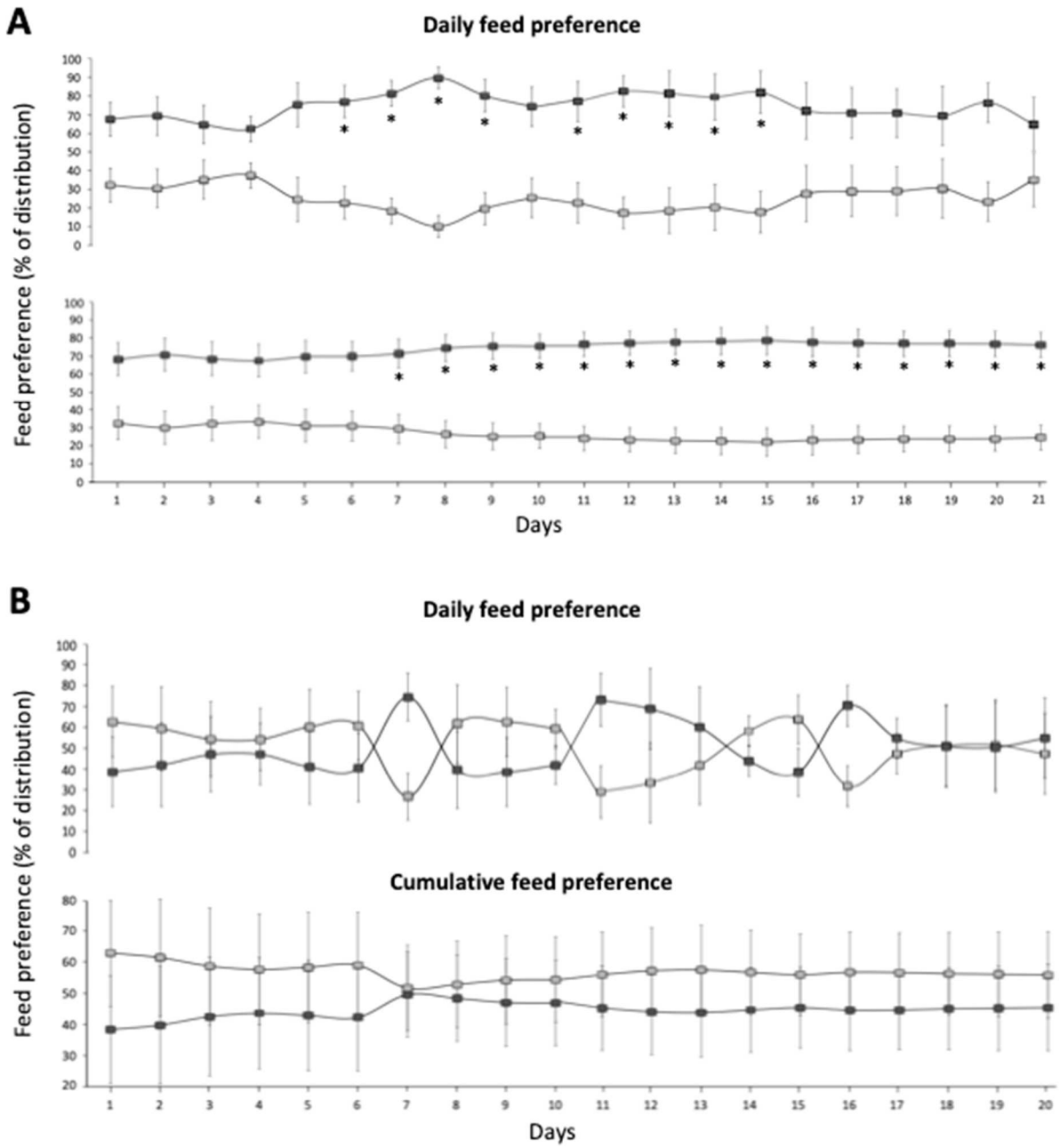
□ LOW ω -3 ■ MEDIUM ω -3



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Figure 2

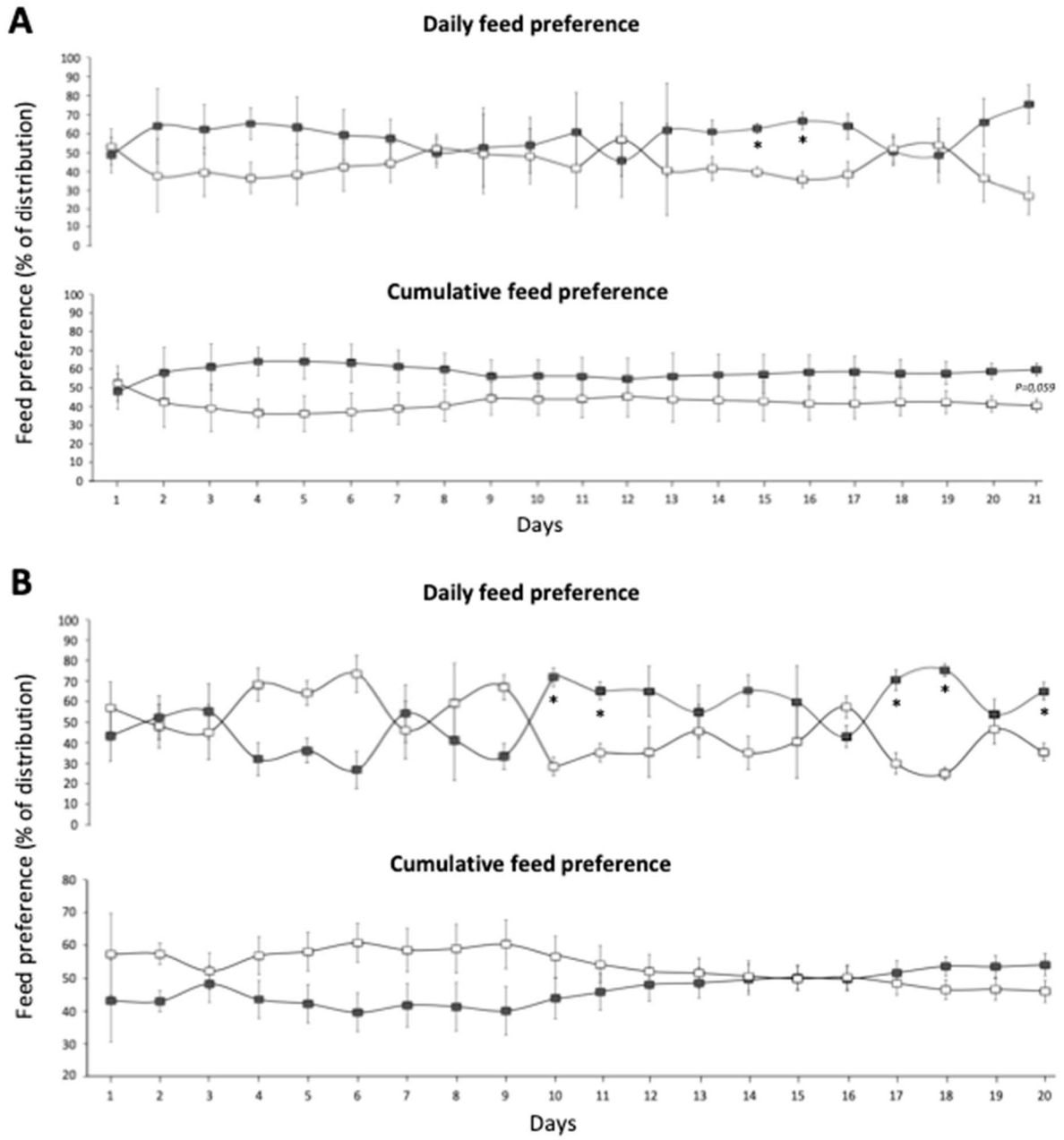
MEDIUM ω -3
 HIGH ω -3



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Figure 3

□ LOW ω -3 ■ HIGH ω -3



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Figure 4

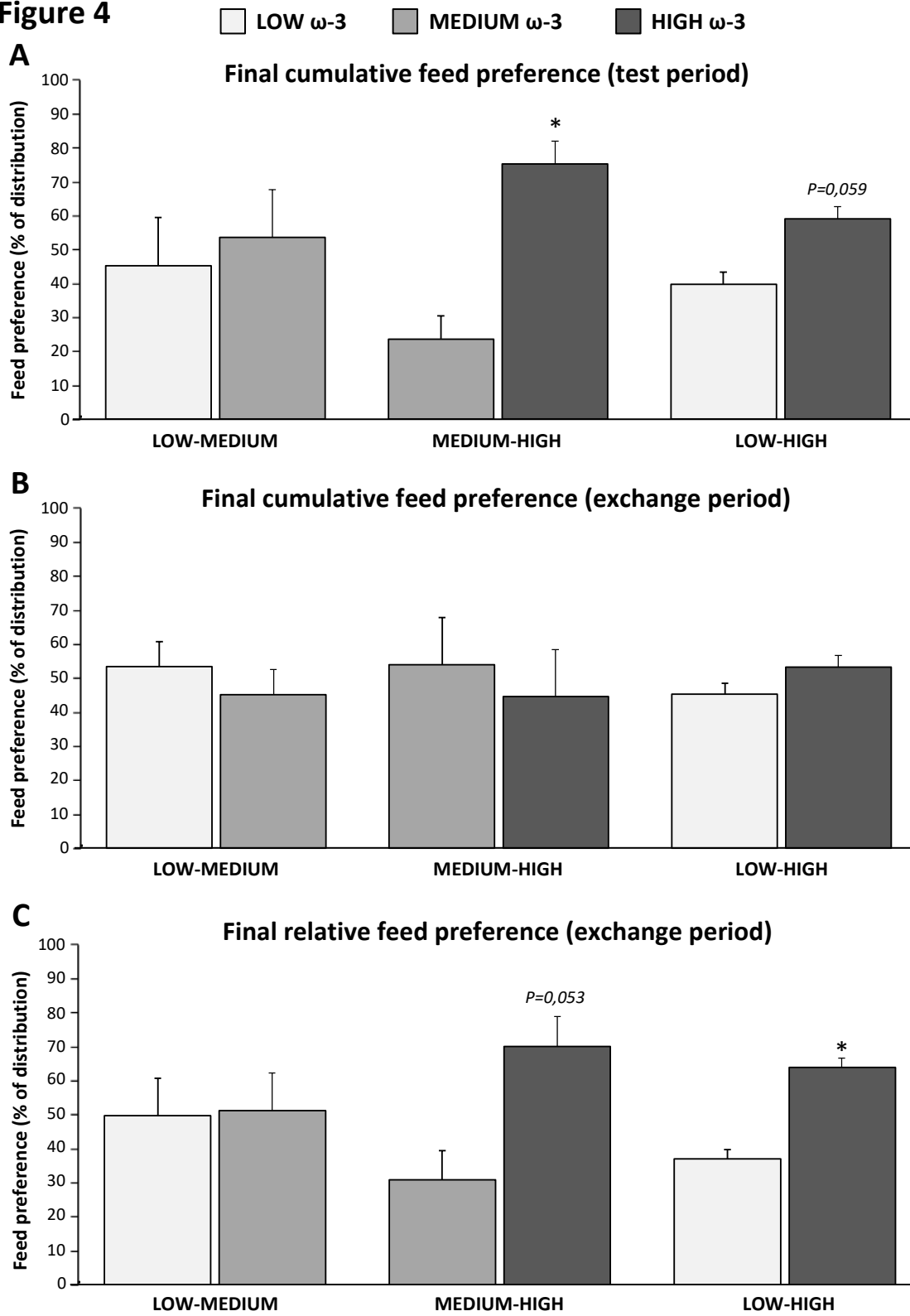
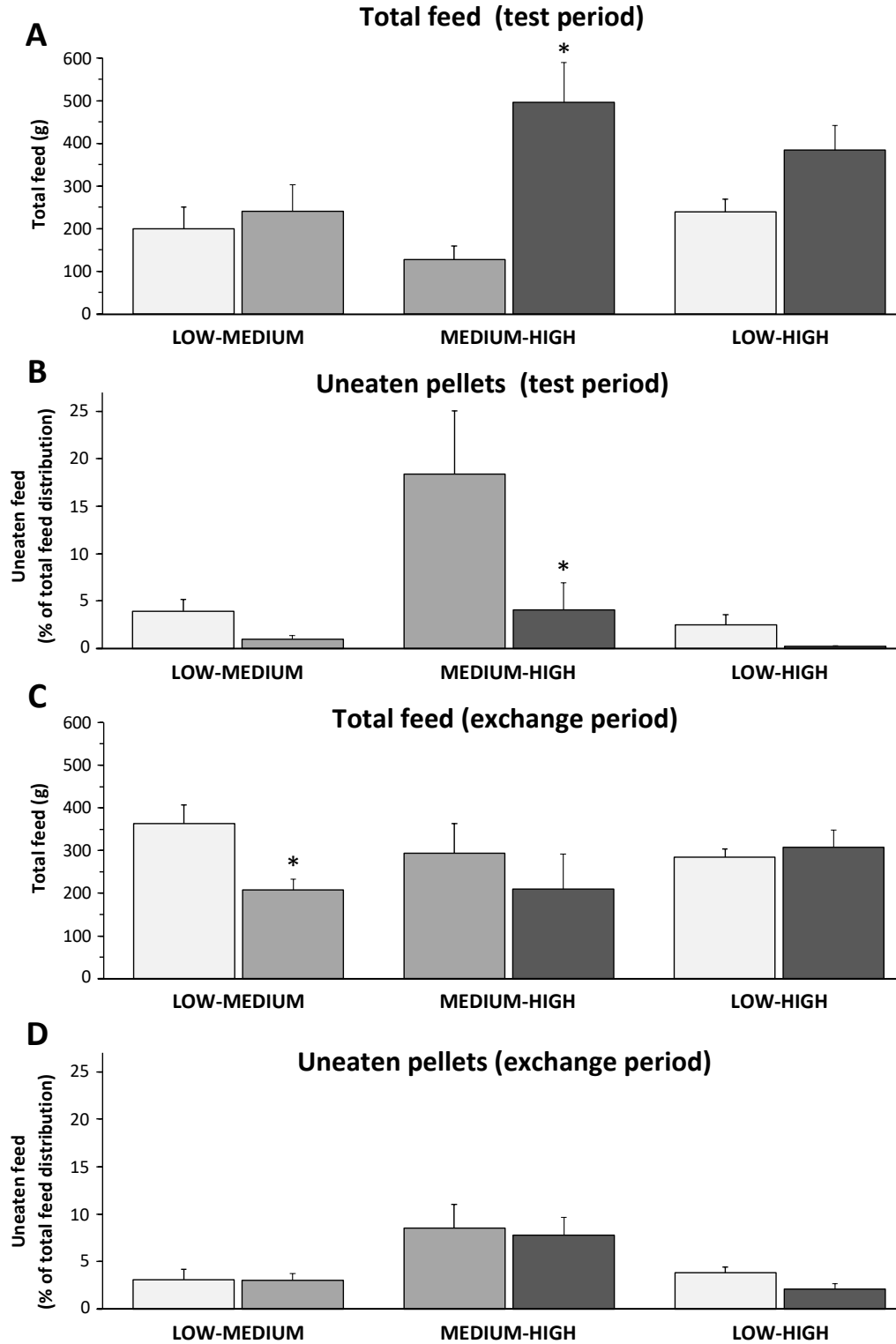


Figure 5

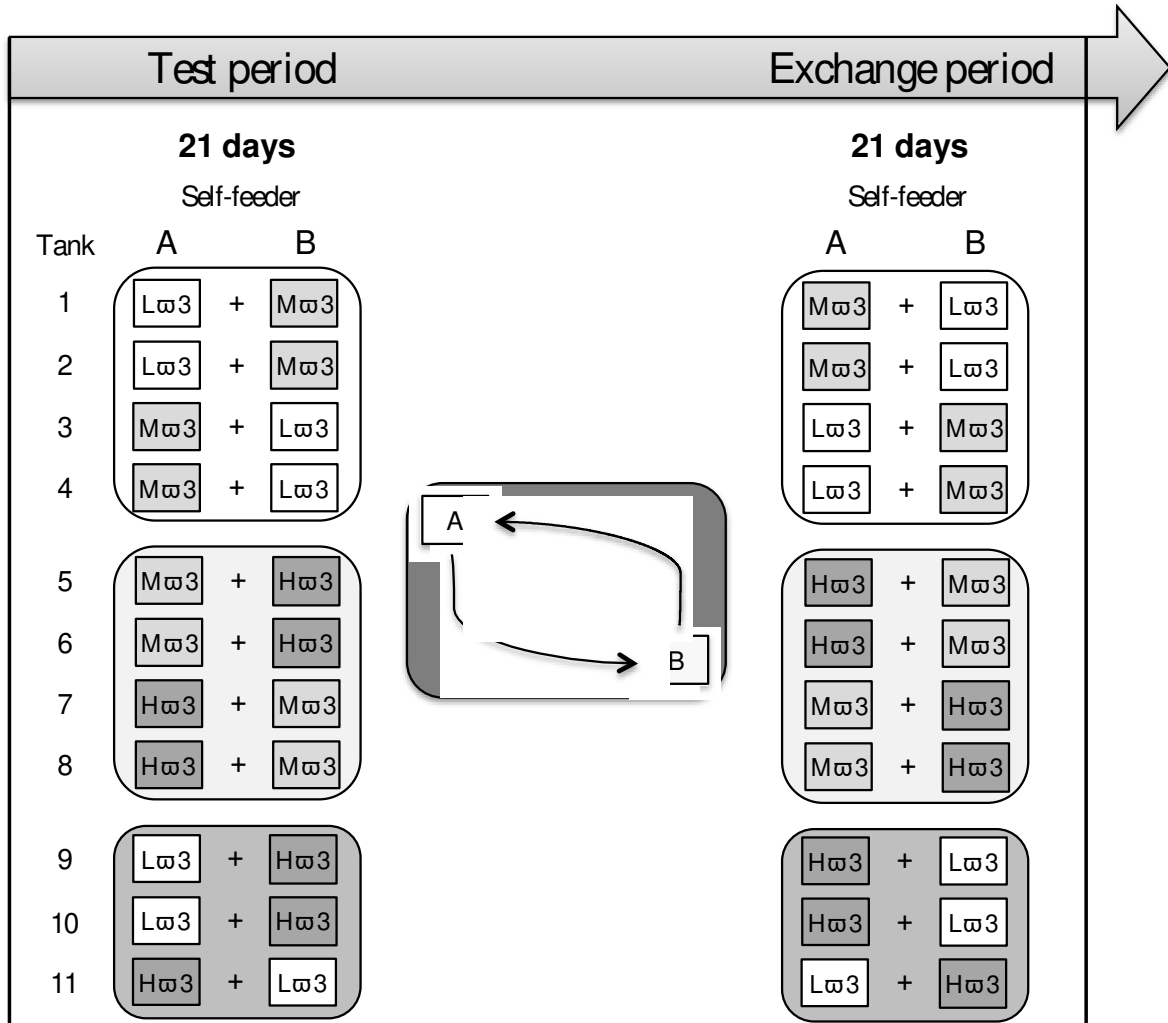
□ LOW ω -3 ▒ MEDIUM ω -3 ■ HIGH ω -3



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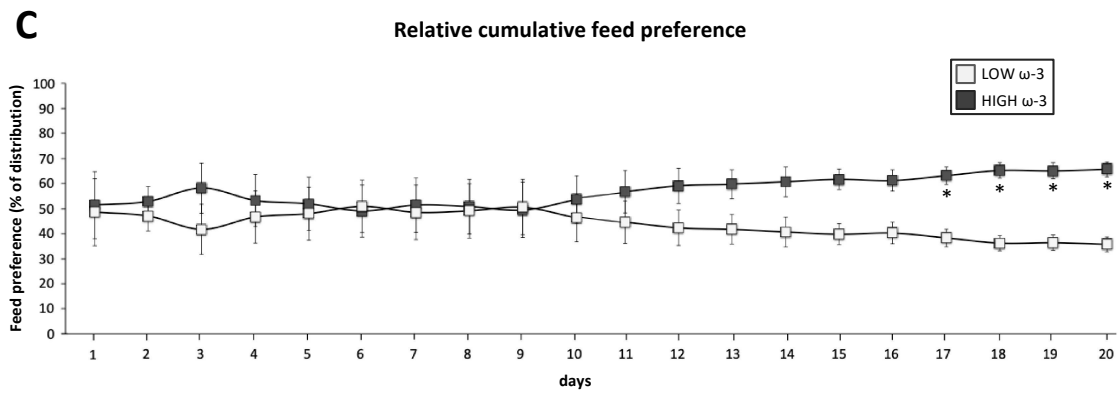
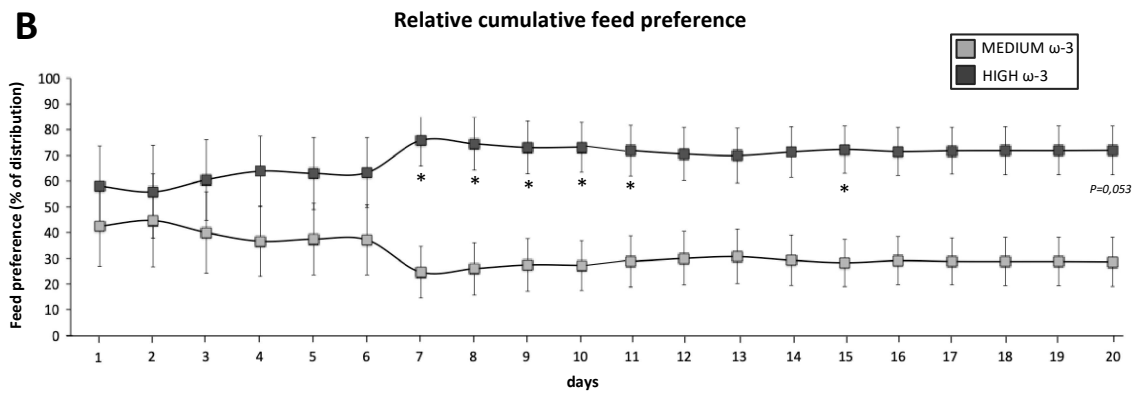
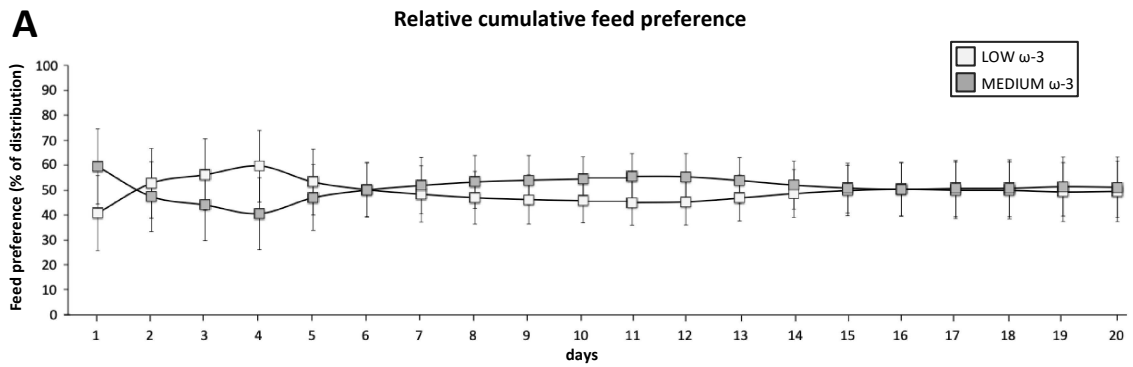
Supporting information 1



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Supporting information 2



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