

1 Novel feed from invasive species is beneficial to walleye aquaculture

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29 **Abstract**

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31 Carnivorous fishes, such as walleye (*Sander vitreus*) are nutritionally demanding for fish
32 meal. A promising alternative to marine-origin fish meal, the supply of which has been stagnant in
33 recent decades, is fish meal derived from undesirable freshwater species, such as the White sucker
34 *Catostomus commersoni*. To evaluate the relative value of such ingredients, we examined the
35 growth performance of Walleye juveniles. Two dietary treatments were tested: an experimental
36 diet (EXP) that was manufactured using White sucker as fish meal in comparison with a
37 commercial (COM) diet, EWOS micro (EWOS Canada Ltd). The protein content was 50.4% and
38 57.6% for EXP and COM diets, respectively. The energy content was $5,098.76 \pm 9.23$ cal/g (mean
39 \pm SD) for the EXP diet and $5,134.47 \pm 10.05$ cal/g for the COM diet. Starting at 27 d posthatch,
40 Walleye juveniles (initial weight [mean \pm SD] = 0.03 ± 0.008 g; initial length = 15.7 ± 1.5 mm)
41 were reared for 6 weeks in three replicate tanks for each treatment. Condition factor (0.83), final
42 weight (1.12 ± 0.3 g), and weight gain (1.09 ± 0.06 g) were higher in EXP fish. Similarly, the
43 energetic lipid content of fish in the EXP treatment group (mean \pm SD = 5.01 ± 0.45 g/kg) was also
44 higher than that of fish fed the COM diet (3.30 ± 0.53 g/Kg). Although the polar lipid content
45 (membrane lipids) was similar in fish from the two treatments, the nutritional ratio for COM
46 juveniles was over 1.5 for arachidonic acid and docosahexaenoic acid, indicating selective
47 incorporation by juveniles and a potential diet \pm imbalance of these fatty acids. Furthermore, the
48 higher observed selective incorporation of oleic acid in juveniles fed the EXP diet suggested that a
49 higher value of this fatty acid in the EXP feed could have increased Walleye growth performance.
50 Threonine was the main essential amino acid (AA; $> 18.5\%$ of total AAs) while serine and glycine
51 contributed the highest percentages of the nonessential AAs ($> 31\%$ and 8.5% of total AAs,

52 respectively). All three AAs, often considered limiting ingredients, are important to support growth
53 and are involved in metabolic processes in some fish species. Our results demonstrate that feed
54 pellets made with white sucker fish meal improved growth in walleye juveniles and can be suitable
55 and probably lower-cost alternative to marine fish meal in feeds for carnivorous fishes.

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58 **Key words:** Walleye (*Sander vitreus*), white sucker (*Catostomus commersoni*), invasive species,
59 juveniles, diet, fatty acid, amino acid.

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67 **Introduction**

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69 Traditional marine-based protein sources derived from pelagic marine fisheries are no
70 longer available in quantities that meet the current increased demands of intensive fish-farm
71 production (FAO, 2016). Stagnant supply, greater demand, and rising prices have prompted the
72 search for substitutes. Indeed, fish meal prices doubled from US\$694 to \$1,379 per metric ton
73 between 2007 and 2008 (Tacon and Metian, 2008).

74 A potential alternative to marine-origin fish meal is a freshwater meal rendered from invasive
75 and/or undesirable species, such as the White sucker *Catostomus commersonii*. Previous studies
76 have evaluated the potential of Asian carp *Hypophthalmichthys* spp. as fish meal in pelleted feeds
77 (Bowzer et al., 2013). Invasive Asian Carp were demonstrated to be a cost-effective alternative
78 protein source (US\$600 per metric ton; Bowzer and Trushenski, 2015) to traditional marine origin
79 fish meal in the diets of carnivorous fishes, such as hybrid Striped Bass (White Bass *Morone*
80 *chrysops* × Striped Bass *M. saxatilis*), Rainbow Trout *Oncorhynchus mykiss*, and Cobia
81 *Rachycentron canadum* (Bowzer et al., 2013; Bowzer and Trushenski, 2015).

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83 The White Sucker is widespread in Canada, the U.S. Midwest, and the U.S. East Coast. It
84 is both a predator and prey of Walleye *Sander vitreus* (Barton, 2011). White Suckers were
85 introduced in Quebec, Canada, by sport fishermen who used it as a baitfish to capture Walleyes or
86 Brook Trout *Salvelinus fontinalis* (Magnan et al., 1990; Duchesne, 1994; St-Laurent, 2002). White
87 Suckers are found in small streams, rivers, and lakes, where they feed on worms, eggs, and fish
88 larvae (e.g., Walleye larvae). The species is tolerant of turbid and polluted waters that are often
89 unsuitable for other fish species (Beamish, 1973; Trippel and Harvey, 1987). This ability allows
90 White Sucker populations to reach high abundances and increase their dispersal. It has been
91 demonstrated that White Sucker is a pest species for Brook Trout and a competitor of Yellow Perch
92 *Perca flavescens* (Duchesne, 1994). White Suckers are found on the same spawning grounds as
93 Walleyes and breed at the same time of the year (Barton, 2011). In 1995, Forêt Faune et Parcs
94 Québec initiated a control program aimed at the mass removal of adult White Suckers in five
95 Quebec lakes (Magnan et al., 1990; Duchesne, 1994; St-Laurent, 2002). Although we do not have
96 a precise estimate for the price per metric ton for this species, we think it is comparable or lower
97 to Asian carp (US\$600 per metric ton), which is considerably lower than current pricing for marine-
98 origin fish meal (US\$1,488 per metric ton, estimated to be 13% higher in 2030) (WBG, 2013).
99 Additionally, an industry must be established to produce the product to supply the market. Due to
100 the initial high risk of investment in a new product, White Sucker meal production facilities have
101 not yet been developed. Based on the potential volumes and the facilities necessary to process
102 White Suckers, it will likely only be a regional alternative to traditional fish meal sources.

103 The Walleye is an important freshwater sport and commercial fish in North America
104 (Hartman, 2009; Johnson and Summerfelt, 2015). Only one available commercial grower feed
105 (Walleye Grower [WG-9206]) has been developed for Walleye larvae and juveniles, and
106 knowledge about their nutrient requirements is still very scarce. Walleyes have been farmed and

107 juveniles have been used in stocking programs for almost 100 years (Webster, 1978). The culture
108 of Walleye juveniles by using pelleted feeds has a much shorter history. Walleyes were first
109 cultured with pelleted feeds in the 1970s using Abernathy Salmon diet and trout granules in private
110 hatcheries and universities in the USA (McCauley, 1970; Beyerle, 1975). The earliest open-
111 formula diet (W series; W7, 14, 15, 16) were developed by the U.S. Fish and Wildlife Service in
112 the 1970s (Beyerle, 1975). The W-16 diet has been used as starter, conversion, and grower diet
113 (Summerfelt and Clayton, 2007, Kuipers and Summerfelt 1994). To date, the grower diet, WG-
114 9206, developed in the early 1990s (Barrows and Lellis, 1996), is the only commercially
115 manufactured diet for walleyes (Nelson & Sons, Inc., Murray, Utah) and has been used
116 continuously to the present at Iowa Department of Natural Resources hatcheries in the USA
117 (Summerfelt and Clay, 2007). Most W-series are available for importation in Canada. However,
118 the cost of importation and the sometimes uncertain availability of pelleted feeds for exportation
119 (i.e. WG 9206) make it hard for farmers in Quebec, Canada, to secure a stable access of Walleye
120 grower feeds. For instance, in 2001, bovine spongiform was found in beef cattle in Japan, and U.S.
121 importation of the BioKyowa FFK formulation (which had been used as habituation diet for
122 Walleyes for 12 years) has halted indefinitely. The same happened with Otohime C2 pellets (used
123 as habituation food for Walleyes) in 2016, where Canadian importation was stopped for an
124 unknown reason. At Most farms in Quebec, Walleye juveniles and broodstock are given frozen
125 fish, and sometimes pelleted diet formulations for salmonids are used to feed juvenile Walleyes
126 (Mejri et al. 2014; *Pêche sportive du Réservoir Baskatong*, Grand Remous , Quebec; personal
127 communication). Thus, development of a pelleted diet is necessary for Walleye production in
128 Canada to increase. Ideally, such a diet should not only be more sustainable, but should also allow
129 faster and more efficient weight gain than the diets currently in use. We tested the potential use of
130 an undesirable species (the White Sucker) as fish meal for the culture of an economically important

131 fish species (the Walleye). The objective of this study was to test whether White Sucker can be
132 used as a main ingredient in feed for Walleye juveniles, thus making use of the removed fish and
133 creating a more sustainable approach. We tested the hypothesis that an artificial diet based on White
134 Suckers can support or improve juvenile Walleye development compared to the commercial diet
135 developed for salmonids that is currently used in some Canadian farms. This approach would make
136 it possible not only to valorize an undesirable species that competes with species of high economic
137 importance but also to produce an affordable and nutrient-dense feed.

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139 **Materials and Methods**

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141 **Fish culture**

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143 All experimental procedures were evaluated and approved by the Ethical-Scientific
144 Committee for Animal Experimentation of the Université du Québec à Rimouski (Certificate
145 number CPA-65-16-176).

146 The 6-week experiment was carried out at the PSRB aquaculture facility, Grand Remous,
147 Quebec. Eggs were collected from wild broodstock in the *Philomène* River, Grand Remous, and
148 then were incubated and hatched at PRSB facilities. Larvae started feeding at 3-4 d post hatch (dph)
149 on Otohime B-1, B-2, C-1, and C-3 (premium Japanese fish nutrition, Japan; pellets used here were
150 imported by PSRB in 2015, with some left to be used in 2016; Canadian importation of Otohime
151 was halted in 2016]). The fish were then switched to EWOS Micro (0.5, 0.7 mm, complete fish
152 feed for salmonids; EWOS Canada Ltd) for 3 weeks. Juveniles used in this experiment were 27

153 dph when the feeding trial started; and initial average weight was 0.03 ± 0.01 g (mean \pm SD) and
154 initial average length was 15.7 ± 1.5 mm.

155 At the start of the trial, approximately 200 fish/ tank were randomly distributed into six
156 900-L circular tanks to obtain three replicate tanks for each dietary treatment. Tanks were provided
157 with natural freshwater from an external pond and connected to a recirculation system, where water
158 was drum filtered, sand filtered, and vacuum degassed before use. The water within each tank was
159 completely exchanged every 2 h, 16 min. The water renewal of the total system was 7.2% daily.
160 Water temperature (range = 21–24 °C) and dissolved oxygen (≥ 7 mg/L) were monitored daily in
161 each tank throughout the experiment. The photoperiod applied was constant (16 h light: 8 h dark),
162 and the light intensity at the surface of the rearing tanks was 630 lx (artificial blue light). Fish were
163 fed daily using an automatic belt feeder that supplied a constant feed ration over two 8-h periods
164 (i.e., from about 0700 to 1500 hours and from 1500 to 2300 hours). Fish were fed to satiation, as
165 indicated by the presence of excess feed in tanks after the two feeding cycles. Tanks were cleaned
166 daily, and dead fish were removed and weighed each day.

167

168 **Experimental feeds**

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170 After the 3-week acclimation period, two dietary treatments were tested: an experimental
171 (EXP) diet and a commercial (COM) diet. The EXP diet was formulated to meet or exceed all
172 known nutrient requirements for Walleye juveniles (Summerfelt and Johnson, 2015) and was
173 prepared at the Département des Sciences Animales at Université Laval, Quebec, Canada,
174 following the recipe of Bharadwaj et al. (2002) for Walleye juveniles (Walleye grower pellet) but
175 substituting the fish meal (menhaden) with White Sucker fish meal. To make the fish meal, filets
176 were removed from White Suckers (20-22 adults weighing 0.894 ± 0.086 kg) captured in the

177 Philomène River during late April 2016. The heads, skin, and viscera were discarded. Briefly,
178 fillets were washed, autoclaved for 1.5 h at 120 °C, dried for 48 h at 65 °C, and ground to make
179 the fish meal. Ingredients for the EXP pellets (size = 1.0–1.2 mm) are listed in Table 1. The dry
180 ingredients were mixed and steam pelleted; the pellets were then dried in a forced-air oven (30 °C,
181 24 h), sieved, coated with mackerel oil, and stored at -20 °C until used. The ingredients of the COM
182 pellets (EWOS micro, 1.0–1.2 mm) are listed in Table 1. Both pellets were slightly pulverized
183 during the first days of feeding to ensure that small individuals had access to the feed.

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185 **Sampling and data collection procedures**

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187 At the beginning and at the end of the experiment, all fish from each tank were individually
188 weighed and their TLs were recorded. From each tank (6 tanks total; 3 tanks/treatment), five
189 replicate samples of five juveniles were frozen in liquid nitrogen and stored at -80 °C for further
190 biochemical analysis.

191 The following indices were calculated:

192

- 193 • Weight gain, $WG = \text{final weight (g)} - \text{initial weight (g)}$
- 194 • Feed conversion ratio, $FCR = \frac{\text{Weight of feed consumed (g)}}{\text{Weight gain (g)}}$
- 195 • Specific growth rate, $SGR = 100 \times \frac{\ln \text{ final body weight (g)} - \ln \text{ initial body weight (g)}}{\text{rearing time (days)}}$
- 196 • Survival (%), $S = 100 \times \frac{\text{Starting N individuals}}{\text{Final N individuals}}$
- 197 • Condition factor, $K = \frac{\text{Mass}}{\text{Length}^3} \times 100$

198

199 To obtain an indicator of nutritional quality of the two diets, we used the ratio of the polar
200 fraction of fatty acids (phospholipids constituting cell membranes) in juveniles to the polar
201 fraction of fatty acids present in the diets. This ratio indicates the juveniles' selective
202 incorporation or elimination of a given dietary fatty acid in polar lipids in the cell
203 membranes.

204

205 **Chemical analysis**

206

207 Walleye body water content was determined by drying the samples to a constant weight for
208 24 h at 70 °C. Proximate analyses were performed in accordance with standard methods (AOAC
209 International 2012; ash [Mthod 942.05], crude protein [Method 990.03], fat [Method 954.02], and
210 moisture [Method 930.15] by New Jersey Feed Laboratory (Trenton, New Jersey).

211 Lipids were extracted using the Folch method (Folch et al., 1957). Lipids were separated
212 into neutral and polar lipid fractions using silica gel (30 × 5 mm internal diameter, packed with
213 Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) hydrated with 6% water and were eluted
214 with 10 mL of chloroform : methanol (98:2 volume/volume) for neutral lipids followed by 20 mL
215 of methanol for polar lipids (Marty et al., 1992). The neutral lipid fraction was further eluted on an
216 activated silica gel with 3 mL of hexane and diethyl ether to eliminate free sterols. All fatty acid
217 methyl esters (FAMES) were prepared as described by Lepage and Roy (1984) and analyzed in
218 MSMS scan mode (ionic range = 60–650 m/z) on a Polaris Q ion trap coupled to a Trace GC
219 (Thermo Finnigan, Mississauga, Otario) equipped with a Valcobond VB-5 capillary column (Valco
220 Instruments Co. Inc., Broakville, Ontario). The FAMES were identified by comparison of retention
221 times with known standards (37-Component FAME Mix, PUFA-3, BAME, and menhaden oil;
222 Supelco Bellefonte, Pennsylvania) and quantified with tricosanoic acid (23:0; i.e. 23 carbon atoms

223 and zero double bonds) and nonadecanoic acid (19:0) as internal standards. Chromatograms were
224 analyzed using the Xcalibur version 1.3 (Thermo Scientific, Mississauga, Ontario).

225 For amino acid (AA) analysis, samples of either whole body tissues of juveniles or pellets
226 were diluted with 2 mL distilled water and hydrolyzed with equal parts of 12-N HCl plus 0.1%
227 phenol at 110 °C for 24 h. After HCl removal by evaporation under vacuum, determination of o-
228 phthalaldehyde derivatives of AAs were made by high-performance liquid chromatography
229 separation, as detailed in Kaushik et al. (1994).

230 Total energy (cal/g) was analyzed using a bomb calorimeter (Parr 6200; Preiser Scientific,
231 Inc., St. Albans, West Virginia), employing benzoic acid as a standard with a known weight and
232 energy content.

233

234 **Statistical analysis**

235

236 Weight, weight gain, length, FCR, SGR, survival, condition factor, and moisture and ash
237 content were tested with one-way analysis of variance (ANOVA) followed by Hsu's a posteriori
238 multiple comparisons tests after assumptions of homoscedasticity and normality had been verified
239 by Levene and Shapiro-Wilk tests, respectively. These analyses were performed with the JMP Pr
240 12 package (SAS Institute Inc., Cary, NC). Permutational multivariate analysis of variance
241 (PERMANOVA with 9999 permutations), including a posteriori pair-wise comparisons, was
242 performed on fatty acid and amino acid profiles in experimental and commercial pellets. Fatty acids
243 from neutral and polar lipid fractions and AA profiles in walleye juveniles were tested with one-
244 factor PERMANOVA (dietary treatment, EXP and COM). Assumptions of homoscedasticity were
245 verified with a PERMDISP test and data were transformed (arcsine square root) when necessary.
246 To analyze the similarity between the profiles, non-metric multi-dimensional scaling (n-MDS) and

247 SIMPER analyses were performed using a Bray-Curtis similarity matrix with PRIMER 6 (v.
248 6.1.12) and PERMANOVA+ (v. 1.0.2) (Anderson, 2001).

249

250 **Results**

251

252 **Diets**

253

254 The dry weight percentage from White sucker filets was $20.8 \pm 0.5\%$. (mean \pm SD).

255 Proximate composition of ECP and COM pellets is presented in Table 1.

256

257 **Growth and fish quality parameters**

258

259 At the end of the 6-week feeding period, no significant difference was observed between

260 the two dietary treatments for Walleye TL, *S*, moisture content, or and ash content (Table 2).

261 Survival percentages decreased significantly 4 weeks after the start of the experiment (Fig. 1). No

262 significant difference was seen for FCR or SGR values. However, *K*, final weight, and WG were

263 significantly higher in fish fed that were fed the EXP diet (hereafter “EXP fish”). Similarly, the

264 neutral lipid content (energetic lipids) of EXP fish (mean \pm SD = 5 ± 0.5 g/kg) was also found to

265 be higher than that of fish receiving the COM diet (hereafter, “COM” fish”; 3.3 ± 0.5 g/ kg), while

266 the polar lipid content (lipids in cell tissues) was similar between fish from the two treatments.

267 Total protein content was not different between the two dietary treatments (EX fish; mean \pm SD =

268 115 ± 10 g/kg; COM fish: 107 ± 11 g/kg).

269

270 **Nutrient composition of the diet**

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272 Percentages of different fatty acids in the EXP and COM diets are presented in Table 3.
273 The AA percentages of COM and EXP pellets are presented in Table 4. Threonine (THR) was the
274 most prominent of the essential AAs (EAAs; Table 3). Serine (SER), glycine (GLY), proline,
275 and taurine quantitatively dominated the nonessential AAs (NEAAs; Table 4).

276
277 **Nutrient composition of juveniles**

278
279 Neutral lipids

280
281 At the start of the experiment (day 0), the fatty acid composition of the neutral lipids was
282 similar in all Walleye juveniles ($p = 0.69$). However, this composition varied according to diet at
283 the end of the experiment (pseudo- $F_{\text{diet}[1,4]} = 16.34$, $P = 0.005$; Table 5). The fatty acid profiles of
284 COM fish did not resemble those of the EXP fish, with both profiles resembling those of the diets.
285 Oleic acid (18:1[n-9], where the number to the left of the colon is the number of carbon atoms, the
286 number to the right of the hyphen is the position of the first double bound from the methyl end),
287 docosahexaenoic acid (DHA; 22:6[n-3]), and total polyunsaturated fatty acids (PUFAs) were
288 highest in COM juveniles. However, 20:1(n-9), 22:1(n-9), 20:4(n-6), and total monounsaturated
289 fatty acids (MUFAs) were significantly higher in EXP fish than in COM fish.

290
291 Polar lipids

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293 The same tendency was observed in the polar lipid fraction as in the neutral lipid fraction:
294 there was no difference in fatty acid composition between juveniles at the start of the experiment
295 ($P = 0.56$), whereas the fatty acid composition in juveniles at the end of the experiment varied
296 according to the diet they were fed (pseudo- $F_{\text{diet}[1, 4]} = 17.71$, $P = 0.003$; Table 5). For polar lipids,
297 20:1(n-9), 22:1(n-9), MUFA, and 20:4(n-6) were significantly higher in EXP juveniles, while DHA
298 was higher in COM juveniles.

299

300 Nutritional ratio

301

302 For COM juveniles, the observed juvenile : diet ratio was above 1.5 for ARA and DHA
303 (Fig. 2). indicating selective incorporation of these fatty acids. For EXP fish, the was around 2 for
304 oleic acid, indicating potential selective retention of this fatty acid by Walleye juveniles and
305 suggesting its importance for cell membranes integrity under the rearing conditions. Results for
306 18:2(n-6) and 18:3(n-3) showed no difference between diets, with the ratio less than 0.5 for both
307 diets, indicating elimination of this fatty acid in the cell membrane.

308

309 Amino acids

310

311 Both at the start and at the end of the experiment, the AA composition of juvenile tissues
312 did not vary with diet (start: $P = 0.329$; end: $P = 0.196$). Threonine, an EAA, represented more than
313 18% of the total AAs. Serine and GLY (NEAAs) constituted over 31 and 8% of total AAs,
314 respectively (Table 6).

315

316 **Discussion**

317

318 Because of the continuous expansion and development of aquaculture worldwide, it is
319 urgent that sustainable alternatives for pelagic fish meal be investigated, developed, and introduced
320 for use in feed formulations. The use of an undesirable and/or invasive fish species as feed for the
321 culture of the economically important Walleye is a very interesting sustainable approach. The
322 White Sucker is a regionally feasible alternative to traditional fish meal sources. The results from
323 this study indicate that the White Sucker, which is considered an undesirable species in Quebec
324 lakes, has the potential to be used as such. Pellets incorporating White sucker as the fish meal were
325 readily accepted by Walleye juveniles, who showed better WG, higher K, and significantly higher
326 energy lipid accumulation compared to those that received the COM diet. Our results suggest that
327 the EXP pellet generally was nutritionally balanced but could have been improved with additional
328 oleic acid. In contrast, the COM diet showed some potential imbalanced proportions of DHA and
329 ARA, and COM juveniles had lower WG than EXP fish..

330

331 **Growth**

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333 Growth was better in the EXP diet, indicating that White Sucker fish meal was readily
334 accepted by Walleye juveniles. Bharadwaj et al. (2002) indicated that salmon and trout grower
335 diets could be used satisfactorily for Walleye juveniles pending more information on their
336 nutritional needs. In our study, final weight, WG, and K were higher in juveniles that were fed the
337 EXP diet compared to those receiving the COM diet formulated for salmonids diet. The SGR was
338 not different between the two dietary treatments, ranging between 8.6% and 8.9% per day, and was

339 similar to observations reported for other percid juveniles (Hilge and Steffens, 1996; Kestemont
340 and Mélard, 2000; Mandiki et al., 2004; Nyina-Wamwiza et al., 2005; Schulz et al., 2006; Schulz
341 et al., 2007; Lepič et al., 2017). However, in contrast to our results, previous investigations on
342 percid fish have shown relatively low SGRs. Indeed, SGR values ranged from 2.1% to 3.5% per
343 day for Zander *Sander lucioperca* juveniles that either were given commercial diets formulated for
344 Rainbow trout or were fed natural diets (Zakes and Demska-Zakes, 1996; Schulz et al., 2006). High
345 FCR may be related to the territorial behavior of dominant individuals, therefore a high daily
346 feeding ratio (25% of total biomass per 24 h) was chosen to ensure adequate feed intake for all fish.
347 However, this resulted in excessive amounts of uneaten feed and probably led to the overestimation
348 of FCR values. A negative impact on FCR by possible aggressive feeding behavior of dominant
349 individuals has also been described for other percid fish, such as the Eurasian Perch *Perca*
350 *fluviatilis* (Schulz et al., 2007). Survival rates of Walleye juveniles in this study were less than
351 50%. Such high mortality was most likely caused by the small initial size of the juveniles (15.7
352 mm; 0.03 g). In agreement with our results, Summerfelt and Johnson (2015) observed low survival
353 rates (ranging from 26 to 69.7%) in Walleye juveniles. Malison and Held (1996) also reported that
354 35- 46 mm (0.3-0.5 g) fish had a survival rate of 33.9%.

355

356 **Fatty acid and nutritional ratio**

357

358 The body fatty acid profiles reflected those of the diets (Tables 3.). Although the
359 percentages of ARA were relatively low in both treatments, tissue levels were influenced by levels
360 supplied in the diet. A higher proportion of ARA in the EXP diet was reflected in a higher
361 percentage of ARA in the polar and neutral lipids of the whole fish. Furthermore, we observed the
362 selective incorporation of ARA in COM juveniles, suggesting that this fatty acid is essential for

363 juvenile Walleye growth. In comparison, the EXP diet showed no selective incorporation of ARA,
364 indicating that this diet meets the physiological ARA needs of juvenile Walleyes. Several studies
365 have indicated the importance of ARA in fish metabolism, and it is known to be the main fatty acid
366 precursor of eicosanoids in fish (Bell et al., 1983; Henderson and Tocher, 1987; Bell and Dick,
367 1990; Bell et al., 1994). In addition, research has been conducted on dietary ARA in several fish
368 species, and these studies have confirmed that elevated ARA can improve growth and survival
369 (Bessonart et al., 1999; Bae et al., 2010) as well as resistance to handling stress (Koven et al.,
370 2001). In addition to the selective incorporation of ARA in COM Walleye juveniles, we also
371 observed a higher selective incorporation of DHA in COM fish compared to EXP fish. These
372 results suggest that ARA and DHA levels may be imbalanced in the COM diet. In comparison,
373 juveniles receiving the EXP diet showed a weak selective incorporation of DHA and EPA but a
374 marked selective retention of oleic acid. Thus the use of White Sucker fish meal in the EXP diet
375 seems so support the physiological essential fatty acid needs, but oleic acid could be increased as
376 a potential energy source for Walleyes. Previous studies have demonstrated that oleic acid is a good
377 energy substrate for *Totoaba macdonaldi* juveniles (Zapata et al. 2016) and Walleye larvae
378 (Mejri et al. 2014). Thus, the EXP diet, as prepared in our study, should be adjusted to contain
379 higher proportion of oleic acid.

380
381 Most PUFAs were found in the polar lipids. The preferential conservation of PUFAs in the
382 polar portion indicated a strong metabolic response of Walleye juveniles. Polyunsaturated fatty
383 acids are the major constituents of structural lipids and are therefore important in maintaining the
384 structural integrity of biological membranes. Previous studies have shown that when PUFAs are
385 insufficient or limited, they are removed from the neutral lipids and conserved in the polar lipids
386 (D'Abramo and Sheen, 1993; Mejri et al., 2014).

387 Our results clearly demonstrate that feeding juvenile Walleyes the EXP pellets resulted in
388 significantly higher energy lipid (neutral lipid) accumulation, better *K*, and modified fatty acid
389 composition in the juveniles. The higher energy reserves of EXP juveniles indicates that these fish
390 have a higher nutritional condition compared to those fed the COM diet.

391

392 **Amino acids**

393

394 The AA composition, especially EAA, is another excellent indicator of how the nutritional
395 needs are satisfied. Amino acids have essential roles in fish growth and diet costs (Wilson and Poe,
396 1985; Conceição et al., 2003). High percentages of THR, SER, and GLY in the EXP and COM
397 pellets were reflected in juvenile AA profiles. Studies conducted on early stages of Walleye have
398 determined the importance of methionine (MET) and SER during embryogenesis, suggesting the
399 need for minimal levels of 0.1% MET and 6% SER in the total AAs (Mejri et al., 2014; Mejri et
400 al., 2017). Compared to the other AAs, SER levels were the highest in Walleye juveniles from both
401 treatments, suggesting its potential role as an energy source. The MET levels were over 2% of total
402 AAs, which should meet the nutritional requirement for Walleyes (Mejri et al., 2017). After lysine
403 and MET, THR is generally one of the most limiting EAAs in feed ingredients (Saldana et al.,
404 1994; Yu et al., 2015). In our study, THR was the most prominent EAA (> 18% of total AAs),
405 showing no significant difference between the COM and EXP groups. Threonine deficiency
406 resulted in retarded growth and poor feed efficiency values in Red Drum *Sciaenops ocellatus*
407 juveniles (Boren and Gatlin, 1995), Olive Flounder, *Paralichthys olivaceus* (Alam et al., 2003),
408 and Rainbow Trout (Rodehutschord et al., 1997). Feng et al. (2013) showed that THR enhanced
409 growth and increased the digestive and absorptive capacities in Jian Carp (a variant of the Common
410 Carp *Cyprinus carpio*).

411

412 **Conclusions**

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414 This study has demonstrated the beneficial effects of using White Sucker as the main fish
415 meal in feed pellets. Reducing feed costs by the substitution of marine fish meal (expensive
416 ingredient) with more cost-effective White Sucker meal can also assist the growth of the
417 aquaculture industry, mainly on a regional scale. The EXP feed resulted in improved growth of
418 Walleye juveniles, and its use may contribute to reducing costs related to the elimination of this
419 undesirable fish species, thus contributing to environment protection. Additional work should be
420 done to optimize the fatty acid composition of pellets made using White Sucker fish meal by
421 supplementing with oleic acid.

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426

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599 **Figure captions**

600 **Fig. 1** Survival of walleye juveniles fed an experimental diet based on the use of white sucker
601 (*Catostomus commersoni*) as fish meal or a commercial diet (EWOS micro; Ewos Canada Ltd)
602 during a six weeks feeding trial.

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605 **Fig. 2** Ratio of polar fatty acids in walleye (*Sander vitreus*) juveniles to dietary fatty acids.
606 Measurements were made six weeks after juveniles were fed an experimental diet based on the use
607 of white sucker (*Catostomus commersoni*) as fish meal or a commercial diet (EWOS micro; Ewos
608 Canada Ltd). The dashed line indicates equal amounts of fatty acids in the juveniles and in the diet.
609 PUFA: polyunsaturated fatty acid.

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1 Table 1. Composition (%) of experimental (EXP; size = 1.0–1.2 mm) and commercial (COM;
 2 EWOS Micro, 1.0–1.2 mm) diets fed to juvenile Walleyes.

Ingredient or component	EXP diet	COM diet	6
			7
White Sucker fish meal	49.5	-	8
Fish meal (mix of anchovies, menhaden, pollock, white fish, and herring trimmings from US and Canadian Pacific Coast fisheries)	-	58.8	9
Blood meal	4.8	6.7	10
Soybean meal*	12		11
Corn gluten meal*	8.3		
Wheat flour*	12.1	11.6	12
Wheat gluten	-	2.5	
Fish oil	11.8	10	13
Vitamin mix*	0.8	-	
Ascorbic acid	0.2	-	14
Choline chloride	0.5	-	
Yeast-amino acid premixes-vitamin and mineral premix	-	10.4	15

16 ^asoybean meal in the EXP diet was obtained from Jefo, St-Hyacinthe, Quebec.

17 ^bCorn gluten meal in the EXP diet was obtained from Meunerie Gérard Soucy, Inc., Ste-Croix,
 18 Québec.

19 ^cWheat flour in the EXP diet was obtained from la Seigneurie des Aulnaies, Inc., Saint-Roch-des-
 20 Aulnaies, Québec.

21 ^dVitamin mix in the EXP diet was obtained from Corey Feed Mills Ltd., Fredericton, New-
 22 Brunswick (Vitamin Mix 30): vitamin A, 650; vitamin D3, 1,000; vitamin E, 50 %, vitamin K,
 23 MSBC50%, 16.5; vitamin B12, 10%; biotin, 2%, folic acid 20%, niacin 50%; vitamin B6,
 24 pyridoxine hydrochloride, 99%; vitamin B2, 96%, BHT-KI, 76%; FeSO₄ 7H₂O (20% Fe); MnSO₄
 25 7 H₂O (36% Mn); ZnSO₄ 7 H₂O (40% Zn); CuSO₄ 5H₂O (25% Cu); Na₂SeO₃ (45.6% Se); CoSO₄
 26 (21% Co) and starch or cellulose.

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30 Table 2. Growth and condition indices (mean \pm nSD) of Walleye juveniles fed a commercial
 31 (COM) diet (EWOS Micro; EWOS Canada Ltd.) or an experimental (EXP) diet (containing White
 32 Sucker as fish meal) over a six-week study period. Within a given row, means with different letters
 33 are significantly different (ANOVA: $P < 0.05$)

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Growth and condition indices	EXP diet	COM diet	One-way ANOVA (P-value)
Initial weight (g)	0.03 \pm 0.008	0.03 \pm 0.009	0.787
Final weight (g)	1.12 \pm 0.36 ^z	0.87 \pm 0.35 ^y	< 0.0001
Weight gain (g)	1.09 \pm 0.06 ^z	0.86 \pm 0.15 ^y	0.042
Final TL (mm)	50.92 \pm 5.99	49.81 \pm 6.72	0.082
Feed conversion ratio	2.85 \pm 1.12	2.21 \pm 1.06	0.145
Specific growth rate	8.87 \pm 0.16	8.58 \pm 0.43	0.413
Survival (%)	43.11 \pm 10.96	46.23 \pm 9.34	0.727
Condition factor	0.83 ^z	0.67 ^y	< 0.0001
Moisture (g/kg)	208.61 \pm 2.73	203.38 \pm 1.75	0.150
Ash (g/kg ¹)	20.27 \pm 1.65	24.97 \pm 2.21	0.138
Neutral fatty acids (g/kg)	5.01 \pm 0.45 ^z	3.30 \pm 0.53 ^y	0.013
Polar fatty acids (g/kg)	2.02 \pm 0.47	1.27 \pm 0.43	0.116
Proteins (g/kg)	115.23 \pm 9.99	107.17 \pm 10.53	0.227

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38 Table 3. Fatty acid composition (percent of total fatty acids; mean \pm SD) of the experimental (EXP)
 39 diet (containing White Sucker as fish meal) and the commercial (COM) diet (EWOS Micro; EWOS
 40 Canada Ltd) fed to juvenile Walleyes.

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Fatty acid	EXP diet	COM diet
14:0	2.14 \pm 0.40	3.84 \pm 0.38
16:0	16.82 \pm 1.79	22.55 \pm 1.15
18:0	3.97 \pm 0.67	5.70 \pm 0.90
Σ SFA α	25.39 \pm 4.46	34.56 \pm 6.02
16:1	4.80 \pm 0.57	3.98 \pm 0.43
18:1 n-9	8.44 \pm 0.29 ^y	15.64 \pm 0.85 ^z
20:1 n-9	8.82 \pm 2.04 ^z	4.76 \pm 1.58 ^y
22:1 n-9	14.62 \pm 3.68 ^z	4.52 \pm 1.29 ^y
24:1 n-9	0.49 \pm 0.09	1.02 \pm 0.13
Σ MUFA β	37.48 \pm 5.48	30.43 \pm 5.20
18:2 n-6	10.01 \pm 0.72	7.53 \pm 0.31
18:3 n-3	1.64 \pm 0.12	1.36 \pm 0.08
18:4 n-3	0.44 \pm 0.03	1.53 \pm 0.18
20:4 n-6	4.94 \pm 0.67 ^z	1.11 \pm 0.32 ^y
20:5 n-3	5.98 \pm 0.21	9.11 \pm 0.72
22:6 n-3	13.21 \pm 2.10	13.65 \pm 1.21
Σ PUFA δ	37.13 \pm 4.61	35.01 \pm 4.12
Σ n-3	21.33 \pm 2.71	25.68 \pm 4.12
Σ n-6	15.48 \pm 4.65	9.13 \pm 3.52

66 α Sum of saturated fatty acids (SFA) includes 11:0, 12:0, 13:0, 15:0, 17:0, 20:0, 21:0, 22:0, 23:0,
 67 24:0, for which the combined percentages are \leq 0.5% of total fatty acids
 68 β Sum of monounsaturated fatty acids (MUFA) includes 14:1 and 17:1, for which the combined
 69 percentages are \leq 0.5% of total fatty acids
 70 δ Sum of polyunsaturated fatty acids (PUFA) includes 20:2, 18:3(n-6), 20:3(n-6), 20:3(n-3), for
 71 which the combined percentages are \leq 0.5% of total fatty acids.
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73 Table 4. Amino acid composition (percent of total amino acids; mean \pm SD) in the experimental
 74 (EXP) diet containing White Sucker as fish meal) and the commercial (COM) diet (EWOS
 75 Micro; EWOS Canada Ltd) fed to juvenile Walleyes. Amino acids constituting less than 1% are
 76 not listed

Amino acid	EXP diet	COM diet
<i>Essential amino acids</i>		
Threonine, THR	15.04 \pm 2.05	15.54 \pm 1.94
Methionine, MET	3.30 \pm 0.19	2.79 \pm 0.35
All-Leucine type, AILE	3.49 \pm 0.60	5.29 \pm 0.43
Phenylalanine, PHE	4.11 \pm 0.20	3.30 \pm 0.26
<i>Non-essential amino acids</i>		
Taurine, TAU	6.41 \pm 4.32	9.64 \pm 7.00
Serine, SER	21.80 \pm 7.84	21.04 \pm 12.49
Tyrosine, TYR	4.34 \pm 0.27	2.01 \pm 0.89
Alanine, ALA	8.59 \pm 3.41	8.68 \pm 5.00
Sarcosine, SAR	3.64 \pm 4.28	3.20 \pm 3.31
Glycine, GLY	15.46 \pm 4.89	14.24 \pm 2.41
Hydroxyproline, HYP	1.04 \pm 0.52	1.02 \pm 0.29
Proline, PRO	7.71 \pm 1.12	7.80 \pm 1.36
Trimethylglycine, TMG	3.89 \pm 0.36	4.52 \pm 1.40

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80 Table 5. Fatty acid composition of neutral and polar lipids in Walleye juveniles (percent weight of
 81 weight of total neutral and polar lipids; mean \pm SD) after 6 weeks of feeding with either the
 82 experimental (EXP) diet (containing White Sucker as fish meal) or the commercial (COM) diet
 83 (EWOS Micro; EWOS Canada Ltd). Different letters indicate significant differences between
 84 treatments (ANOVA: $P < 0.05$), with polar and neutral fatty acids being tested separately.

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Fatty acid	Neutral fraction		Polar fraction	
	EXP diet	COM diet	EXP diet	COM diet
14:0	4.11 \pm 0.13	4.04 \pm 0.96	1.92 \pm 0.09	1.66 \pm 0.31
16:0	14.86 \pm 2.23	15.02 \pm 0.65	23.08 \pm 0.22	25.45 \pm 0.73
18:0	2.15 \pm 0.36	3.13 \pm 0.79	5.66 \pm 0.63 ^y	7.96 \pm 0.60 ^z
Σ SFA α	22.65 \pm 3.98	24.14 \pm 4.02	32.92 \pm 6.15	38.32 \pm 6.85
16:1	10.61 \pm 0.42	7.73 \pm 0.53	4.78 \pm 0.34 ^z	2.76 \pm 0.25 ^y
18:1 n-9	16.76 \pm 0.92 ^y	24.19 \pm 2.19 ^z	16.52 \pm 1.13	14.97 \pm 0.33
20:1 n-9	12.62 \pm 0.29 ^z	5.62 \pm 2.06 ^y	6.27 \pm 0.35 ^z	2.19 \pm 0.50 ^y
22:1 n-9	16.05 \pm 0.60 ^z	4.19 \pm 1.65 ^y	3.91 \pm 0.26 ^z	1.14 \pm 0.22 ^y
24:1 n-9	0.63 \pm 0.05	0.94 \pm 0.14	0.79 \pm 0.10	0.97 \pm 0.20
Σ MUFA β	56.84 \pm 7.62 ^z	43.23 \pm 8.11 ^y	33.01 \pm 5.54 ^z	23.15 \pm 4.96 ^y
18:2 n-6	5.97 \pm 1.98	7.34 \pm 1.40	3.06 \pm 0.29	2.79 \pm 0.39
18:3 n-3	1.04 \pm 0.11	1.50 \pm 0.18	0.53 \pm 0.04	0.69 \pm 0.08
18:4 n-3	0.69 \pm 0.06	1.42 \pm 0.14	0.37 \pm 0.04	0.59 \pm 0.05
20:4 n-6	1.33 \pm 0.12 ^z	0.73 \pm 0.24 ^y	3.64 \pm 0.24 ^z	1.76 \pm 0.22 ^y
20:5 n-3	5.17 \pm 0.39	7.12 \pm 1.66	7.39 \pm 0.37	8.80 \pm 0.89
22:6 n-3	5.57 \pm 0.87 ^y	13.39 \pm 1.53 ^z	18.09 \pm 1.00 ^y	22.78 \pm 1.28 ^z
Σ PUFA δ	20.50 \pm 2.42 ^y	32.62 \pm 4.39 ^z	34.06 \pm 5.48	38.53 \pm 6.88
Σ n-3	12.47 \pm 2.34 ^y	23.48 \pm 3.14 ^z	26.40 \pm 3.55	32.87 \pm 4.20
Σ n-6	7.82 \pm 2.73	8.82 \pm 3.43	7.40 \pm 1.75 ^z	5.26 \pm 1.20 ^y

88

89 ^{α} Sum of saturated fatty acids (SFA) includes 11:0, 12:0, 13:0, 15:0, 17:0, 20:0, 22:0, 24:0, for
 90 which the combined percentages are $\leq 0.5\%$ of total fatty acids.

91 ^{β} Sum of monounsaturated fatty acids (MUFA) includes 14:1 and 17:1, for which the combined
 92 percentages are $\leq 0.5\%$ of total fatty acids.

93 ^{δ} Sum of polyunsaturated fatty acids (PUFA) includes 20:2, 18:3(n-6), and 20:3(n-6), for which
 94 the combined percentages are $\leq 0.5\%$ of total fatty acids

95

96 Table 6. Amino acid composition (percent of total amino acids; mean \pm SD) of Walleye juveniles
 97 at the end of the 6-week experiment during which they were fed the experimental (EXP) diet
 98 (containing White Sucker as fish meal) or the commercial (COM) diet (EWOS Micro; EWOS
 99 Canada Ltd). Amino acids constituting less than 1% are not listed. Amino acid codes are defined
 100 in Table 4.

101

Amino acid	EXP diet	COM diet
	<i>Essential amino acids</i>	
THR	18.97 \pm 2.49	19.76 \pm 2.52
MET	2.41 \pm 0.60	3.18 \pm 0.21
AILE	7.17 \pm 1.12	5.55 \pm 0.94
PHE	7.14 \pm 0.82	4.18 \pm 0.28
	<i>Non-essential amino acids</i>	
TAU	4.82 \pm 3.39	11.07 \pm 3.81
SER	34.08 \pm 9.82	31.94 \pm 4.46
TYR	4.25 \pm 0.85	2.18 \pm 2.09
ALA	1.95 \pm 0.25	3.04 \pm 0.23
SAR	2.11 \pm 0.27	3.28 \pm 0.25
GLY	9.30 \pm 4.48	8.55 \pm 2.03
PRO	4.35 \pm 2.96	3.21 \pm 1.16
TMG	2.02 \pm 0.26	2.57 \pm 0.36

102



