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Growth, Survival and Whole-body Proximate and Fatty Acid Composition of Haddock, Melanogrammus aeglefinus L. Post-Larvae Fed a Practical Microparticulate Weaning Diet.

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Abstract

Further development of high-quality feeds for hatchery-reared haddock in the North Atlantic would benefit from a standard formulation that can be used as a reference for hatcheries and laboratory studies. A practical marine diet (PMD) developed and evaluated with newly metamorphosed juvenile haddock, Melanogrammus aeglefinus L., post-larvae is proposed. Survival of fish fed PMD was just as high (88-89%; P>0.05) as those fed a high-quality imported feed (Biokyowa). Alternatively, fish fed PMD had higher (P<0.05) final fork lengths (39.5 vs. 35.1 mm), wet weights (851.3 vs. 580.2 mg) and weight gains (1637.2 vs. 1115.7%). No differences (P>0.05) in whole-body moisture (846-857 g/kg), ash (17-18 g/kg) or protein (101 g/kg) contents were found. Lipid content of fish fed PMD (26 g/kg) was higher (P<0.05) than those fed Biokyowa (21 g/kg) despite PMD containing 15 g/kg lower dietary lipid; suggesting higher intake and/or lipid retention. The PMD formulation proved to be a highly-suitable weaning diet for haddock post-larvae based on high feed acceptance, survival and fish growth. Given the economic and logistical difficulties associated with importing commercial weaning feeds, this easily-produced practical weaning diet has good potential for use by laboratory researchers and farm managers for hatchery-based nutrition research with haddock post-larvae.

Haddock, Melanogrammus aeglefinus L., is a coldwater marine white fish, which will reproduce in captivity and appears to adapt and grow well in salmon cages, making it a potential candidate species for aquaculture in Atlantic Canada, Eastern USA and Norway (Frantsi et al. 2002; Paisley et al. 2010; Tibbetts 2012). While still modest relative to other cultured finfish, the number of fingerlings stocked into sea pens has grown to 21 million in recent years (Paisley et al. 2010). Several studies have been directed to determine the utilization of certain nutrients by a similar gadoid fish species, Atlantic cod, Gadus morhua L. (Jobling et al. 1991; Dos Santos et al. 1993; Lall and Nanton 2002; Hamre 2006; Hamre and Mangor-Jensen 2006; Tibbetts 2012). However, very little information is available for haddock (Kim and Lall 2001; Lall et al. 2003; Treasurer et al. 2006; Treasurer 2008; Tibbetts 2012). In addition, knowledge gaps still exist for both gadoid species with regard to ontogeny of the digestive tract and associated organs as well as nutrient utilization during the larval and post-larval stages, particularly for haddock (Kjørsvik et al. 1991; Hamlin et al. 2000; Perez-Casanova et al. 2004, 2006; Kvåle et al. 2007) and other farmed marine fish species (Zambonino Infante et al. 2008; Micale and Muglia 2011; Hamre et al. 2013; Rønnestad et al. 2013; Borsky and Bricknell 2016). Nutritional data obtained with fish at the grower stage is often of little value when studying the requirements of fish at the larval and post-larval stages since mechanisms of digestion and absorption change during their development and, thus, nutritional requirements and tolerance for various ingredients also change (see reviews of Rønnestad et al. 2013 and Hamre et al. 2013). As a result, a major obstacle for haddock culture is low feed acceptance, poor growth and high mortality associated with weaning from live food to formulated diets; rendering ‘total’ replacement largely unsuccessful for most marine fish including haddock. Given that the digestive tract of most marine fish is still under development during the earliest larval phases, a deficiency of

appropriate digestive enzymes is likely to account for poor weaning of fish from live foods to formulated diets. While earlier reports proposed that exogenous enzymes supplied by consuming live prey have an essential role in marine fish larval digestion (Munilla-Moran et al. 1990), this does not appear to be entirely the case for haddock. Perez-Casanova et al. (2006) demonstrated that while α-amylase activity in haddock larvae was enhanced when consuming rotifers, their contribution to protein and lipid digestion was negligible. Another reason for the lack of success in the weaning of most marine fish is that, aside from essential fatty acids (EFAs), very little is known of their nutrient utilization from formulated feeds and quantitative nutrient requirements at this stage. As a result, commercially available feeds may be marginal in certain essential dietary nutrients and trace elements. Recent reviews have summarized a significant amount of new data on the qualitative and quantitative nutritional requirements of various marine species such as Senegalese sole, gilthead seabream, European seabass, Atlantic cod, Atlantic halibut, turbot and Japanese flounder (Rønnestad et al. 2013; Hamre et al. 2013). However, the essential micronutrient requirements for most other marine fish, including haddock, remains to be investigated.

In order to increase fingerling production of haddock through enhanced feed acceptance, survival and growth rate, it is necessary to have high quality formulated diets available locally for laboratory or hatchery-based nutrition research as importation of small quantities of commercial feeds is costly (Yúfera et al. 2005) and logistically difficult due to the different hatching periods and earlier larval feed production in Europe and Asia. Significant research has been published on weaning diets for cod in Norway (Kvåle et al. 2006, 2009; Hamre 2006; Hamre and Mangor-Jensen 2006; Bogevik et al. 2012; Hamre et al. 2013; Chauton et al. 2015). Haddock diet development research was initiated in our laboratory in 2002 (Frantsi et al. 2002;

Lall et al. 2003). Biokyowa is widely used for the early feeding of warm water marine fish in Asia. In an exploratory study in our laboratory, Biokyowa exhibited suitable physical properties as compared to other commercial feeds, such as uniformity of particle size and stability in the water column and it was palatable to haddock post-larvae resulting in high growth rates. The use of this product in Canada, however, is limited due to its high cost of importation and currently under Canadian Feeds Regulations it cannot be imported for commercial use due to the potential presence of banned ingredients. To undertake research for this study, an exemption under the provisions of the Feeds Act had to be obtained from the Canadian Food Inspection Agency (CFIA). There are also concerns that the quality of imported commercial weaning diets may vary significantly from year-to-year, batch-to-batch and within batch during transport. Companies producing these types of products experience high costs with poor returns, since the demand for weaning diets is limited and their shelf-life is short. For these reasons, it is imperative that countries involved in haddock aquaculture develop formulations for high quality and effective diets that can be produced locally for laboratory or hatchery-based nutrition research. While the Biokyowa diet used in this study is not commercially available for use in Canada, our preliminary studies have demonstrated its effectiveness for successful weaning of haddock. Thus, for the purposes of this study, it provides an ideal 'benchmark' as locally-sourced weaning diets are further developed. The objective of this study was to develop and evaluate the suitability of a formulated practical microparticulate weaning diet that promotes good feed acceptance, high survival and growth rate of haddock post-larvae that can be adopted by laboratory researchers and farm managers as a tool for hatchery-based research, development and benchmarking of other commercially available weaning feeds.

Materials and Methods

Practical Microparticulate Diet (PMD)

The formulation of the PMD diet is shown in TABLE 1. All dry ingredients were finely ground and mixed with micronutrients and lipid components. Once all ingredients were thoroughly blended, 200 g/kg boiling distilled water was incorporated and the dough was passed through a Hobart meat grinder (Model H600T, Rapids Machinery Co., Troy, OH, USA) with a 2 mm die to produce long strands which were immediately frozen at -20ºC and then freeze-dried. The freeze-dried strands were crumbled using a Roskamp Grappler (Series 6.5, Roskamp Manufacturing, Inc., Waterloo, Iowa, USA) and the resulting particles were roughly size-graded through a Sweco Vibro-Energy Particle Separator (Model LS1884443, Sweco Inc., Florence, Kentucky, USA) and then finely graded by hand with laboratory sieves. Particles between 400-600 and 600-800 µm were used in the experiment to match those of the commercial Biokyowa weaning feed.

Experimental Conditions

Fertilized haddock eggs received from the St. Andrews Biological Station (Fisheries and Oceans Canada, St. Andrew's, New Brunswick) were incubated at 7ºC for 14 d. Newly hatched larvae were then transferred to a 3500-L larval tank where they were acclimated to a temperature of 11ºC where they stayed for 40 d. During this period, they were fed algae-enriched rotifers from 0 to 10 d post-hatch (DPH), Algamac 2000-enriched rotifers from 10 to 33 DPH and Algamac 2000-enriched Artemia from 33-54 DPH. At 54 DPH, the post-flexion larvae were transferred into nine, 350-L dark green tanks, each receiving filtered (30 µm) seawater (28-30 g/L salinity) at a flow rate of 1 L/min. The salinity optima for survival and growth of haddock

larvae, has been reported at 25-30 g/L (Opstad 2003). At this developmental stage (e.g., 54 DPH, 52 mg live weight, 17 mm fork length) the fish's fin rays, swim bladder, notochord, teeth and digestive organs should all be developed (Auditore et al. 1994; Perez-Casanova et al. 2006). The 9 tanks were each stocked with 500 fish (stocking density, 1.43 post-larvae/L) and fed 300,000 live Artemia twice daily under continuous dim light (~60 lux at water surface). The experiment evaluated a commercially-produced imported weaning diet (Biokyowa, Kyowa Hakko Kogyo, Tokyo, Japan) and a practical microparticulate diet (PMD) formulated and produced in our laboratory. The nine experimental tanks (Biokyowa was fed to triplicate tanks and PMD was fed to six replicate tanks) contained 500 fish each (initial mean weight, 52.2±0.2 mg) at a water temperature of 12ºC over a period of 20 d. After the pre-trial weaning phase (54 DPH), the experiment began. The selection of the post-larval developmental stage for the beginning of the study was based on the results of our previous pilot feeding trials and light microscopy information which involved investigations related to intake and assimilation of macronutrients from live organisms and food particles within the digestive tract of the fish (unpublished data). Beginning at 55 DPH, the test diets (400-600 µm size class) were introduced and were fed in excess every h between 0900 and 1700 h with a subsequent feeding at 2200 h each night. Concurrently, the amount of live Artemia fed to each tank was reduced from 300,000 daily at 54 to 56 DPH to 200,000 daily at 57 DPH, 150,000 daily at 58 DPH, 100,000 daily at 59 DPH, 50,000 daily at 60 DPH and 0 at 61 DPH. The diet particle size was gradually increased as the fish grew so that the final food particle sizes ranged between 600-800 µm. Mortalities were collected daily from the experimental tanks and dissolved oxygen levels (9.5±0.1 mg/L, 106±1% saturation) and water temperatures (11.9±0.1ºC) were monitored daily. Tank bottoms were gently siphoned and the surface water skimmed every second d. At the end of the feeding trial

(74 DPH), the fish were unfed for 24 h and live counted. At the beginning of the feeding trial (54 DPH), 100 fish were sacrificed with an overdose of tricaine methanesulfonate (TMS) after 24 h food deprivation and individual fork lengths (mm) and wet weights (mg) were recorded. These fish were pooled into three groups, frozen on dry ice, stored at -80ºC, freeze-dried and then finely ground for subsequent whole-body compositional analysis. At the end of the experiment (74 DPH), 25 fish from each tank (225 in total) were collected and measured in the same manner. Initial and final fish samples were analyzed in triplicate for whole-body proximate composition (moisture, ash, protein and lipid) and fatty acid profile using procedures described in the following section.

Analytical Methods

Test diets and freeze-dried whole fish were analyzed in triplicate by the same procedures. Lipid content was determined according to Bligh and Dyer (1959). Fatty acid methyl ester (FAME) derivatives were prepared using 7% boron trifluoride in methanol and heating to 100ºC for 1 h (Christie 1982). Individual FAMEs were separated by gas chromatography (Hewlett Packard 6890 GC system equipped with a flame-ionization detector) using an Omegawax 320 capillary column (Supelco) and identified by comparison of retention times with those of known standards (Supelco 37, Menhaden Oil). The total extracted lipids were further separated into polar and non-polar fractions according to Nanton et al. (2001) using a silica gel column comprised of a Pasteur pipette plugged with glass wool, a thin layer of anhydrous sodium sulphate and silica gel (40 µM flash chromatography packing; J.T. Baker Inc., Phillipsburg, NJ, USA). The polar and non-polar lipids were separated using chloroform followed by methanol with the FAME compositions determined using the procedure described above. Following

AOAC (1990) methods, the moisture content was determined by drying in an oven for 24 h at 110ºC and ash by incineration in a muffle furnace at 550ºC for 24 h. Crude protein (N × 6.25) was measured using a nitrogen determinator (model FP-528, Leco Corporation, St. Joseph, Michigan, USA). Gross energy content of the diets was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA) and carbohydrate was calculated by difference (1000 – [moisture + ash + protein + lipid]). All samples were analyzed in triplicate.

Statistical Analyses

Mean±SE was calculated from the average of multiple tanks receiving each test diet. Statistical analyses were performed using ANOVA with a 5% level of probability and in the case of a statistically significant difference, treatment means were differentiated using Tukey's test (SYSTAT® 8.0). Correlations between response variables were calculated by Pearson correlation analysis (*r*) using Microsoft Excel.

Results

Experimental Diets

The test feeds had similar levels of dietary crude protein (608-626 g/kg), lipid (154-169 g/kg) and gross energy (22-23 MJ/kg). However, dietary ash content of Biokyowa (140 g/kg) was higher than PMD (73 g/kg) while carbohydrate levels were higher for PMD (146 versus 83 g/kg). Total and polar fatty acid compositions of PMD and Biokyowa are shown in TABLES 3 and 4. The ratio of polar to non-polar lipids was similar between the test diets at 37:63 (PMD) and 35:65 (Biokyowa). As for individual fatty acids, Biokyowa contained higher levels than PMD of

16:0, 18:1n-9, 18:2n-6, 20:4n-6 and 20:5n-3 and lower levels of 20:1n-9 and 22:1n-11. Fatty acid levels were similar for 14:0, 16:1n-7, 18:0, 18:1n-7, 18:3n-3, 20:4n-3, 22:5n-3 and 22:6n-3. As for fatty acid groups, Biokyowa contained higher levels than PMD of total SFA (28 vs 22%), total PUFA (44 vs 36%), total n-3 PUFA (17 vs 15%), total n-6 PUFA (13 vs 7%) and lower levels of total MUFA (29 vs 45%). The DHA:EPA ratio was higher for PMD (1.3) than Biokyowa (1.1) and this was also the case for the EPA:ARA ratio (16.3 and 10.2, respectively). In terms of the polar fatty acids, Biokyowa contained higher levels than PMD of 16:0, 18:1n-9, 18:1n-7 and 20:5n-3 and lower levels of 18:2n-6 and 22:6n-3. Fatty acid levels were similar for 14:0, 16:1n-7, 18:0, 18:3n-3, 18:4n-3, 20:1n-9, 20:4n-6, 20:4n-3, 22:1n-11 and 22:5n-3. As for fatty acid groups, Biokyowa contained higher levels than PMD of total SFA (31 vs 28%), total MUFA (22 vs 18%) and total n-3 PUFA (19 vs 14%) and lower levels of total PUFA (50 vs 57%) and total n-6 PUFA (16 vs 19%). The DHA:EPA ratio was higher for PMD (2.3) than Biokyowa (1.0) while the EPA:ARA ratio was similar (10.2-10.7, respectively).

Fish Performance

Survival was high throughout the experiment at 88.2-89.1% and there were no significant differences ($P>0.05$) between PMD and Biokyowa (FIGURE 1). The majority of mortalities occurred within the first 3 d after introduction of the weaning diets and then stabilized at a low rate (generally <10 fish/d) for the duration of the experiment and at similar levels for PMD and Biokyowa. With regard to growth performance, haddock fed PMD outperformed those fed Biokyowa (TABLE 5). Final fork lengths and wet weights of fish fed PMD (39.5 mm and 851.3 mg) were significantly higher ($P<0.05$) than those fed Biokyowa (35.1 mm and 580.2 mg)

231 resulting in significantly higher (P<0.05) weight gains for fish fed PMD (1637% of initial
232 weight) than those fed Biokyowa (1116% of initial weight).

233 Whole-Body Composition

234 As expected, all aspects of final whole-body proximate composition, regardless of diet,
235 changed from initial values in a predictable manner with final fish containing significantly lower
236 (P<0.05) moisture levels and significantly higher (P<0.05) levels of ash, protein and lipid. Final
237 whole-body moisture, ash and protein levels of fish fed PMD and Biokyowa were statistically
238 the same (P>0.05%) at 846-857 g/kg, 17-18 g/kg and 101 g/kg, respectively. However, whole-
239 body lipid content of fish fed PMD (26 g/kg) was significantly higher (P<0.05) than those fed
240 Biokyowa (21 g/kg).

241 Final lipid profiles of the fish highly reflected those of the diets with correlation (*r*) values
242 between fatty acid composition of the diet and those of the final fish of 0.80-0.87. No significant
243 differences (P>0.05) in whole-body fatty acid composition were observed between fish fed PMD
244 or Biokyowa for 18:3n-3, 20:4n-3, 20:5n-3 and 22:5n-3. Haddock fed PMD contained
245 significantly higher (P<0.05) levels than Biokyowa of 14:0, 16:1n-7, 18:1n-9, 18:4n-3, 20:1n-9
246 and 22:1n-11 and significantly lower (P<0.05) levels of 16:0, 18:0, 18:1n-7, 18:2n-6, 20:4n-6
247 and 22:6n-3. As for fatty acid groups, fish fed PMD contained significantly higher (P<0.05)
248 levels than those fed Biokyowa of total SFA (47 vs 35%) and significantly lower (P<0.05%)
249 levels of total PUFA (41 vs 53%) and total n-6 PUFA (1.9 vs 3.2%) while there were no
250 significant differences (P>0.05) observed in the levels of total MUFA (5.8-6.2%) and total n-3
251 PUFA (13.9-14.8%). The DHA:EPA ratio was lower for fish fed PMD (1.5) than Biokyowa (2.0)
252 while the EPA:ARA ratio was higher for PMD (11.0) than Biokyowa (5.5).
253

Discussion

While a limited amount of data is available on weaning diets for haddock (Hamlin and Kling 2001; Blair et al. 2003), reference formulations for this species for further nutritional work do not exist. Not surprisingly, attempts to raise hatchery-reared haddock from first feeding through metamorphosis on formulated diets have not provided a high rate of survival under commercial conditions and, indeed, hatchery mortalities have been high in published laboratory studies (36-98%). The high mortalities during early weaning of haddock are related to a number of factors. The fish likely do not have a fully functional digestive system prior to introduction of microparticulate diets, the feeds lack the attractive chemosensory properties required to promote rapid ingestion of the particles and the nutrient composition is likely unbalanced as a result of knowledge gaps on specific dietary requirements and also as a result of leaching of essential nutrients while in the water column.

This study demonstrates that high survival (>88%) of haddock can be obtained through the weaning stage from 54-61 DPH. The survival rates found in our study are much higher than those previously reported for haddock of 35% (Hamlin and Kling 2001) and 2-5% (Blair et al. 2003). In addition to the dietary formulation, the major difference is the period of co-feeding of live feed and dry feed. Where we co-fed the larvae from 54-61 DPH (88-89% survival), these studies co-fed from 30-37 DPH (35% survival) and 25-29 DPH (2-5% survival). Thus, it is clear and somewhat predictable that the earlier the co-feeding period occurs, the less likely it is that high survival rates will be possible. This was also the case for summer flounder, Paralichthys dentatus and southern flounder, P. lethostigma where it was found that larval survival was significantly improved by weaning older larvae (Bengtson et al. 1999; Alam et al. 2015). The

277 fact that we could achieve such high survival success by beginning weaning at 54 DPH is
278 supported by Hamlin et al. (2000), Hamlin and Kling (2001) and Otterå and Lie (1991) who
279 reported that differentiation of the larval gadoid digestive system into one that resembles that of
280 the adult does not fully occur until at least 50-53 DPH when the larvae are at least 15 mm in
281 length. The weaning period in this experiment began at 54 DPH with fish at a length of 16.7 mm.
282 This is also the case for greenback flounder, Rhombosolea tapirina where highest larval survival
283 (>80%) occurred when weaning was done at 23 DPH, roughly the same time that the stomach of
284 the flounder is fully differentiated (20 DPH) (Hart and Purser 1996). The high haddock survival
285 rates found in this study (>88%) are also much higher than those reported for Atlantic cod
286 (<40%) under several co-feeding period variations (Baskerville-Bridges and Kling 2000a,b;
287 Callan et al. 2003). The initial spike in mortality of fish fed PMD between 54-56 DPH is likely
288 due to handling stress of moving the larvae into the experimental tanks as this was also observed
289 in similar studies with haddock (Blair et al. 2003) and gilthead seabream, Sparus aurata larvae
290 (Koven et al. 2001). Interestingly, this initial mortality spike was less pronounced in the larvae
291 fed Biokyowa. This may be attributed to the fact that the Biokyowa contained more than double
292 (240%) the concentration of arachidonic acid (ARA, 20:4n-6) than PMD. Koven et al. (2001)
293 found that gilthead seabream larvae showed increased resistance to transportation stress and
294 subsequently lower mortality when fed ARA-enriched rotifers.

295 Growth performance of haddock in this study was high with specific growth rates of over
296 13%. Lower growth rates (7-9%) have been reported for cod (Otterå and Lie 1991; Baskerville-
297 Bridges and Kling 2000a; Callan et al. 2003). In some studies of weaning cod and seabream,
298 microdiets with a higher moisture content generally provided highest survival rates (Otterå and
299 Lie 1991; Yúfera et al. 2015). This is likely related to increased olfactory attractiveness and a

300 greater ease of particle disintegration in the developing gut; however moist feeds are logistically
301 problematic. In the present study, PMD and Biokyowa were dry feeds, yet still promoted good
302 feed acceptance and provided high survival and growth rates throughout the experimental
303 weaning period. While we cannot be sure of the ingredients within Biokyowa, the attractiveness
304 of PMD is likely related to the use of krill and squid hydrolysates, both of which are known as
305 potent feed attractants for fish and specifically for marine gadoids (Lie et al. 1989). The
306 relatively low survival and growth of haddock in previous studies conducted in North America is
307 likely related to a lack of these chemosensory-rich ingredients in the experimental diets used.
308 Only one of the studies with a related gadoid species (Atlantic cod) used experimental diets
309 containing krill protein; and feed acceptance, growth and survival were still poor (Baskerville-
310 Bridges and Kling 2000b). As such, it may be that the most potent feed attractant for young
311 gadoid fish is squid protein. This is consistent with a recent report by Alam et al. (2015) who
312 observed significantly higher survival and growth rates of larval southern flounder fed microdiets
313 containing squid meal when compared to microdiets containing only krill meal as an attractant.
314 In addition to other key components having chemo-attractant properties, squid protein is
315 uniquely rich in taurine and betaine which have been correlated with enhanced feed intake by
316 other species of marine fish larvae such as Asian sea bass, Lates calcarifer, summer flounder,
317 gilthead seabream and Japanese flounder, Paralichthys olivaceus (Lee et al. 1996; Kolkovski and
318 Tandler 2000; Kim et al. 2005; Lian et al. 2008). These marine products presented in hydrolysate
319 form as opposed to native form have also been shown to improve development of hatchery-
320 reared marine fish, presumably through an increased ease of absorption of short-chain peptides
321 and free amino acids through the immature intestinal microvilli (Zambonino Infante et al. 2008).
322 Anecdotally, we suspect this to be the case for other marine fish species as well where we have

observed similar highly encouraging results using a modified PMD formulation for hatchery-reared Atlantic halibut, Hippoglossus hippoglossus, bluefin tuna, Thunnus thynnus and sablefish, Anoplopoma fimbria. In addition to its possible feed attractant properties, taurine is increasingly becoming considered as a conditionally-essential amino acid for many farmed fish species and recently Zheng et al. (2015) have suggested a dietary requirement of 10 g/kg (1% of the diet) for marine fish post-larvae feeds.

In the present study, the significantly higher growth rate found for haddock fed PMD also corresponds to significantly higher whole-body lipid content; despite the fact that PMD contained 15 g/kg less dietary crude lipid than Biokyowa. Presumably, fish fed PMD ingested more total digestible energy than those fed Biokyowa, which may have been stored as liver lipid. However, by the end of this experiment, the fish were still too small to effectively remove the livers in order to calculate the hepatosomatic index or determine the liver composition. The lower levels of total n-3 fatty acids and total PUFA in PMD resulted in fish having lower levels of total PUFA but no appreciable decrease in total n-3 PUFA. Alternatively, fish consuming PMD had higher levels of total SFA and a reduced DHA/EPA ratio. In comparison of the initial haddock samples to the final samples, the deposition of total SFA, MUFA and n-3 PUFA followed the same pattern as European sea bass, Dicentrarchus labrax where there was an overall increase in deposition of SFA and an overall decrease in MUFA and n-3 PUFA (Fontagné et al. 2000). Of course, it is not only the fatty acid profile, but the form in which the lipid is supplied in the diet which is an important consideration for early developmental stages. Previous work indicates that marine fish larvae may utilize phospholipids more efficiently than triglycerides (Shields et al. 1999) and, as such, dietary inclusion of phospholipid-rich ingredients has been recommended for weaning feeds (Zambonino Infante et al. 2008). However, it should be noted

346 that many marine fish weaning studies to date have utilized soybean lecithin as the
347 supplementary source of dietary phospholipids. It has been demonstrated that phospholipids from
348 marine sources are more highly assimilated by marine fish than those from soybean lecithin
349 (Leifson et al. 2003; Saleh et al. 2015). It appears that, in addition to enhanced digestion and
350 absorption, marine-derived phospholipids may improve larval survival, stress resistance, growth
351 rate and bone mineralization compared to diets formulated with soybean lecithin-derived
352 phospholipids. In the present study, in addition to soybean lecithin, PMD was also supplied with
353 freeze-dried fish roe, a highly rich source of marine-based phospholipids.

354 In conclusion, high survival (~90%) of haddock post-larvae fed PMD was possible under
355 laboratory conditions when the transition from live food to formulated diets occurred after 53
356 DPH when the digestive system was thought to be fully differentiated. The PMD formulation
357 used in the present study promoted good feed acceptance, high survival and rapid growth; greatly
358 exceeding those of previous studies and these findings are likely the result of several factors.
359 Incorporation of feed ingredients with strong chemoreceptive properties (e.g., krill and/or squid
360 hydrolysates) is important to promote rapid ingestion of microparticles by young haddock and
361 that higher levels (>1%) of dietary arachidonic acid (AA) may help reduce larval stress and
362 subsequent mortality during the first few d of transition from live food to formulated weaning
363 diets. Additionally, the high survival and growth rates of haddock fed PMD may provide
364 additional evidence for the importance of incorporating phospholipid-rich marine sources (e.g.,
365 cod muscle and herring roe) into microparticulate weaning diets for hatchery-reared haddock.
366 Additional work is required to better define the nutrient requirements of haddock, specifically at
367 the larval and post-larval stages, and to improve weaning feed production technologies that
368 minimize leaching of essential nutrients into the water column. Overall, given the logistical and

economic difficulties associated with importation of small quantities of commercially-produced marine fish weaning feeds, the good feed acceptance, high survival and rapid growth of haddock fed PMD demonstrated in this study suggests that it is a highly suitable, easily produced practical weaning diet formulation that can be used by laboratory researchers and farm managers for hatchery-based nutrition research with haddock.

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603 TABLE 1. Formulation (as-fed basis) of the practical microparticulate diet (PMD).

604

Ingredient	g/kg
Herring meal[a]	313
Cod muscle (freeze-dried)	150
Pre-gelatinized corn starch[b]	130
Herring roe (freeze-dried)	120
Soluble fish protein concentrate[c]	80
Herring oil[d]	50
Gelatin[e]	50
Krill hydrolysate[f]	25
Squid hydrolysate[a]	20
Molasses	20
Soy lecithin[g]	20
Mineral premix[h]	10
Vitamin premix[i]	9
Choline chloride[e]	2
Ascorbic acid (Stay-C)[j]	1
Total	1000

[a] Corey Nutrition Ltd. (Fredericton, NB, Canada).

[b] National Starch and Chemical Company (Bridgewater, NJ, USA).

[c] Sopropêche CPSP-G (Northeast Nutrition, Truro, NS, Canada).

[d] Comeau Seafoods (Saulnierville, NS, Canada).

[e] United States Biochemical (Cleveland, OH, USA).

[f] Specialty Marine Products Ltd. (Vancouver, BC, Canada).

[g] LV Lomas (Montreal, QC, Canada).

[h] Manganous sulfate, 40 mg/kg; ferrous sulfate, 30 mg/kg; copper sulfate, 5 mg/kg; zinc sulfate, 75 mg/kg; sodium selenite, 1 mg/kg; cobalt chloride, 2.5 mg/kg; sodium fluoride, 4 mg/kg; ground wheat.

[i] Vitamin A, 8000 IU; vitamin D_3, 4500 IU; vitamin E, 300 IU; vitamin K, 40 mg/kg; thiamin, 50 mg/kg; riboflavin, 70 mg/kg; pantothenate, 200 mg/kg; biotin, 1.5 mg/kg; folic acid, 20 mg/kg; vitamin B_{12}, 0.15 mg/kg; niacin, 300 mg/kg; pyridoxine, 20 mg/kg; ascorbic acid, 300 mg/kg; inositol, 400 mg/kg; butylated hydroxy toluene, 15 mg/kg; butylated hydroxy anisole, 15 mg/kg; ground wheat.

[j] DSM Nutritional Products Canada Inc. (Ayr, ON, Canada).

643 TABLE 2. Proximate composition (dry matter basis) of the practical microparticulate diet (PMD)
644 and a commercial control diet (Biokyowa).
645

Proximate composition	PMD	Biokyowa[a]
Crude protein (g/kg)	626±1	608±1
Lipid (g/kg)	154±1	169±3
Carbohydrate[b] (g/kg)	146±3	83±1
Ash (g/kg)	73±1	140±3
Gross energy (MJ/kg)	22.8±0.2	21.7±0.2

[a] Kyowa Hakko Kogyo, Tokyo, Japan.

[b] Carbohydrate = (100 – [moisture + protein + lipid + ash]).

659 TABLE 3. Total fatty acid composition of the practical microparticulate diet (PMD) and a
660 commercial control diet (Biokyowa).
661

	PMD	Biokyowa
Total lipid (%, dry matter basis)	15.4±0.1	16.9±0.3
Polar lipids (% of total FAME)	36.7±0.3	35.5±0.2
Non-polar lipids (% of total FAME)	63.3±0.3	64.5±0.2
Fatty acid[a]		
14:0	5.0±0.2	4.2±0.1
16:0	13.7±0.4	18.9±0.2
16:1n-7	5.7±0.1	4.7±0.1
18:0	1.9±0.1	3.4±0.1
18:1n-9	8.5±0.1	14.6±0.4
18:1n-7	2.5±0.0	4.3±0.1
18:2n-6	5.9±0.0	10.6±0.2
18:3n-3	1.0±0.0	1.5±0.0
18:4n-3	1.5±0.0	1.4±0.0
20:1n-9	11.0±0.1	1.9±0.1
20:4n-6 (ARA)	0.5±0.0	1.2±0.1
20:4n-3	0.4±0.0	0.5±0.0
20:5n-3 (EPA)	9.2±0.1	11.4±0.7
22:1n-11	15.4±0.4	1.6±0.1
22:5n-3	1.3±0.1	0.8±0.0
22:6n-3 (DHA)	11.6±0.2	13.1±0.7
Σ SFA	21.7±0.5	27.6±0.1

Σ MUFA	45.0±0.3	28.9±0.8
Σ PUFA	35.6±0.5	44.3±1.0
Σ n-3 PUFA	14.5±0.2	16.6±0.7
Σ n-6 PUFA	7.1±0.1	13.0±0.2
DHA:EPA ratio	1.3±0.0	1.1±0.0
EPA:ARA ratio	16.3±0.1	10.2±0.1

[a] Expressed as area percentage of total FAME.

695 TABLE 4. Fatty acid composition of the polar lipid FAMEs of the practical microparticulate diet
696 (PMD) and commercial control diet (Biokyowa).
697

	PMD	Biokyowa
Fatty acid[a]		
14:0	2.2±0.1	1.8±0.1
16:0	21.7±0.4	23.8±0.1
16:1n-7	2.3±0.1	2.8±0.1
18:0	3.4±0.1	3.9±0.1
18:1n-9	7.8±0.1	11.0±0.1
18:1n-7	3.1±0.1	4.7±0.2
18:2n-6	16.9±0.2	13.8±0.1
18:3n-3	2.0±0.0	1.6±0.0
18:4n-3	0.5±0.0	0.7±0.0
20:1n-9	2.0±0.1	1.4±0.4
20:4n-6 (ARA)	0.9±0.0	1.5±0.0
20:4n-3	0.3±0.0	0.3±0.0
20:5n-3 (EPA)	10.0±0.0	14.7±0.3
22:1n-11	1.0±0.1	0.5±0.0
22:5n-3	0.2±0.0	0.4±0.1
22:6n-3 (DHA)	22.7±0.4	14.1±0.3
Σ SFA	28.5±0.6	30.6±0.3
Σ MUFA	17.6±0.4	22.3±0.4
Σ PUFA	56.8±0.6	50.3±0.3
Σ n-3 PUFA	14.2±0.0	18.7±0.3

Σ n-6 PUFA	18.6±0.2	16.1±0.2
DHA:EPA ratio	2.3±0.0	1.0±0.0
EPA:AA ratio	10.7±0.0	10.2±0.3

[a] Expressed as area percentage of FAME.

728 TABLE 5. Survival and growth performance of haddock post-larvae fed the practical
729 microparticulate diet (PMD) and a commercial control diet (Biokyowa) until 74 DPH[a,b].
730

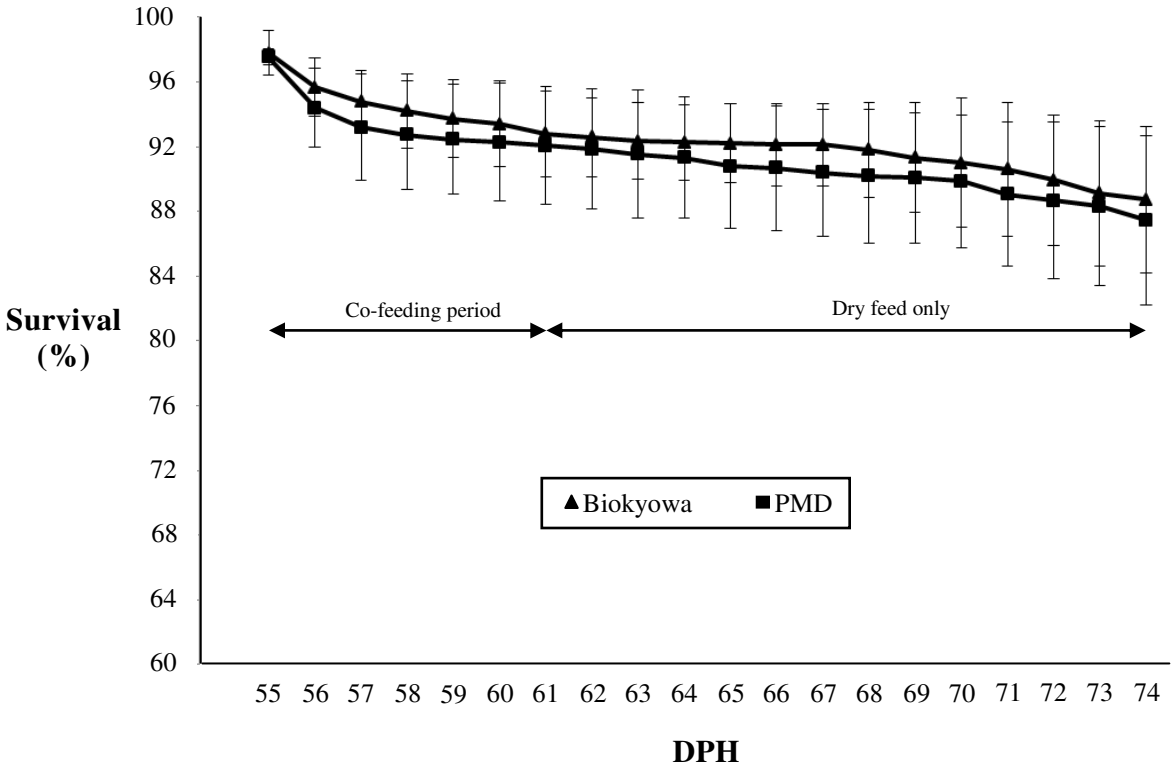
Diet	Survival (%)	Final fork length (mm)	Final wet weight (mg)	Weight gain (%)
PMD	88.2±1.7[ns]	39.5±0.5[a]	851.3±33.0[a]	1637.2±63.1[a]
Biokyowa	89.1±1.5	35.1±0.7[b]	580.2±24.3[b]	1115.7±46.6[b]

[a] Mean±standard error of replicate tanks (PMD, n=6; Biokyowa, n=3) and values within the same column with different superscripts are significantly different (P<0.05).

[b] Average initial fork length = 16.7±0.2 mm and wet weight = 52.2±0.2 mg (n=100).

[ns] Not significant.

743 FIGURE 1. Survival (%) of fish fed the practical microparticulate diet (PMD) and a commercial
744 control diet (Biokyowa) until 74 DPH.
745


100
96
92
88
84
80
76
72
68
64
60
Survival (%)
Co-feeding period
Dry feed only
▲Biokyowa
■PMD
55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74
DPH

760 TABLE 6. Whole-body proximate composition (wet weight basis) of haddock post-larvae fed the
761 practical microparticulate diet (PMD) and a commercial control diet (Biokyowa) until 74 DPH[a].
762

Diet	Moisture (g/kg)	Ash (g/kg)	Protein (g/kg)	Lipid (g/kg)
Initial fish	881±6[a]	13±1[a]	91±5[a]	18±1[a]
PMD	846±1[b]	17±1[b]	101±1[b]	26±0[c]
Biokyowa	857±1[b]	18±1[b]	101±0[b]	21±0[b]

[a] Mean±standard error of replicate tanks (PMD, n=6; Biokyowa, n=3) and values within the same column with different superscripts are significantly different ($P<0.05$).

774 TABLE 7. Whole-body fatty acid composition of haddock post-larvae fed the practical
775 microparticulate diet (PMD) and a commercial control diet (Biokyowa) until 74 DPH[a].
776

Fatty acid[b]	Initial fish	PMD	Biokyowa
14:0	0.7±0.0[a]	2.0±0.1[c]	1.1±0.0[b]
16:0	15.0±0.1[b]	13.2±0.3[a]	15.4±0.5[b]
16:1n-7	1.6±0.0[a]	4.0±0.1[c]	2.2±0.1[b]
18:0	6.5±0.0[c]	3.4±0.1[a]	5.4±0.2[b]
18:1n-9	14.9±0.1[b]	14.0±0.4[b]	12.1±0.4[a]
18:1n-7	5.9±0.1[c]	3.3±0.1[a]	3.8±0.1[b]
18:2n-6	3.3±0.0[a]	4.7±0.1[b]	5.6±0.2[c]
18:3n-3	7.0±0.1[b]	0.8±0.0[a]	0.7±0.0[a]
18:4n-3	1.0±0.0[b]	0.9±0.0[b]	0.5±0.0[a]
20:1n-9	0.8±0.0[a]	8.8±0.3[c]	2.2±0.1[b]
20:4n-6 (ARA)	4.1±0.0[c]	0.9±0.0[a]	2.0±0.0[b]
20:4n-3	0.7±0.0[a]	0.6±0.1[a]	0.7±0.1[a]
20:5n-3 (EPA)	9.3±0.0[a]	9.7±0.4[ab]	10.9±0.3[b]
22:1n-11	0.1±0.0[a]	5.8±0.3[c]	1.1±0.1[b]
22:5n-3	0.2±0.1[a]	0.4±0.1[b]	0.3±0.0[ab]
22:6n-3 (DHA)	13.5±0.3[a]	14.9±0.7[a]	22.0±0.7[b]
Σ SFA	23.7±0.0[a]	47.4±0.9[c]	35.4±0.8[b]
Σ MUFA	24.5±0.2[a]	5.8±0.2[b]	6.2±0.4[b]
Σ PUFA	49.0±0.2[b]	41.0±0.9[a]	53.4±0.6[c]
Σ n-3 PUFA	20.0±0.2[c]	13.9±0.3[b]	14.8±0.2[b]

Σ n-6 PUFA	13.0±0.1[c]	1.9±0.3[a]	3.2±0.5[b]
DHA:EPA ratio	1.5±0.0[a]	1.5±0.0[a]	2.0±0.0[b]
EPA:ARA ratio	2.2±0.0[a]	11.0±0.3[c]	5.5±0.1[b]

[a] Mean±standard error of replicate tanks (PMD, n=6; Biokyowa, n=3) and values within the same row with different superscripts are significantly different ($P<0.05$).

[b] Expressed as area percentage of FAME.