fishes

Review

In Pursuit of Fish-Free Feeds: A Multi-Species Evaluation

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Abstract: The future growth and sustainability of fed aquaculture, and especially that for carnivorous species, will be highly dependent upon the industry stepping away from its reliance upon forage fishes as major feed ingredients. With this goal in mind, the F3 Feed Innovation Network—a consortium of researchers; businesses, including feed manufacturers and ingredient providers; NGOs; and others—energizes industry to adopt novel and promising aquafeed ingredients and formulations. All evaluated formulae are open-source and freely available on the F3 website. Moreover, the F3 diets can be readily retailored to suit user demands and/or local conditions (i.e., ingredient availability/restrictions). This presentation summarizes completed F3 trials undertaken with five species of cultured and candidate fishes. With reference to eight studies, findings are compared against conventional fishmeal (FM)/fish oil (FO)-based feeds. The described research documents the response of test animals to aquafeeds containing traditional FM/FO alternatives (e.g., soybean meal and poultry by-product meal) as well as innovative ingredients (e.g., microalgae and single-cell proteins). Depending on the species examined, account is given to the overall growth performance, health aspects, and product quality. The F3 trials demonstrate the feasibility of the complete removal of FM/FO from the diets of the tested animals.

Keywords: largemouth bass; pompano; amberjack; red drum; algal oil

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1. Introduction

The desire to optimize aquafeeds has a long history. Like today, although not necessarily directly articulated, there was an aspiration to develop a more sustainable aquaculture from as early as the 1920s. Concerns included reducing water pollution caused by raw meats (fish, horse, seal, and sheep) and offal (liver, spleen, heart, and lungs), which were commonly used as hatchery feeds. Around the same time, and especially during war years, fish and meats employed as feeds were also rationed and or becoming more expensive [1–4]. Concurrently, culturists sought to confront problems related to the effective storage and dissemination of feed [5–8] and disease transmission from trash to cultured fish [9]. Thus, cheaper feeds based on alternative ingredients were sought. Investigations with animal-protein-free diets, however, resulted in inferior growth and feed conversion, changes in animal physiology, and increased mortalities (e.g., [10,11]). These adverse reactions were generally attributed to plant-derived toxins and nutritional inadequacies, such as vitamin deficiencies [12,13], and were so commonly described that some suggested the use of plant meals, especially in fingerling feeds, was inadvisable [14].

In the intervening years, various dietary formulations were evaluated [15,16], with pelleted feeds such as the Oregon Moist Pellet [17] and dry preparations being used in US state hatcheries and at commercial farms in the 1950s [18–21]. In the late 1950s, Edward Grassl [22–24] evaluated the use of dry diets both as feeds and medicated diets. He compared the growth of trout fed either wet chopped meats or dry pelleted animal/vegetable feeds and reported identical growth even when the pellet was fed at 50% of the amount recommended for raw feeds, provided that chopped liver was fed once a month or so. Implementation of the pelleted feeds by state hatcheries resulted in 60% improved production and a 40% reduction in food costs. Use of a vitamin mixture in the dry pellet, as recorded by Phillips et al. [25], eliminated the need for chopped liver supplements and formed the basis for trout dry pellet formulations and the development of mechanized feeders [26,27]. Across the Atlantic, similar trials were being undertaken with salmon [28]. These pioneering studies, together with the elucidation of the nutritional requirements of some species, represent foundational moments in the elaboration of the global aquafeed industry.

Between 2001 and 2010, global aquaculture production increased at an annual average of 5.8%, and between 2011 and 2018, by 4.5%. This astonishing rate of expansion, while having moderated to around 3% during 2020, continues to grow [29]. Significantly, much of the growth experienced this century has occurred in the fed aquaculture sector, which accounted for 86% and 73% of global FM and fish oil (FO) supplies, respectively, in 2020 [29]. Future projections suggest that if the use of FM/FO is to remain as-is in aquafeeds, demand will outstrip supply by 2030 [30]. This scenario has led to major reductions in the use of FM/FO over the last two decades through wiser use allocations and their replacement with alternatives, including fish processing by-products among many others [31]. Together with enhanced feed conversion efficiencies, genetic selection programs [32], and even commodity price risk hedging [33], among other strategies, feed costs have been reduced, with some of the alternative products illustrating adequate environmental performance [34–36], thereby having potential to improve industry sustainability.

The drive to replace FM/FO from aquafeeds is based not only on projected availability, which also influences raw material prices, but also on the growing concerns of active environmental and consumer lobbies. These groups point to the fact that forage fisheries influence the health and sustainability of marine and coastal ecosystems, while their prey are vital to the sustenance of marine predators including other fishes, birds, marine mammals, and humans. Seafood buyers are also becoming more knowledgeable of the range of potential contaminants that may impact food safety, including those of raw materials used during aquafeed production (review: [37]). Well-informed consumers are learned of human rights infractions that occur in some industrial fisheries and across aquaculture supply chains [38] and aware of the negative consequences of at-sea discarding [39], ghost nets [40], harmful fisheries subsidies [41], carbon emissions from fleets and feed manufacturers [42,43], and animal welfare issues [44]. These worries have resulted in the creation of a sustainability imperative driven by consumers who demand safe and ethically and environmentally responsive food production systems and base purchase decisions on these principles. The aquafeed industry is in an influential position to ensure that consumer and environmental desires are achieved. In the interim, the search for and the evaluation of suitable alternatives to FM and FO must be unwavering.

Since 2014, the F3 Feed Innovation Network (f3fin.org (accessed on 11 November 2022)) has encouraged sustainable initiatives to reduce the dependence of the aquafeed industry on forage fishes and embolden the sector to adopt novel and promising ingredients and formulations. One way in which the F3 consortium accomplishes this is through openly sharing recipes and experimental findings through its website and publications. Aquafeeds do not have a requirement for any specific ingredient but must satisfy the nutritional prerequisites of the target animal. Feeds, therefore, must provide a combination of nutrients in the correct proportions to fulfill the metabolic needs of the species in question [45]. Bearing this in mind, the F3 consortium has completed several trials in efforts to eliminate FM/FO from the feeds of a variety of widely cultivated and candidate species. Here, we recount the findings of eight of these trials. The formulations considered herein for each species studied are all open-source and freely available on the F3 website. All F3 diets may be retailored to suit the user's demands; other tested formulations are similarly deposited on the F3 website.

2. Species Evaluated

Five well-established and candidate cultured species of teleost were examined for their sensitivity to fish-free feeds (F3). They included the largemouth bass *Micropterus salmoides*, which is the preeminent farmed Perciformes, representing over 50% or around 432,000 tons of total production [46]; the Florida pompano *Trachinotus carolinus*, a strong candidate species for US aquaculture; and other members of the family Carangidae, including two species of amberjack, namely the California yellowtail *Seriola dorsalis* and kampachi *S. rivoliana*. More than 170,000 tons of pompano is cultured annually, with most production being in Asia [47], while 150,000 tons of amberjack is farmed globally, with most production being dependent on raw fish, although the availability of formulated diets has recently increased. As a major portion of compounded feeds used in Chinese mariculture is taken by farmers of pompano and red drum *Sciaenops ocellatus* [48], the latter species was also evaluated. Global production of Sciaenids exceeds 340,000 tons, with the red drum representing around 25% of the total [49]. As adults, the species evaluated herein are considered as obligate carnivores. For many marine carnivores, only a few studies have investigated the potential for concurrently replacing FM/FO in diets.

3. Selection of Dietary Ingredients

The ingredients employed in the various diets are presented in Table 1. All experimental feeds were evaluated against the response of the test species to an FM/FO-based diet. The least expensive and best performing experimental feeds from each reference were chosen for comparison for the sake of this paper; however, additional diets and corresponding performance data are available in the references listed. Dietary protein for the investigational diets was derived from both animal and plant sources and based on availability, demonstrated utility, and/or promise as a dietary component. For example, poultry by-product meal (PBM) represents a resource of considerable potential as an FM alternative. Global production of chickens is estimated to be 33 billion individuals, equivalent to 101 million tons for 2022 [50], with the largest producers being the USA, Brazil, China, and the EU Raw materials leftover from slaughterhouses and processing facilities represent about 30% of liveweight [51] or around 30 million tons. The clean unused parts of butchered poultry, including the voided intestines and culled laying hens, are ground and then rendered into meal. Corn gluten meal (CGM), a by-product of corn processing containing about 65% crude protein, and corn protein concentrate (CPC), comprising around 67% protein, have both enjoyed success as components of a variety of commercial and investigational aquafeeds, including those for amberjack [52], Florida pompano [53], red drum [54], and largemouth bass [55]. No negative effects of CGM or CPC have been reported, even when used at relatively high levels of dietary incorporation. Soybean meal (SBM; ~50% crude protein) is an excellent substitute for animal proteins in aquafeeds, even though the presence of anti-nutritional factors (ANFs) may disrupt gut function in some species [56]. Nevertheless, SBM and soy products, such as soy protein concentrate (SPC; ~63% crude protein), which has a reduced concentration of ANFs, have garnered wide use and are well represented in commercial and experimental aquafeeds across the board [57,58]. MrFeed Pro 50 (~51% crude protein), a bacterial hydrolysate made from soybean-derived cellulosic sugars, and similar products have received increased attention due to their availability, high digestibility, lower costs, safety, and sustainability [59,60]. *Spirulina* expresses 55%+ crude protein and an elevated PUFA content and, when used at high levels of supplementation, has been observed to provide a beneficial effect on animal growth, body composition, pigmentation, immunity, and reproductive performance [61]. A wide variety of FO alternatives have been assessed with a broad range of species, and flax, canola, and algal oils are not exceptional, being widely available and competitively priced. Each has been successfully used as an FO substitute with pompano, yellowtail, largemouth bass, and others [62–64].

Table 1. Formulations of experimental diets in which fishmeal and fish oil were replaced with a combination of different protein sources and oils. GMO = Genetically Modified Organism; ARS = Agricultural Research Service. For formulation information on FM/FO-based diets, the reader is directed to f3fin.org/resources/open-feed-formulas/ (accessed on 11 November 2022).

Ingredient	Kampachi [65]	Yellowtail [66]	Largemouth Bass 1 [67]	Largemouth Bass 2 [68]	Largemouth Bass 3 [46]	Red Drum [49]	Pompano 1 [47]	Pompano 2 [69]
Poultry by-product meal	36.12	23.12	25.62	25.62	28.80	28.8	36.12	23.12
Wheat, whole ground	20.53	16.75	20.43	22.7	18.41	18.41	17.77	16.75
Corn gluten meal	-	-	8.16	8.16	-	-	-	-
Corn protein concentrate	13.56	7.14	-	-	-	-	8.22	7.14
Non-GMO soybean meal	-	-	11	11	-	-	-	-
Soy protein concentrate	7.86	-	17.93	-	24.32	24.32	5.96	-
MrFeed Pro50	-	-	-	15	12.5	12.5	12.5	-
Algae meal	-	-	-	6	-	-	-	-
Spirulina	-	30	-	-	-	-	-	30
Algal oil, Veramaris	5.32	10.80	-	-	2.28	2.28	2.13	10.80
Flax oil	2.71	-	-	-	-	4.52	4.86	-
Non-GMO soy oil	-	-	4.73	2.7	-	-	-	-
Canola oil	2.38	-	-	-	5.42	0.9	1.32	-
Fish oil—Menhaden	-	-	3	-	-	-	-	-
Dicalcium phosphate	3.1	4.16	-	-	-	-	3.10	4.16
Monocalcium phosphate	-	-	1.97	1.35	1.8	1.8	-	-
Lysine-HCL	2.67	2.68	1.66	1.97	1.62	1.62	2.27	2.68
Taurine	2	2	1	1	-	-	2	2
DL-Methionine	0.69	0.64	0.64	0.64	0.74	0.74	0.77	0.64
Threonine	0.46	0.31	0.31	0.31	0.21	0.21	0.38	0.31
Choline CL	0.6	0.6	0.6	0.6	0.6	0.6	0.60	0.60
Lecithin	-	-	2	2	2	2	-	-
Stay-C	0.2	0.2	0.2	0.2	0.2	0.2	0.20	0.20
Vitamin Premix ARS 702	1.5	1.5	0.5	0.5	1	1	1.50	1.50
Trace min premix ARS 1520	0.10	0.10	0.25	0.25	0.10	0.10	0.10	0.10
Trace min premix F3	0.20	-	-	-	-	-	0.20	-
TOTAL	100	100	100	100	100	100	100	100

4. Fish Holding and Husbandry

Other than for a study with kampachi, all feeding trials were undertaken in tanks configured as recirculating systems. The study lengths, which varied from 56 to 126 days; water quality parameters, including temperature, salinity, and dissolved oxygen levels; and start weights of experimental animals are summarized in Table 2. Water quality parameters were collected using standard methods. The feeding schedule for the experimental and control diets for each species is likewise presented in Table 2. All studies were executed with appropriate regard to Institutional Animal Care and Use Committee regulations and complied with all relevant international animal welfare laws, guidelines, and policies.

Table 2. Experimental systems employed, stocking densities, starting weights, study lengths, water quality parameters, and feeding schedules in various studies undertaken to evaluate the impact of dietary fishmeal and fish oil replacement on the performance of established and candidate species of teleost for aquaculture.

	System	# of Fish/Tank	Start Weight (g)	Study Length (d)	Temperature (°C)	DO_2 (mg L^{-1})	Salinity (g L^{-1})	Daily Feed Schedule
Kampachi	Tanks	30 → 15	282	84	-	-	-	2× → 1× to satiety
Yellowtail	RAS	15	20	64	22	10-12	34.5	5–10% body wt
Largemouth bass 1	RAS	20	25	84	28	7.7	3.1	3× to satiety
Largemouth bass 2	RAS	60–64	48	126	28	8.2	3.7	3× using feed tables
Largemouth bass 3	RAS	20	15.2	70	28	6.0	1.2	2× to satiety
Red drum	RAS	15	3	56	27.6	6.9	5.4	2× to satiety
Pompano 1	RAS	10	15	84	26.6	7.4	3	2× for 5 min
Pompano 2	RAS	20	4.1	84	28	8.0	34	4× up to 5% body wt

5. Data Collection

Details relating to the precise procedures employed in data acquisition for each species may be found in the relevant publications (see Table 1 for references). Depending on the trial under consideration, the following information was compiled to assess the performance of experimental animals with each dietary treatment:

$$\text{Weight gain (\%)} = [(\text{Final body weight} - \text{initial body weight})/(\text{initial body weight})] \times 100;$$

$$\text{Survival (\%)} = [\text{final population}/\text{initial population}] \times 100;$$

$$\text{Feed efficiency (FE)} = \text{weight gain (g)}/\text{dry feed consumed (g)};$$

$$\text{Feed conversion ratio (FCR)} = \text{weight of feed consumed (g)}/\text{weight gained by the animal (g)};$$

$$\text{Protein efficiency ratio (PER, \%)} = [\text{weight gain (g, wet weight)}/\text{protein intake (g, dry weight)}] \times 100;$$

$$\text{Fillet yield (\%)} = [\text{fillet weight (g)}/\text{gutted weight (g)}] \times 100;$$

$$\text{Hepatosomatic index (HSI, \%)} = [\text{liver weight (g)}/\text{body weight (g)}] \times 100;$$

$$\text{Interperitoneal fat ratio (IPF, \%)} = [\text{IPF weight (g)}/\text{body weight (g)}] \times 100;$$

$$\text{Fulton condition factor (K)} = [\text{fish weight (g)}/(\text{fish length, cm})^3] \times 100;$$

$$\text{Viscerosomatic index (VSI, \%)} = [\text{weight of viscera (g)}/\text{body weight (g)}] \times 100.$$

After gauging the above-mentioned indices, all the remains of fish samples ($n \geq 5$) were homogenized as a composite sample and analyzed for proximate composition, when measured, using established methods: the Dumas protocol for crude protein (6.25 × N) [70], and chloroform–methanol (4:1) extraction for crude lipid [71]. A lipid droplet subsample was isolated from these ingredients and conserved in N_2 at −80 °C for identification of their fatty acid profile by flame ionization gas chromatography. Fatty acid methyl esters (FAMEs) were prepared as described previously [72] and modified to include an additional saponification step [73]. Ash was determined after heating samples at 650 °C in a muffle furnace for 3 h [70].

Histological analyses were undertaken on the guts and livers of California yellowtail and largemouth bass. Samples were collected immediately following gross necropsies for performance characteristics ($n \leq 6$ per treatment). Sections of liver and distal intestine (2 cm × 2 cm) were preserved in Bouin's fixative for 24 h and subsequently transferred to 70% ethanol for final fixation. Tissues were then dehydrated, embedded in paraffin, and sectioned at 5 µm before staining with H&E using standard procedures. Rankings were then performed to differentiate histopathologic changes in the liver and intestine between diets.

Criteria assessed included intestinal goblet cell density and inflammation, hepatic glycogen content, and cellular changes [66]. For largemouth bass, spleen samples were stained using Gomori's modified iron procedure for hemosiderin [74] to evaluate the staining intensity of melano-macrophage centers (MMCs), which were graded from 0 to 2 for low, medium, and high, respectively.

Taste tests of largemouth bass were informal and used 25 active consumers who were provided with blind samples and asked to prepare fish using plain methods. Each was then requested to determine whether there were differences in taste, texture, or aroma between samples. Similar studies were undertaken with kampachi. The collected data were subjected to various statistical analyses with significance set at the $p < 0.05$ level. Readers are directed to the papers noted in Table 1 for complete details.

6. Observations and Discussion

Weight gain in all marine species fed F3 diets, except pompano 2, was less than that achieved by animals fed conventional FM/FO feeds (Table 3). However, there was no impact discerned on FCR, survival, fillet yield, HSI, or *K*. In kampachi, a significantly higher VSI in the control group accounted for the increased weight gain such that once this was taken into account, no differences in weight were apparent. In largemouth bass, the only freshwater species examined, the weight gain in fish fed F3 and F2 (FO included) was equivalent to that in animals fed the conventional diet (Table 3). The FCR in F3/F2-fed largemouth bass was equivalent to that in the conventional group in two of the three trials and was elevated in one of the trials, while survival was lower in one study. Accordingly, the trials described here illustrate the potential to severely reduce, and perhaps eliminate, FM/FO from aquafeeds of facultative carnivores. Importantly, the evidence presented to support this statement originated from investigations that employed a constrained list of possible FM/FO alternatives. Additionally, the F3 recipes used were derived from a formulator's experience rather than from experiments designed to determine the optimal inclusion rates for specific ingredients. Undoubtedly, with dietary refinement, perhaps involving the inclusion of other proteins and oils or modification to their concentrations/combinations, even greater benefits than those achieved will accrue. This supposition is supported by the findings of other researchers who have successfully replaced FM/FO in diets for an ever-increasing number of species (e.g., [75–81]).

The results considered here with the F3 feeds, together with the experience of others, imply that marine species will be more demanding than freshwater fishes regarding the complete removal of dietary FM/FO. It is probable that the largemouth bass were indifferent to lipid exchange due to their essential fatty acid (EFA) requirements being met by dietary 18:3n-3 and/or 18:2n-6 PUFA [82]. Similar observations have been made with other species of freshwater fish, where a wide variety of alternative dietary lipids have been shown to facilitate growth [83–85]. These results thus provide support for the idea that FO can already be totally removed from largemouth bass diets. However, a precautionary approach should be taken since some substitute oils have been demonstrated to cause physiological disturbance [86,87]. Marine species lack the enzymatic machinery necessary to elongate or desaturate PUFAs, such that EFA requirements are met by long-chain PUFAs, *viz.* 20:5n-3 and/or 22:6n-3 [88], which, in some diets, may have been limiting. Nonetheless, the substitution of fish oil with vegetable and/or algal oil in all species examined had no significant impact on survival, suggesting that the dietary fatty acid composition, even though varying, achieved the n-3 HUFA requirements of the species examined, at least over the study length. Importantly, lipid exchange had either no impact or only a marginal impact on feed palatability, thereby underscoring the flexibility that exists for the substitution of dietary lipids. An additional advantage of using *Schizochytrium* sp.-derived algal oil, produced by controlled heterotrophic fermentation, is its contaminant-free status, which contrasts to that of some FOs [89].

Table 3. Response of various species to experimental diets in which fishmeal and fish oil were replaced with alternatives. FCR = feed conversion efficiency; FE = feed efficiency; HSI = hepatosomatic index; IPF = intraperitoneal fat ratio; *K* = condition factor; PER = protein efficiency ratio; VSI = viscerosomatic index. Up- and downward-pointing arrows indicate significant differences ($p < 0.05$) from fish fed a control diet.

Ingredient	Kampachi [65]	Yellowtail [66]	Largemouth Bass 1 [67]	Largemouth Bass 2 [68]	Largemouth Bass 3 [46]	Red Drum [49]	Pompano 1 [47]	Pompano 2 [69]
Wt gain (%)	419↓	633.6↓	201.4	149.3	398	666↓	243.1↓	1149
FE	-	-	-	-	-	1.09	0.52	-
FCR	1.31	1.33	1.28	1.95↑	0.89	-	-	1.6
PER	-	-	-	-	2.34	-	-	1.2↓
Survival (%)	-	100	99	84↓	100	90	-	100
Fillet yield (%)	60.9	-	-	-	32.3	31.5	31.2	-
HSI (%)	-	-	1.49	1.66	3.0	1.99	2.60	-
IPF ratio (%)	-	-	-	-	3.0	0.39↓	0.01	-
K factor	-	-	1.16	1.19	1.29		1.34	1.59
VSI (%)	5.7↓	-	4.55	1.93	-	-	-	-
				Proximate composition				
Moisture	-	70.9	-	-	68.8	74.9	68.6	-
Protein	-	20.98	45.4	41.5	17.9	17.4	18.1	-
Lipid	-	7.37↓	14.7	15	8.8	3.87↓	9.72	-
Ash	-	2.46↑	6.96	7.39	4.0↓	3.90	3.29	-

As recorded previously for a wide variety of species [90,91], the fatty acid profiles of fillets of the assessed fish correlated well with those of their feeds (Table 4, Figure 1). One negative aspect of this trait, however, was that while n6:n3 ratios remained stable, the EPA/DHA fractions were inferior to those of control fillets. Fish oil substitution, therefore, may negatively affect the nutritional value of fillets [92,93]. Were it to be considered necessary, fillet lipids (types and levels) might be tailored to a specific use with finishing diets [94,95]. Such an eventuality might occur where significant changes in flesh quality, including firmness, juiciness, and fresh oily taste, deviate following large fluctuations in proximate composition, or, for example, when higher fillet lipid levels are required for reasons of processing, such as smoking [96]. Even given differences between the control and treatment group fillet fatty acid profiles, and subtle modifications to proximate the composition of largemouth bass, organoleptic evaluation by 25 habitual consumers resulted in 48% preferring the fishmeal–fish-oil-fed fish based on the taste, texture, and aroma, while 40% favored the F3-fed animals and 12% indicated no preference [68]. Thus, for largemouth bass, the deletion of FO from their diet had no apparent impact on consumer acceptance. Similarly, a blind taste test of kampachi resulted in 62% of participants preferring the F3-fed fish, 19% having a preference for *S. rivoliana* fed on a traditional diet, and 19% being unable to discriminate between the two dietary groups [65].

Table 4. Identified feed and fillet fatty acids of largemouth bass, Florida pompano, and red drum following 56–84 days of feeding with fishmeal- and fish-oil-free diets. Values are expressed as a percentage of total fatty acids. Up- and downward-pointing arrows indicate significantly ($p < 0.05$) higher and lower values, respectively, than control fillet levels.

	Largemouth Bass 3		**Pompano 1**		**Red Drum**	
	F3 Feed	**F3 Fillet**	**F3 Feed**	**F3 Fillet**	**F3 Feed**	**F3 Fillet**
C14:0	1.10	1.72↓	1.32	2.07↓	1.12	0.87↓
C14:1	0.05	0.31	-	0.58	-	-
C16:0	16.74	18.13	15.85	21.42	17.3	17.6↓
C16:1	2.23	4.02↓	2.96	3.40↓	2.13	2.65↓
C18:0	4.42	2.81	4.51	5.65	5.90	5.72
C18:1n9	25.55	34.35↑	24.28	41.32	20.80	24.60↑
C18:2n6	19.47	17.80↑	17.44	19.74	20.7	17.3↑
C18:3n3	13.89	2.97↑	19.29	5.46	14.7	9.81↑
C20:0	0.42	0.31	0.51	0.58	0.48	0.33
C20:1n9	0.55	1.62	0.36	1.13	-	-
C20:2n6	0.07	0.57	0.04	0.71	0.12	0.28
C20:3n3	-	0.36	-	0.81	-	-
C20:4n6	0.86	0.89↑	0.83	0.87	1.06	0.95
C20:5n3	4.13	1.52↓	3.57	0.87↓	4.10	2.66↓
C22:0	0.31	0.31	0.32	0.58	0.37	0.32
C22:1	-	-	-	0.58	-	-
C22:6n3	7.51	8.09↓	6.36	6.08↓	7.14	5.39
C24:0	0.56	0.31	0.39	0.58	-	-
C24:1n9	0.44	0.31	0.43	0.58	0.41	1.85
Total ω-3 Isomers	25.53	22.53	29.22	19.58	26.0	17.9
Total ω-6 Isomers	20.40	18.62	18.31	16.64	21.9	18.6
EPA/DHA	0.55	0.19	0.56	0.14	0.57	0.49
n3:n6	0.78	0.83	0.63	0.85	1.19	0.96

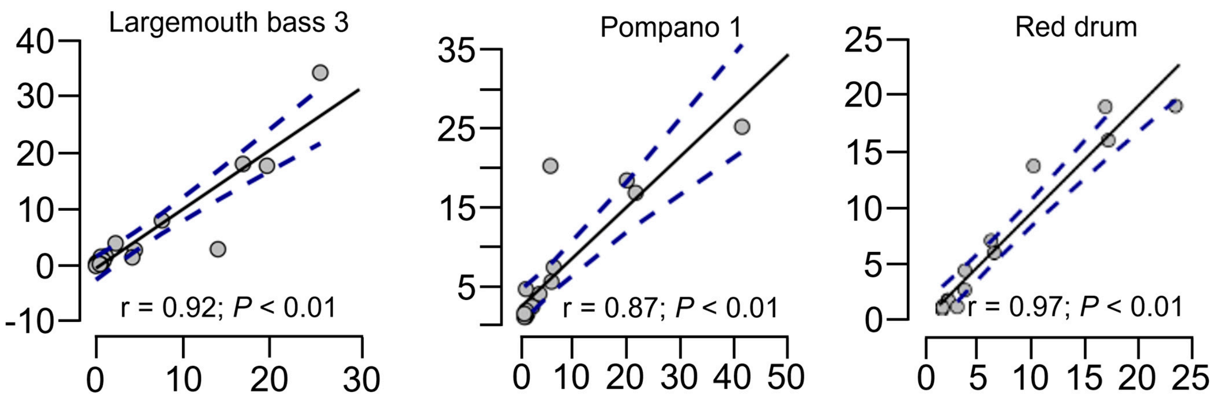

Figure 1. Scatter plots depicting the relationship between measured feed (y-axes) and fillet (x-axes) fatty acid content and 95% confidence intervals (dashed lines) for largemouth bass, Florida pompano, and red drum (see Table 4 for data).

Although the main goal of the F3 initiative is to eliminate the use of forage fishes in aquafeed production, an aspiration that is close to attainment for the species evaluated, some still question the practice of using animal by-products as alternative proteins. While this may be achievable with lower trophic species, a consistent observation with carnivores has been poorer overall performance when diets comprise vegetable proteins only. This is undoubtedly related to the presence of poorly digested carbohydrates and imbalances in essential amino acids (EAAs), the presence of a wide variety of anti-nutritional factors, and structural differences between plant and animal proteins [97]. These have negative impacts on growth, feed efficiency, metabolism, and health [31], and it is feasible that these effects may partly account for the reduced growth observed in the described trials herein. However, even given the presence of PBM in all F3 feeds, the marine test species failed to attain the growth recorded by control groups. Due to the variety of generally unsegregated material that is employed in PBM production, together with differences in processing and equipment, meals vary widely in their protein content and nutritional quality, lacking certain EAAs, being high in ash, and expressing variable digestibility [98,99]. Nonetheless, PBM has been successfully employed to replace relatively high levels of FM [100], although growth penalties coupled with higher FCRs and changes in body composition are known to occur in various species (e.g., [101–103]), and this may have been witnessed here.

The new and emerging technologies that modify raw materials, together with advances in process engineering, are starting to overcome many of the constraints encountered with alternative vegetable proteins, which bodes well for the future. For example, the production of plant protein concentrates and isolates removes carbohydrates, fiber, and anti-nutritional factors, resulting in products that, while more expensive, generally express an augmented EAA balance and have enhanced digestibility. However, the use of plant proteins for aquafeeds is disapproved by some who raise concerns relating to forest transitions, displacement of land use, increased use of fertilizers, eutrophication, environmental degradation, carbon footprinting, and others [104]. Given the current production strategies of established and emergent alternative proteins and their projected growth potential, it has been suggested that no single substitute protein will be able to source future demands of the animal feed industry, just as reliance on a few sources of ingredients, namely FM/FO, has created the bottlenecks we see today. Accordingly, the availability of a broad range of replacement proteins represents the soundest approach to overcome future supply constraints. Indeed, today, feed formulation scientists have a wide assortment of FM/FO alternatives [31,51,105]. Nevertheless, the aquafeed sector retains a significant dependency upon marine products [106], and it is likely that this addiction will remain for some time. Although their use will probably continue to decline in grower feeds, FM/FO will remain significant ingredients in specialty feeds, as exemplified by broodstock diets, and, perhaps, finishing feeds that may overcome fillet quality issues.

To date, most successful FM/FO replacement trials with carnivores have used diets containing blends of proteins and/or lipids that have been formulated to meet the nutrient requirements of the target species [77–79,107–110]. The broad range of potential aquafeed ingredients currently available, however, while providing strategic opportunities for formulating FM/FO-free feeds, also brings headaches for predicting optimal nutritional and economic blends, especially when mixtures might include a range of functional ingredients. Methods for overcoming some of these complexities are considered elsewhere [111–113]. One aspect of feed blending that has received limited attention is the potential to impact gut flora and fauna colonization and how this may influence nutrient absorption, etc., leading to potential for gut dysfunction. Clearly, there must be no consequences to the health of the target species when using alternative dietary ingredients. In one study with largemouth bass (Table 3), however, survival was apparently compromised by F3 feeds, although in a further two studies, no such effect was observed. Nonetheless, the detected anomaly prompted more detailed analyses of fish health. One indicator of immune function in teleosts is the status of splenic MMC [114], but evaluations thereof failed to detect differences between control and F3 treatments [67]. Moreover, the splenic index and hematocrit levels in examined fish were similar, and histological observations of the liver and distal intestine did not reveal any microscopic changes for the F3-fed group. In California yellowtail, slight hepatic inflammation and microscopic structural changes were encountered, with F3-fed animals also expressing higher glycogen accumulation. In contrast, control fish exhibited increased hepatocellular vacuolization and eccentric nuclei, together with a higher number of goblet cells in the distal intestine [66]. The decreased presence of goblet cells in F3 fish was not associated with inflammation, which the authors suggested might have indicated a protective effect of the *Spirulina* and/or algal oils incorporated into the diets. Notable is that the inclusion of soybean meal and concentrate in great amberjack *S. lalandi* diets was also associated with increased goblet cell numbers [115].

Since it is likely that animals cultured using sustainable marine-resource-free diets, such as organically certified and other premium foods, will represent quality products [116], methods for verifying their authenticity and traceability will become an imperative [117,118]. Animal tissue $\delta^{15}N$ is commonly employed to designate trophic position in food [119–121], and the technique has been applied to examine the relative contributions of plant and animal proteins in feeds for crustaceans [122–124] and fishes [117,125,126]. Thus, when the contribution of dietary FM declines, a corresponding decline in $\delta^{15}N$ is encountered. This response thereby potentially provides a method for verifying the integrity of animals reared using F3 diets. To substantiate this possibility, a study was undertaken with largemouth bass [68] (Figure 2). The trial examined fish fed a commercial feed, an FM/FO-based control diet, an FM-free feed containing FO, and an F3 diet. The FM control and commercial feeds both expressed final $\delta^{15}N$ values that were significantly higher than those for the FM-free feeds (Figure 2), no doubt reflecting the relative proportion and isotopic values of their ingredients. Substitution of the PBM from the F3 feed with another plant protein would likely shift the $\delta^{15}N$ values lower still. The use of stable isotope ratios to discriminate between aquacultured animals fed on more sustainable feeds, therefore, is apparently operational but should probably be restricted to animals reared in contained environments.

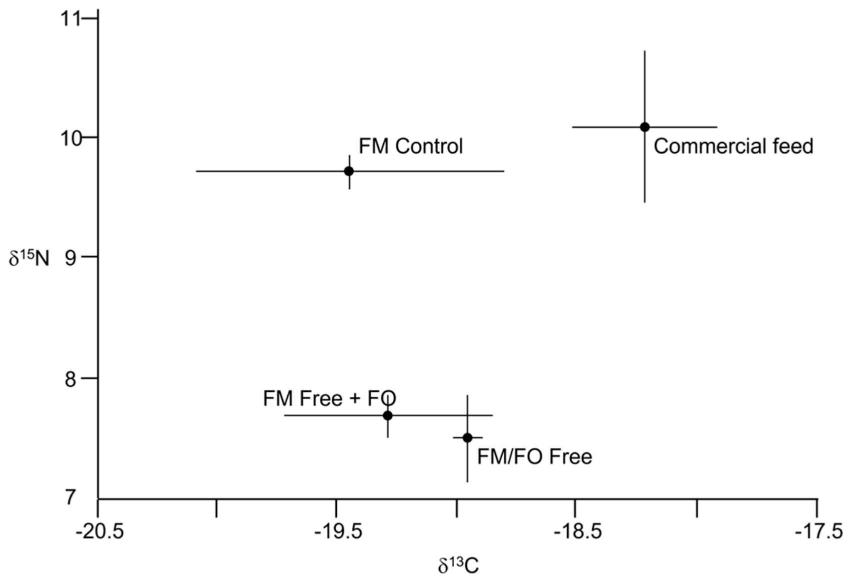

Figure 2. Isotope values for largemouth bass fed one of four diets. Values are means ± 95% confidence intervals (redrawn from [68]).

Based on the findings presented using essentially carnivorous species of cultured fish, total replacement of FM/FO appears more than just a convincing and economically viable proposition. Even so, further production-length research, perhaps with adjusted dietary formulae, is warranted to ensure that such diets have no negative consequences to the overall health and welfare of farmed animals. The potential adverse outcomes that dietary changes may have on various quality attributes, which may influence wholesale, retail, and consumer purchasing choices, also demand greater attention. Lucid though, from the considered trials, is that replacement protein/oil combinations provide products that are more secure in terms of food safety and more acceptable to discriminating consumers. The use of such nutrients will bridge gaps between the future supply and demand for FM/FO while serving global sustainability initiatives. While this might appear an over-enthusiastic conclusion, we have already demonstrated the potential for aquafeed mindset change with Pacific whiteleg shrimp *Litopenaeus vanammei* production [127,128], where F3 feeds are now firmly placed in the production sector. Similar success has been achieved with trout, largemouth bass, yellow croaker, and red seabream [129].

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