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Is removing marine ingredients from largemouth bass diets possible?

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14 December 2020 Ewen McLean, Ph.D. Luke Fredriksen Kelly Alfrey Michael F. Tlusty, Ph.D. Steven R. Craig, Ph.D. Frederick Barrows, Ph.D.

Complete elimination of fishmeal and fish oil is possible without affecting growth, health or consumer acceptance

The results of these studies decidedly demonstrate that marine ingredients can be totally removed from largemouth bass diets without negatively affecting fish growth, health, or consumer acceptance, and that it is not feed ingredients that matter, but the balance of nutrients that are of greatest importance. Photo by Prairie Aquatech.

According to the Food and Agriculture Organization of the United Nations, there was a more than **2,000-fold increase** in global production of largemouth bass (*Micropterus salmoides*) from 1999 to 2018. The most recent data suggest that around 435,000 metric tons of largemouth bass were farmed in 2018, with a value over (U.S.) $1.24 billion. Almost all (99.5 percent) farm-raised largemouth bass originates from China, with the majority being reared in ponds. Critical to future growth and sustainability of this aquaculture, however, will be the development of diets that are less dependent on fishmeal and other marine resources.

Despite this, and as with other carnivorous species, there has been a reticence to move away from fishmeal-based feeds due to reports suggesting that its replacement depresses feed intake and growth, and has detrimental effects on animal health and welfare (DOI: 10.1016/j.oneear.2019.10.018). Another facet that works against replacing marine ingredients in aquafeeds is the suspicion that alternative ingredients may adversely affect product quality. This includes issues such as fillet yields; body and flesh pigmentation; processability (e.g., smoking, freezing, shelf-life); and most important, consumer acceptance (safety, traceability, texture, flavor).

As part of the **F3 Future of Fish Feed** initiative – which has the goal of eliminating marine ingredients from aquafeeds – we designed and carried out studies to examine the possibility of removing marine ingredients from largemouth bass diets, and results were published in the peer-reviewed *International Journal of Fisheries and Aquatic Studies*. The alternative proteins used in the present studies included a poultry by-product meal (PBM), hydrolyzed soybean meal (SBM) and a SB protein concentrate. Furthermore, the trials explored the possibility of replacing fish oil using an omega-3 rich algal product (AlgalPrime™), thereby achieving the F3 goal of eliminating fishmeal and fish oil from the diet.

Experience with other, essentially carnivorous fish (DOI: 10.1016/j.aquaculture.2009.11.003; 10.1371/journal.pone.0186705) teaches that this challenge can, with due care, be conquered. Furthermore, previous studies with largemouth bass have demonstrated that substantial proportions of fishmeal can be substituted without undue effects on growth, feed conversion, protein digestibility, or body composition (DOI: 10.111/jwas.12415; 10.111/j.1365-2095.2007.00533.x).

https://www.aquaculturealliance.org/advocate/testing-diets-without-fishmeal-and-fish-oil-for-kampachi/

We also evaluated three other concerns in these trials. First, the health of experimental animals was assessed with reference to structural changes to the intestine and liver, while the spleen was appraised for the staining intensity of melanomacrophage centers, MMC [microscopic structures possibly involved in activation of adaptive immunity].

Second, customer endorsement of the final product was examined through a user-based taste trial; and third, substantiation of the fact that animals were reared using marine resource-free feeds was assessed using stable isotope ratio mass spectrometry. The former issue has obvious implications whereas the latter two have become of increasing importance to consumers more attuned to social concerns surrounding sustainability. These same individuals are prepared to pay more for organic and environmentally sound products (DOI: 10.3390/su11061577; 10.1016/j.marpol.2020.104176).

Setup of studies

Two trials were undertaken, the objective of both being not only to evaluate fishmeal- and fish oil-free feeds, but also to reduce cost of the feed to make new formulas more acceptable to producers. The studies were completed using recirculating aquaculture systems at the Aquatic Research Laboratory, Prairie Aquatech (Brookings, South Dakota USA; Fig. 1). Water quality (dissolved oxygen, DO_2: ~8 mg/L; temperature, 28-30 degrees-C; salinity, ~3.5 mg/L; pH ~8.4; total dissolved solids ~4 g/L; NH_3, 0.33 mg/L; NO_2, ~0.5 mg/L; NO_3, ~25 mg/L) values throughout the studies was suitable for largemouth bass cultivation (Tidwell. J., Coyle, S., Bright, L.A. (Editors). *Largemouth bass aquaculture*. Wellcome Trust, London, U.K., 2018; 272 pp).

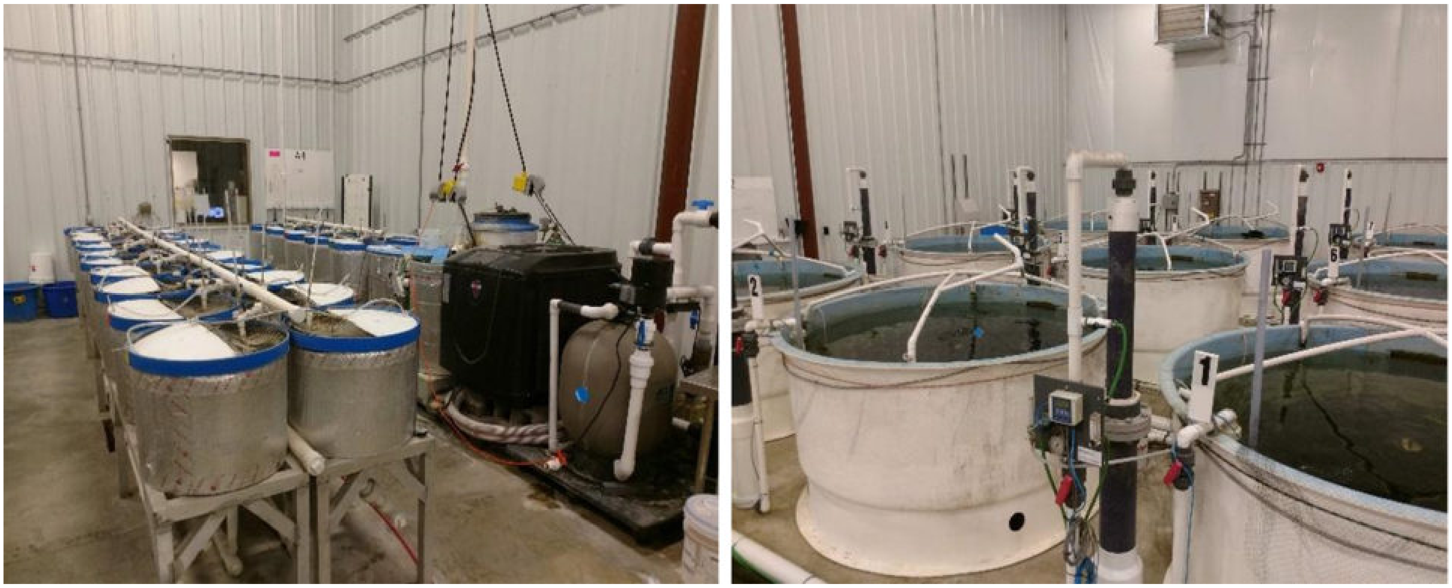

Fig. 1: Experimental recirculating aquaculture systems of the Aquatic Research Laboratory, Prairie Aquatech (Brookings, South Dakota, USA) used in the trials. The systems comprised tanks (30 × 113 liters and 8 × 757 liters) with a flow rate of 2 liters per minute, equipped with biological and mechanical filtration, UV sterilization, temperature control and pure oxygen supplementation.

First study

The first study assessed the response of fish – growth and health – to the different formulations (Table 1), while the second study examined fish taste and traceability. Diets included a commercially available feed (COM) presently used by LMB producers (Classic Bass®, extruded, floating; protein/fat: 48/18; Skretting Tooele, Utah, USA) and three experimental feeds, varying in protein and lipid levels.

McLean, largemouth bass diets, Table 1a

Ingredients	FMC	FMF	FFF
Algae meal-1	–	–	0.06
Hydrolyzed soy meal-2	–	0.15	0.15
Corn gluten meal	0.0816	0.0816	0.0816
Whole cleaned wheat	0.2219	0.254	0.227
Poultry meal-3	0.2082	0.2562	0.2562
Fishmeal-4	0.263	0	0
Vitamin premix	0.005	0.005	0.005
Lysine	0.0135	0.0197	0.0197
Methionine	0.0034	0.0064	0.0064
Choline chloride	0.006	0.006	0.006
Mineral premix	0.0025	0.0025	0.0025
Stay C (L-Ascorbat-2-Mono)	0.002	0.002	0.002
Soy oil (non-GMO)	0.031	0.03	0.027
Fish oil, menhaden-5	0.03	0.03	0
Monocal phosphate, 21%	0	0.0135	0.0135
Taurine	0	0.01	0.01
Threonine	0.0019	0.0031	0.0031
Soybean meal (non-GMO)-6	0.11	0.11	0.11

Ingredients	FMC	FMF	FFF
Lecithin	0.02	0.02	0.02
TOTAL	1	1	1

Table 1. Formulation and composition of experimental diets.
1-Profine VF®, Dupont Nutrition and Biosciences, 2-AlgaPrimeTM, Corbion Inc., San Francisco, CA., 3-MrFeed Pro50 S®, Menon Renewable Products Inc., Escondido, CA., 4-Tyson River Valley Animal Foods, Texarkana, AR., 5,6-Daybrook Fisheries, New Orleans, LA., 7-South Dakota Soy Processors, Volga, SD.

McLean, largemouth bass diets, Table 1b

Proximate composition	FMC	FMF	FFF	COM-7
Dry matter	92.27	92.1	90.28	92.86
Crude protein	46.8	42	41.5	50.8
Fat (acid hydrolysis)	13.7	14.5	15	17.2
Ash	9.25	7.2	7.39	7.37
Fiber (crude)	1.03	1.53	1.17	<0.20
Phosphorus (total)	1.54	1.26	1.26	1.18

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All experimental fish were fed to apparent satiation three-times daily and group weighed at three-week intervals, with feeding rates following commercial feed tables. Feed consumption was monitored for determination of feed conversion ratio (FCR = grams fed/grams gained) and mortalities recorded daily. At the end of the first trial fish were weighed and measured individually and a sub-sample from each treatment (n=3 fish/tank, 12 per treatment) euthanized (MS-222; Tricaine S, Western Chemical Inc., Ferndale, Wash., USA) and bled via caudal venipuncture for hematocrit determination (Fisher Scientific, Pittsburgh, Pa., USA).

These fish were also used for tissue analyses, including collection of liver, intestine, and spleen for histological evaluations and determination of somatic (= weight of tissue (g)/weight of fish (g) x 100) and visceral fat (VFI = weight of fatty tissue (g)/weight of viscera (g) x 100) indices. Other performance indicators included:

1. Biomass gain, Fulton's condition factor **(k = wt/L^3 x 100000)**

Relative growth rate = **(wt – wi)/100** , wi * where wt was final weight and wi initial weight; and

2. Specific growth rate = **((ln(final weight/length) – ln(initial weight/length))/number of days**

Tissue samples from liver, spleen, head kidney, proximal and distal intestine were prepared for histological analyses using standard methods. Analyses of these samples were used to determine impacts, if any, of the experimental diets on targeted organs. Liver lipid, glycogen and glucose were quantified using standard methods.

Second study

For the second trial, 60 to 64 fish from each treatment group were randomly distributed into eight larger tanks (densities of 3.11 ± 0.29 kg per square meter; biomass 2970.3 ± 275.7 grams; Fig. 1). Fish were weighed as a group every three weeks for a further 18 weeks, and their performance monitored in terms of survival and FCR.

A preliminary taste trial was undertaken using fish derived from the commercial and F3 dietary groups. Twenty-five active consumers of LMB were sent color-coded samples and asked to prepare their fish using plain methods. Each was then asked to establish whether there were differences in taste, texture, or aroma between the samples.

Finally, the muscle (taken dorsal to the midline between the second dorsal and caudal fins) of three fish per treatment were also sampled for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios, as well as strontium analysis. Samples were collected and sent to the Marine Biological Laboratory, Woods Hole, Mass., USA, Stable Isotope Laboratory, where they were processed and analyzed.

All statistical analyses were performed using JASP software (JASP Team, 2019, Version 0.11.1) at the α=0.05 level of significance. Differences between treatment means were examined by one-way ANOVA and significant differences isolated using Tukey's studentized range (honestly significant difference) test. Any potential tank effect or associated handling/treatment stress was assumed to be identical for each dietary group.

Fish in both studies were regularly measured and weighed.

Results

During the first trial, which evaluated the growth response of largemouth bass to the experimental feeds, fish weight increased between 200 and 230 percent, while length increased between 119 and 130 percent. There were no differences in weight, weight specific growth rate, length, length specific growth rate or Fulton condition factor, *k* [which relates fish length and weight, and is considered a health index], although the FCR for the COM diet was lower ($P < 0.05$) than the FFF diet.

Likewise, hematocrit [proportion of red blood cells by volume in the blood], visceral somatic index, VSI [weight of the viscera as a percentage of total body weight] and splenosomatic index, SSI [weight of the spleen expressed as a percentage of total body weight] did not differ between treatment groups but visceral [body fat in abdomen that cushions organs] fat index, VFI, was lower in the FFF diet group when compared to fish fed on the FMF feed. Hepatosomatic index, HSI [ratio of liver weight to total body weight; used as a measure of the energy reserves of an animal, especially in fish] was highest in the FMC group, with livers being larger ($P < 0.05$) than those observed in the COM fed fish. Liver vacuolization, glucose, lipid, and glycogen levels and (non)-MMC hemosiderin [an iron storage complex within cells] were equivalent across all groups.

In the second trial, there were no differences between dietary groups for feed consumption, but by trial end, the largemouth bass fed the COM diet were heavier ($P < 0.05$) with percent increase in biomass shown in Fig. 2. The FCR values also differed with the order FFF 1.95 ± 0.09^{c} > FMF $1.95 \pm 0.13^{b,c}$ > FMC 1.78 ± 0.06^{b} > COM 1.67 ± 0.02^{a}. VSI, SSI, HSI and *k* did not differ between groups at trial end (Fig. 3). However, visceral fat index was lower in fish fed the FMC diet when compared to all other feeds ($P < 0.05$).

Fig. 2: Hematocrit and percent increase in biomass of largemouth bass, fed three experimental feeds and a commercial feed during the second trial. Data with a different superscript were significantly different (P < 0.05). For dietary formulation details see Table 1.

Fig. 3: Somatic indices and condition factor (k) of largemouth bass fed three experimental feeds and a commercial feed. Data with a different superscript were significantly different ($P < 0.05$). For dietary formulation details see Table 1. VFI = visceral fat index, VSI = visceral somatic index; HSI = hepatosomatic index; k = condition factor.

Hematocrit was similarly impacted by diet as depicted in Fig. 2. Stable isotope ratio mass spectrometry [a technique to measure the relative abundance of isotopes (atoms of the same element that have different numbers of neutrons but the same number of protons and electrons) in a given sample] for carbon and nitrogen isotopes demonstrated the feasibility of distinguishing fish fed FM-free diets (Fig. 4); these expressed lower levels of the nitrogen isotope $\delta^{15}N$ compared against the FMC and COM fed fish. Levels of the carbon isotope $\delta^{13}C$ varied, with values for COM fed fish being greater than recorded in other diets.

Fig. 4: Isotope values for d 15N and d 13C for largemouth bass fed one of four diets. Values are means ± 95 percent confidence intervals. For dietary formulation details see Table 1.

On the organoleptic evaluation, of the 25 participants 12 stated – based on taste, texture and aroma – a preference for the FFF fed fish, three indicated no preference and 10 preferred fish raised on the COM diet.

Perspectives

Results from the trials described above unambiguously demonstrate that marine ingredients can be totally withdrawn from largemouth bass dietary formulations without negatively influencing fish growth, health, or consumer acceptance. The trials thereby support the recent viewpoint of an ***Advocate* article** that it is not feed ingredients that matter, but the balance of nutrients that are of greatest importance.

Nevertheless, even though the current trial series was successful, there remains a need to continue to develop and fine-tune diets that incorporate a fusion of terrestrial proteins and oils that work synergistically to the benefit of animal performance, welfare, product safety and quality and, where needs be, processability.

Commercial dietary formulations already include a wide variety of plant and animal proteins and oils, supplemented with pre- and probiotics, essential amino acids, vitamins, minerals and other constituents. An important facet of the presented trials was the application of stable isotope ratio mass spectrometry (SIRMS) to authenticate that fish fillets were derived from fishmeal and fish oil-free fed animals and this proved successful. The study confirms and extends the findings with salmon, shrimp, and other species (DOI: 10.1007/s00217-014-2298-5; 10.1016/j.foodcont.2015.01.003) and thereby supports the use of SIRMS for verification purposes of the presence of marine ingredients. Other methods for establishing the origin and authenticity of aquacultured products are nonetheless deserved of evaluation (DOI: 10.1016/j.tifs.2019.07.010).

It has been suggested that alternative proteins, and especially those of vegetable origin, influence textural and organoleptic qualities of fillets. These suspicions are supported by measured changes in fillet composition, and especially that of fat, deviations in muscle cell/fiber size, mechanical evaluations of fillet texture and gene expression analyses (DOI: 10.1016/j.aquaculture.2004.01.006; 10.111/jwas.12452; 10.1016/j.aquaculture.2006.12.012; 10.1016/j.aquaculture.2015.11.034).

These findings are of critical importance since alterations in fillet quality characteristics may influence not only consumer acceptance and fish processing, but market demand. Herein, fishmeal and fish oil replacement had no discernable impact of the eating quality of largemouth bass. Similar findings have been made for a broad variety of species including, but not limited to, blunt snout bream and gilthead sea bream (DOI: 10.1111/anu.1258110; 1016/j.aquaculture.2012.07.009) and, using various fishmeal replacers, rainbow trout (DOI: 10.1016/0044-8486(94)00403-B; 10.1111/j.1749-7345.2010.00441.x; 10.1046/j.1365-2095.1998.00077.x). Upcoming studies, with the objectives of the F3 in mind, have already been planned using other carnivorous species that rely heavily on fishmeal and fish oil inputs.

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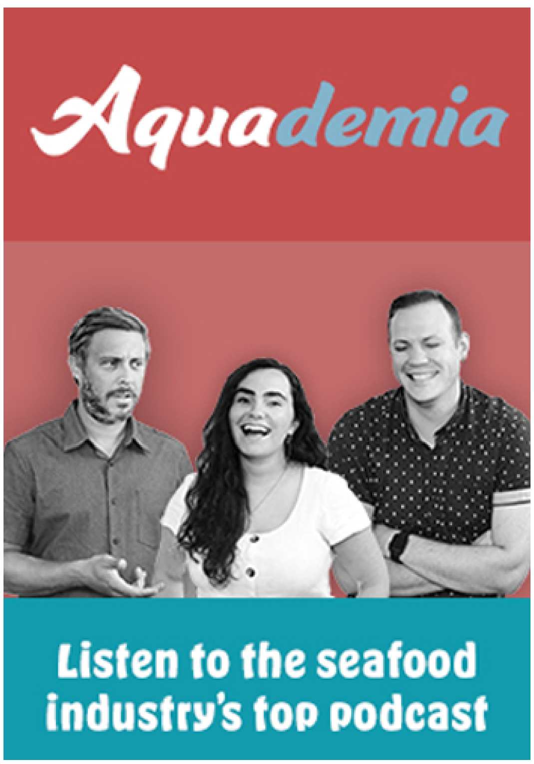

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