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## Growth, integrity, and consumer acceptance of largemouth bass, *Micropterus salmoides* (Lacépède, 1802), fed marine resource-free diets

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### Abstract

Largemouth bass (LMB) were fed diets developed to substitute fishmeal (FM) using a blend of alternative proteins. Diets included a FM control (FMC), two FM-free formulae (FMF), one of which (FFF) was formulated using an algal oil replacing fish oil (FO) and, for comparative purposes, a commercial LMB feed (COM). Fish (densities of  $3.11 \pm 0.29 \text{ kg m}^{-1}$ ) were arbitrarily distributed into one of eight tanks configured as a recirculating system (RAS;  $28.3 \pm 0.76 \text{ }^\circ\text{C}$ ;  $\text{DO}_2$ :  $7.7 \pm 1.19 \text{ mg L}^{-1}$ ) and tanks randomly assigned to one of the four treatments (i.e. each treatment was tested in duplicate). Animals were fed 3x daily to apparent satiation and group weighed every 3 weeks for 18 weeks. No differences were observed in feed consumption between groups ( $P > 0.05$ ) but LMB fed the COM diet were heavier ( $P < 0.05$ ) than fish fed the FMF and FFF feeds. In efforts to verify whether fish fed the FM/FO-free were authentic, samples were assessed by stable isotope ratio mass spectrometry.  $\delta^{15}\text{N}$  fish muscle isotope values at trial end were indicative of dietary FM substitution. A preliminary blind taste trial undertaken using fish from the FFF and COM diets did not differentiate between treatments ( $P > 0.05$ ). Results from the present study show that, with prudent dietary control, complete replacement of FM/FO in LMB diets is attainable and verifiable, without compromising growth performance or consumer acceptance of the final product.

**Keywords:** Algal oil, alternative proteins, mass spectrometry, taste, provenance

### Introduction

Largemouth bass (LMB) aquaculture has experienced rapid growth over the last decade, especially in China, where production quadrupled between 2000-2018, to 432,000 tons, valued at US\$1.23 billion [1]. As with other types of aquaculture, the highest variable operating cost in LMB farming is feed [2], and the costliest ingredient of feeds is protein; especially when supplied as fishmeal (FM). Critical to future food system sustainability [3] is the need for aquaculture to replace FM and fish oil (FO) in commercial diets. Indeed, traditional supplies of feed protein and oil are now being supplemented and replaced with various by-products and alternative proteins and oils from diverse sources [4]. This shift away from convention has socio-economic, ecological, and moral implications since removal of FM/FO reduces feed costs, decreases pressures on wild-caught fish, and heeds consumers attuned to social issues surrounding the sustainability of fisheries. In fact, recent analyses indicate that reduction fisheries production will likely decline slightly in the near future, while increased price and reduced availability of FM/FO may slow expanded farming of species that traditionally rely on these inputs [5]. Consumer demand for sustainably produced farmed seafood is also increasing [6]. Remarkably, given this landscape, certain commercial LMB feeds still retain high levels of FM. Recently, for the first time, we demonstrated the feasibility of totally removing FM/FO from LMB diets, using blends of alternative proteins and algal oil [7]. However, due to small fish size at trial end, that study was concluded without assessment of consumer perceptions of organoleptic properties. Here we report continued data from this prior study to investigate if the FM/FO substitutes influenced consumer perceptions of fillet eating quality. We also report on carbon and nitrogen stable isotope ratio mass spectrometry (SIRMS) as a tool to authenticate fillets derived from sustainably produced (i.e., without marine feed ingredients) fish.

This method could potentially be employed as a means to verify the integrity of products labeled as environmentally or organically farmed and enhance consumer confidence in their authenticity.

### Materials and Methods

Animals were maintained in a recirculating system (RAS) comprising eight tanks holding 757 L of water with a total system volume of 10,100 L. The RAS was equipped with biological and mechanical filtration, UV sterilization, temperature control and pure oxygen supplementation. Tanks were stocked to with 60-64 fish per tank at a density of  $3.11 \pm 0.29 \text{ kg m}^{-1}$  (biomass of  $2970.3 \pm 275.7 \text{ g} = 60\text{-}64 \text{ fish}$ ) and assigned to one of four diets (Table 1) and fed 3x daily using commercial LMB feed tables. Feed consumption was monitored daily, and fish weighed as a group every 3 weeks for 18 weeks. Performance indicators included biomass gain and Feed Conversion Ratio (FCR = g fed/g gain). At trial end four fish from each treatment group were euthanized by anesthetic overdose (Tricaine S, Western Chemical Inc., Ferndale, WA., USA) and bled via caudal venipuncture for hematocrit determination (Fisher Scientific, Pittsburgh, PA., USA) and to assess Fulton's condition factor ( $k = \text{wt/L}^3 \times 100000$ ) [8]. Fish were also employed for determination of visceral somatic (VSI) and fat (VFI) indices and hepatosomatic (HSI) and splenosomatic (SSI) indices. Somatic indices were calculated according to the following formula: Somatic index = weight of tissue (g)/weight of fish (g) x 100. VFI = weight of fatty tissue (g)/weight of viscera (g) x 100.

A preliminary taste trial was undertaken using fillets derived from FFF (FM/FO-free) and COM 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 and nitrogen isotope ratios, as well as strontium analysis. Samples were collected and sent to the Marine Biological Laboratory, Woods Hole, MA, Stable Isotope Laboratory, where they were dried, pulverized and analyzed for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  using a Europa 20-20 continuous-flow isotope ratio mass spectrometer interfaced with a Europa ANCA-SL elemental analyzer. The analytical precision based on replicate analyses of isotopically homogeneous international standards is  $\pm 0.1\%$  for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  measurements, and about 1% relative on the % N and % C measurements. A subsample from each fish was also sent to the New Jersey Feed Lab Inc. (Ewing, NJ, USA), for strontium analysis.

All statistical analyses were performed using JASP software (JASP Team, 2019, Version 0.11.1) at the  $\alpha=0.05$  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.

**Table 1:** Formulation and composition of experimental diets

Ingredients	FMC	FMF	FFF	COM <sup>7</sup>
Algae meal <sup>1</sup>			0.06	
Hydrolyzed soy meal <sup>2</sup>		0.15	0.15	
Corn Gluten Meal	0.0816	0.0816	0.0816	
Whole Cleaned Wheat	0.2219	0.254	0.227	
Poultry Meal <sup>3</sup>	0.2082	0.2562	0.2562	
Fish Meal <sup>4</sup>	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 <sup>5</sup>	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 <sup>6</sup>	0.11	0.11	0.11	
Lecithin	0.02	0.02	0.02	
Total	1.000	1.000	1.000	
<b>Proximate composition</b>				
Dry matter	92.27	92.10	90.28	92.86
Crude protein	46.8	42.0	41.5	50.8
Fat (acid hydrolysis)	13.7	14.5	15	17.2
Ash	9.25	7.20	7.39	7.37
Fiber (crude)	1.03	1.53	1.17	< 0.20
Phosphorus (total)	1.54	1.26	1.26	1.18

<sup>1</sup> AlgalPrime™, Corbion Inc., San Francisco, CA., <sup>2</sup> MrFeed Pro50 S®, Menon Renewable Products Inc., Escondido, CA., <sup>3</sup> Tyson River Valley Animal Foods, Texarkana, AR., <sup>4,5</sup> Daybrook Fisheries, New Orleans, LA., <sup>6</sup> South Dakota Soy Processors, Volga, SD, <sup>7</sup> Classic Bass®, extruded, floating; protein/fat: 48/18; Skretting Tooele, Utah, USA.

## Results

Water quality parameters throughout the trial were: DO<sub>2</sub>, 8.19±1.05 mg L<sup>-1</sup>; temperature, 28.27±0.75 °C; salinity, 3.72±0.58 mg L<sup>-1</sup>; pH 8.49±0.09; total dissolved solids 4.43±0.65 g L<sup>-1</sup>; NH<sub>3</sub>, 0.32±0.16 mg L<sup>-1</sup>; NO<sub>2</sub>, 0.17±0.32 mg L<sup>-1</sup>; NO<sub>3</sub>, 38.78±12.38 mg L<sup>-1</sup>; values suitable for LMB aquaculture [9]. No deaths befell fish fed the COM diet. Mortalities recorded for other feeds over the duration of the trial were: FMC (2), FMF (14), and FFF (10). Table 2 summarizes the group response of LMB to the 18-week period fed with the experimental and commercial diets. Differences were apparent in group weights at trial start with FMC fed fish being smaller ( $P < 0.05$ ) than the LMB receiving the COM feed. No differences were recorded in feed consumption over the 18-week trial but, by study end, LMB fed the COM feed were heavier ( $P < 0.05$ ) than the FMF and FFF groups and variances were apparent for FCR, favoring diet COM (Table 2). Percent increase in group biomass differed between FMF and COM diets with the latter gaining more weight on a percent basis (Table 2). Biomass gain and

condition factor of randomly sampled fish did not differ between feed groups ( $P > 0.05$ ; Tables 2 and 3), thereby indicating equivalence in overall growth. However, there were differences between feed groups with respect to visceral fat presence with LMB fed on the FMC diet having lower accumulation ( $P > 0.05$ ). Hematocrit, too, was lower ( $P > 0.05$ ) in FMC fed fish (Table 3). The  $\delta^{15}\text{N}$  isotope values in the fish muscle at the end of the trial were indicative of the substitution of the fish meal in the diet (Fig. 1) where FMF and FFF values were lower than those of the FMC or COM fed fish. It was feasible to differentiate fish diet based on carbon and nitrogen isotopes (Fig. 1). Diets including FM had a greater  $\delta^{15}\text{N}$  than those fed alternative diets. There was some variation in  $\delta^{13}\text{C}$ , and the values for the COM fed fish were greater than the other diets. There was no difference in muscle strontium value for the fish fed the different diets (Fig. 1). When presented to 25 participants for organoleptic evaluation, twelve stated, based on taste, texture and aroma, a preference for the FFF fed LMB, 3 indicated no preference and ten preferred fish raised on the COM diet.

**Table 2:** Initial group weights, biomass gain, feed consumed, feed conversion ratios (FCR) and percent increase in group biomass of largemouth bass fed on one commercial and three experimental diets over a period of 18-weeks. Final data is adjusted for mortality weights. Data within a column with a different superscript were significantly different ( $P < 0.05$ ).

Treatment	Initial biomass	Feed consumed	Final biomass	Biomass gain	% increase in biomass	FCR
FMC	2702±127.3 <sup>b</sup>	4454.1±199.1	5289±340.8 <sup>a,b</sup>	2505±201.2	180.4±16.3 <sup>a,b</sup>	1.78±0.06 <sup>b</sup>
FMF	2888±200.8 <sup>a,b</sup>	4608.9±130.0	4239±380.4 <sup>b</sup>	2368±224.5	146.7±2.98 <sup>b</sup>	1.95±0.13 <sup>b,c</sup>
FFF	2963±76.4 <sup>a,b</sup>	4888.7±164.7	4588±48.8 <sup>b</sup>	2515±197.8	169.9±6.22 <sup>a,b</sup>	1.95±0.09 <sup>c</sup>
COM	3355±97.6 <sup>a</sup>	4741.7±131.0	6201±74.9 <sup>a</sup>	2846±42.4	184.9±4.24 <sup>a</sup>	1.67±0.02 <sup>a</sup>

**Table 3:** Viscera, condition factor ( $k$ ), and hematocrit response of largemouth bass to different experimental, and a commercial diet, fed over a period of 18 weeks. Data within a column with a different superscript were significantly different ( $P < 0.05$ ). For dietary formulation details see

Treatment	VSI	VFI	SSI	hematocrit	HSI	$k$
FMC	1.80±0.92	1.97±0.18 <sup>b</sup>	0.09±0.03	37.25±3.78 <sup>b</sup>	1.79±0.92	1.24±0.06
FMF	2.53±1.13	2.64±0.30 <sup>a,c</sup>	0.10±0.03	43.25±3.78 <sup>a</sup>	2.53±1.13	1.20±0.10
FFF	1.93±0.66	2.77±0.52 <sup>c</sup>	0.07±0.03	39.50±2.89 <sup>a</sup>	1.66±1.08	1.19±0.03
COM	1.66±0.48	2.81±0.17 <sup>a</sup>	0.08±0.03	45.75±2.06 <sup>a</sup>	1.66±1.08	1.27±0.14

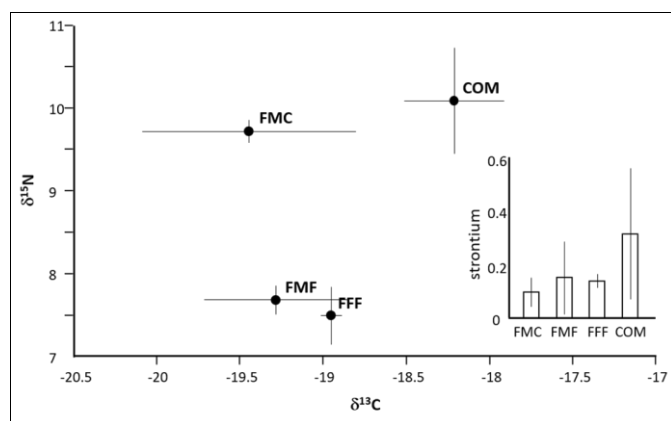
## Discussion

Blends of different proteins were able to replace the FM part of LMB feeds without untoward effects on overall growth performance during an 18-week trial. Nevertheless, fish fed the FMC, FMF and FFF diets returned 3%, 21% and 16% mortality, respectively, compared against 100% survival in animals fed diet COM. The mortality rates experienced in the FMF and FFF diets were similar to those observed by others who fed LMB diets of comparable protein and fat levels in which FM was replaced by poultry byproduct meal (PBM) [10, 11]. However, survival was lower than reported by Tidwell *et al.*, [12] who employed similar protein levels, half the amount of dietary lipid, and a wider range of alternative proteins (corn gluten, SBM, meat, blood, feather and bone meals and PBM). While the reasons for such differences in survival remain obscure, they could reflect dietary inadequacy over the longer-term, differences in ingredient quality, fish strains employed [29], the differential length of trials, or other factors. The contrasting results nonetheless suggest that adjustments to dietary formulæ, or use of specific strains of LMB, may be necessary for effective FM replacement; possibilities that are deserved of future evaluation.

Many species have been examined in FM substitution studies using PBM and SBM, either isolated or as blends [13, 14], and when used at 50% or higher levels, adverse effects have been observed for growth, appetite and FCR [15-17]. The use of PBM

and SBM to replace FM has been observed to decrease feed palatability [19] and this may explain the poorer FCR observed between diets. The lack of impact of experimental feeds on HSI differs to that reported previously for LMB fed diets with protein blends [11, 19], suggesting dietary differences may invoke changes in energy partitioning as also indicated by differences in visceral fat deposition recorded herein. Future studies should evaluate this possibility. Rationally, the elimination of FM from aquafeeds and its replacement with other proteins must not have a negative impact on product quality; either in terms of eating or processability. For example, fillets should not gape, storage qualities of fillets ought to be identical and fillet color should not fluctuate. Fillet texture too, is an important component of consumer mouth feel while inadequate firmness may result in fillet downgrading by the processing industry [20]. Alternative plant proteins increased gel strength, distance to rupture, and breaking force of cobia fillets, indicating a firmer fillet [13]. These changes may be perceived to alter quality by consumers although several studies that substituted FM with plant protein reported no sensorial impact on fillets [21, 22]. Consequently, a rudimentary taste trial was performed to examine whether sensorial differences existed between FM/FO-free fed LMB (FFF) and those on the COM diet. Of 25 participants in the sensory analysis, half stated, based on taste, texture, and aroma, preference for the FFF fed LMB,

three indicated no preference and ten preferred the fish reared with the COM commercial diet. These findings, while requiring more formal taste panel confirmation, indicate that habitual LMB consumers provide favorable outcome for the open formula diet described herein. Clearly, the trial would have benefited with the addition of fish compositional analyses. Nonetheless, the present study provides additional evidence to support the concept that feeds for LMB, and potentially other carnivorous species, can be formulated without the need for marine-derived ingredients. While the development of compounded fish-free feeds requires fine-tuning with respect to ingredient selection and formulation, such dietary manipulations will likely have minimal impact on consumer acceptance and their perceptions of fillet quality. Labeling provides buyers with transparency and trust in determining authenticity and provenance of fish and fish products and ultimately drives purchasing decisions [23]. Nevertheless, mislabeling is surprisingly widespread, often with 30% or more of tested products being incorrect [24, 25]. Previous studies have successfully used SIRMS to authenticate and discriminate between wild, cultivated and organically reared seafood [26-28], and this motivated its application here in attempts to corroborate FM/FO-free production of LMB. Results for the carbon-nitrogen isotope analyses were encouraging, permitting distinction of FM-containing experimental and commercial feeds from non-FM containing feeds.



**Fig 1:** Isotope values for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  and strontium for largemouth bass fed one of four diets. Values are means  $\pm$  95% confidence intervals.

## Conclusion

Ever-more discerning consumers are prepared to pay premium prices for commodities that express specific quality attributes. This is especially so for food, since these products enter the body and, if adulterated or contaminated, may cause harm. The increasing demand for safe, sustainably produced farmed seafood [6], therefore, should not be too surprising. To realize customer requirements, one of the biggest challenges facing the aquaculture industry is to meaningfully reduce or, better yet, eliminate FM/FO from aquafeeds. Here, we achieved this objective with LMB, although there were differences recorded in FCR, final, and percent increase in biomass, between groups. These observations were likely more reflective of differential group mortality rates which may suggest a need for fine tuning of feed ingredients. Our research also substantiated the applicability of SIRMS as a method to verify use of FM/FO-free feeds during production. Moreover, there was no detriment to product eating quality. Further gains in animal performance will undoubtedly accrue

with refinement to dietary formulations and more rigorous selection of farmed stock [29].

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