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# Performance of largemouth bass *Micropterus salmoides* (Lacépède, 1802), fed fishmeal-and fish oil-free diets

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**Abstract**
Diets were manufactured for largemouth bass (LMB) replacing fishmeal (FM) with poultry by-product meal (PBM), soybean meal (SBM), and a hydrolyzed soy meal. Experimental diets included a FM control (FMC), and three FM-free formulations containing equal amounts of PBM and SBM with fish oil (diet $F_2$), Algal meal DHA ($F_3$) or a soy protein concentrate (SPC). A commercial LMB diet was included for reference. Fish (n=20 per group) were randomly dispersed into one of 20 tanks with group weights of ±5%, and densities of 7.39±0.17 kg $m^{-3}$. Tanks were maintained as a RAS (28.3±0.76 ºC, $DO_2$ at 7.7±1.19 mg $L^{-1}$) and randomly assigned to one of the five diets (n=80 fish per diet). Animals were fed to apparent satiation 3x daily for 12-weeks. Groups were weighed every 3 weeks and feed consumption recorded for calculation of FCRs. At trial end all fish were weighed and measured individually and 3 fish per tank employed for various analyses and comparisons against pre-trial samples. At trial end no differences ($P > 0.05$) were observed between groups for growth, SGR, or condition. FCR differed between the commercial and $F_3$ diets ($P < 0.05$). $F_2$ fed fish had higher ($P < 0.05$) visceral fat than $F_3$ fed fish. Survival was 98-100% across all groups. Results indicate that judicious dietary manipulations may allow elimination of FM from LMB diets without compromising overall performance.

**Keywords:** Histology, soybean meal, poultry by-product meal, taste, quality

## 1. Introduction
Global aquaculture production of largemouth bass (LMB), was ~435,000 tons in 2018 and worth ~US$1.2 billion [1]. More than 99% of farmed LMB were produced in China [1, 2], mainly using ponds of 0.3-1 ha [3]. As with other carnivorous species, a vital facet of LMB cultivation is the need to redress the continued use of formulated feeds that incorporate fishmeal (FM) and fish oil (FO) as key components [4]. Indeed, rising costs, stagnant raw material supply, sustainability arguments, feed safety issues, increasing user competition and a host of other biological, technical and ethical issues [5], have led the aquafeed and production sectors, in general, to move away from dependency on dietary marine resources.
Reticence to use FM alternatives with LMB likely arises due to studies that report FM replacement with soybean (SBM), or other meals, depresses feed intake and growth [6-9]. Substitution of FM in LMB diets, therefore, would appear problematic. Nonetheless, experience with other, fundamentally carnivorous species, suggests that FM can be successfully exchanged using various alternative proteins [10, 11]. These, and other studies, imply that with due diligence it might be possible to completely replace FM from the diets of LMB while lessening their cost. The present research was undertaken to examine this proposition using diets varying in dietary protein content. The alternative proteins employed included poultry by-product meal, soybean protein concentrate, and a hydrolyzed SBM. Additionally, we examined the replacement of FO using an omega-3 rich algal product, thereby completely eliminating FM and FO from the diet.

## 2. Materials and methods
The feed trial described herein was undertaken at the Aquatic Research Laboratory, Prairie Aquatech, Brookings, South Dakota. Approximately 1,000 LMB, initial weight of 5-8 g per fish, were acclimated to an experimental system for 3 weeks, at a temperature of 30 ºC, prior to trialing. The system comprised 30 113 L tanks with a flow rate of 2 L $min^{-1}$, connected as part of a recirculating aquaculture system (RAS). The RAS was equipped with biological and mechanical filtration, UV sterilization, temperature control and pure oxygen supplementation.

After acclimation, fish were grouped together and the experimental tanks restocked with 20 randomly dispersed fish, with group weights of ±5%, and densities of 7.39±0.17 kg $m^3$. Each of the 20 tanks were randomly assigned to a dietary treatment as recorded in Table 1 (4 replicates per diet). 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 four experimental feeds, varying in protein and lipid levels (Table 1). The goal of this study was 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. Since protein is a relatively expensive nutrient the concept was that protein could be reduced when the amino acids are balanced. The commercial reference diet contained 48% CP, and the FMC diet was formulated to have 45% CP, and the other two diets were formulated to have 42% CP. The lower CP levels of the three experimental diets were used to lower cost of the diet while supplementing the first three limiting amino acids lysine, methionine and threonine to the balance found in fish muscle [11]. All, except one of the latter, were FM/FO-free, open formulations. Table 2 summarizes the profile of essential amino acids for each of the tested diets.

A random sample of fish (n=10) were appraised for baseline histological and physiological data at trial initiation. Experimental fish were fed to apparent satiation three-times daily and group weighed at three-week intervals Feeding rates followed those developed for commercial LMB production and feed consumption was monitored for determination of feed conversion ratio (FCR). The feeding trial lasted 12 weeks. Mortalities were recorded daily for each diet. At trial termination fish were weighed and measured individually and a sub-sample from each treatment (n=3 fish $tank^{-1}$, 12 per treatment) were euthanized with MS-222 (Tricaine S, Western Chemical Inc., Ferndale, WA., 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. Liver, spleen, gut and visceral fat were weighed for determination of various indices (*vide infra*). Performance indicators included:

$$\text{Relative growth rate} = \frac{(wt - wi)}{wi * 100}$$

where wt was final weight and wi initial weight [14]. Specific growth rate = ((ln(final weight/length) – ln(initial weight/length))/number of days. Feed conversion ratio (FCR = g fed/g gain). Somatic Indices = (tissue weight {g}/total body weight{g}) x 100. Condition factor = ($wt/L^3$) x 100.

### 2.1 Histology

Tissue samples from liver, spleen, head kidney, proximal and distal intestine were fixed in 10% buffered formalin for >24h, cut into smaller sizes, dehydrated in ethanol and cleared using ParaClear™ (Polysciences Inc., Warrington, PA, USA), and embedded in paraffin using standard methods. Sections of ~5 µm were taken and fixed to slides; 4-6 sections per sample were mounted per slide. Tissues were stained with Hematoxylin/Eosin (H&E) or, for hemosiderin (crystalline aggregates of ferritin), Gomori's modified iron stain (Prussian blue) [15]. H&E stained slides were used to determine vacuolization of liver tissue and scored as: 0 = none to a few vacuoles, 1 = low, 2 = medium, or 3 = highly vacuolized. Gomori-stained slides were used to evaluate staining intensity in melanomacrophage centers (MMC), and other splenic cells, independently from each other, being graded as 0 = low, 1 = medium, or 2 = high, by two independent observers. Analyses of these sections were used to determine impacts, if any, of the experimental diets on targeted organs. Liver lipid, glycogen and glucose were quantified using previously described methods [16, 17].

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

| Ingredients | FMC | F2 | F3 | SPC | COM |
|---|---|---|---|---|---|
| Soy protein concentrate[1] | | | | 0.1793 | |
| Algae meal[2] | | | 0.06 | 0 | |
| Hydrolyzed soy meal[3] | | 0.15 | 0.15 | 0 | |
| Corn Gluten Meal | 0.0816 | 0.0816 | 0.0816 | 0.0816 | |
| Whole Cleaned Wheat | 0.2219 | 0.254 | 0.227 | 0.2043 | |
| Poultry Meal[4] | 0.2082 | 0.2562 | 0.2562 | 0.2562 | |
| Fish Meal[5] | 0.263 | 0 | 0 | 0 | |
| Vitamin Premix | 0.005 | 0.005 | 0.005 | 0.005 | |
| Lysine | 0.0135 | 0.0197 | 0.0197 | 0.0166 | |
| Methionine | 0.0034 | 0.0064 | 0.0064 | 0.0064 | |
| Choline Chloride | 0.006 | 0.006 | 0.006 | 0.006 | |
| Mineral Premix | 0.0025 | 0.0025 | 0.0025 | 0.0025 | |
| Stay C (L-Ascorbat-2-Mono) | 0.002 | 0.002 | 0.002 | 0.002 | |
| Soy Oil (Non-GMO) | 0.031 | 0.03 | 0.027 | 0.0473 | |
| Fish Oil – Menhaden[6] | 0.03 | 0.03 | 0 | 0.03 | |
| Monocal phosphate, 21% | 0 | 0.0135 | 0.0135 | 0.0197 | |
| Taurine | 0 | 0.01 | 0.01 | 0.01 | |
| Threonine | 0.0019 | 0.0031 | 0.0031 | 0.0031 | |
| Soybean Meal -Non GMO[7] | 0.11 | 0.11 | 0.11 | 0.11 | |
| Lecithin | 0.02 | 0.02 | 0.02 | 0.02 | |
| Total | 1.000 | 1.000 | 1.000 | 1.000 | |
| Proximate composition | | | | | |
| Dry matter | 92.27 | 92.10 | 90.28 | 92.92 | 92.86 |
| Crude protein | 46.8 | 42.0 | 41.5 | 45.4 | 50.8 |
| Fat (acid hydrolysis) | 13.7 | 14.5 | 15 | 14.7 | 17.2 |
| Ash | 9.25 | 7.20 | 7.39 | 6.96 | 7.37 |

| | | | | | |
|---|---|---|---|---|---|
| Fiber (crude) | 1.03 | 1.53 | 1.17 | 1.74 | < 0.20 |
| Phosphorus (total) | 1.54 | 1.26 | 1.26 | 1.38 | 1.18 |

[1]Profine VF®, Dupont Nutrition and Biosciences, [2] AlgalPrime™, 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.

**Table 2:** Amino acid profile (g 100 $g^{-1}$) of experimental and commercial diets and estimated nutritional requirements [27].

| Diet | Arg | His | Iso | Leu | Lys | Met | Phe | Thr | Tyr | Val |
|---|---|---|---|---|---|---|---|---|---|---|
| COM | 2.63 | 1.43 | 1.52 | 3.64 | 2.79 | 0.96 | 2.14 | 1.53 | 1.29 | 2.56 |
| SPC | 2.63 | 0.78 | 1.67 | 3.35 | 3.36 | 1.42 | 1.89 | 1.38 | 1.28 | 1.92 |
| FMC | 2.50 | 0.77 | 1.58 | 3.34 | 3.27 | 1.19 | 1.80 | 1.28 | 1.27 | 1.84 |
| F2 | 2.34 | 0.71 | 1.42 | 3.09 | 3.50 | 1.48 | 1.67 | 1.17 | 1.25 | 1.65 |
| F3 | 2.20 | 0.70 | 1.38 | 2.98 | 3.31 | 1.45 | 1.65 | 1.14 | 1.16 | 1.65 |
| Requirement | 2.00[1] | 0.50 | 0.90 | 2.00 | 2.10 | 0.70[2] | 0.90 | 1.10 | 0.80 | 1.40 |

[1] Estimated dietary Arg requirement as 1.91% of diet [12].
[2] Estimated dietary Met requirement of 1.22% of diet [13].

## 3. Results

Water quality parameters throughout the trial were: $DO_2$, 7.7±1.19 mg $L^{-1}$; temperature, 28.3±0.76 °C; salinity, 3.12±0.81 mg $L^{-1}$; pH 8.41±0.09; total dissolved solids 3.76±0.91 g $L^{-1}$; $NH_3$, 0.30±0.53 mg $L^{-1}$; $NO_2$, 0.83±1.17 mg $L^{-1}$; $NO_3$, 29.68±12.38 mg $L^{-1}$; values suitable for LMB aquaculture [18]. Throughout the 12-week trial, 4 mortalities were recorded: 1 each for the SPC and COM feed and 2 for diet F3 (separate tanks).

Table 3 summarizes the weight, length and condition factor (*K*) of LMB at the start of the experiment (n=10) and the response of animals to experimental and commercial feeds (n=12/diet) for 12 weeks, including weight and length, Specific Growth Rate (SGR), and FCR data for fed animals. Depending on diet, fish weight increased between 200-230%, while length increased between 119-130%. Condition remained stable throughout the trial and no differences were detected between treatments at 12 weeks for weight, wSGR, length, lSGR or condition ($P > 0.05$; Table 3). However, FCR for the commercial feed was lower ($P < 0.05$) than the F3 diet.

Table 4 summarizes somatic indices for various tissues both at study start and 12 weeks thereafter. Relative to initial values, all treatment groups had higher somatic indices for viscera (VSI) and decreased index for liver (HSI) and spleen (SSI), but similarity for hematocrit readings (Table 4). Among treated fish, those fed the FMC and F2 diets returned highest values for all somatic indices measured, including hematocrit. F3 fed LMB expressed significantly ($P < 0.05$) lower levels of visceral fat when compared against other feed groups at week 12 (Table 4). FMC fed LMB expressed larger ($P < 0.05$) livers than LMB receiving commercial feed. F2 fed fish returned higher ($P < 0.05$) visceral fat than F3 fed animals (Table 4).

Table 5 summarizes the effect of different diets on hepatic structure and composition. Liver mass in fish fed the commercial diet was smaller ($P < 0.05$) than that measured in the FMC group. However, all other treatments returned similar values for HSI (Table 5). The degree of vacuolization recorded was similar across treatments ($P > 0.05$) but, nonetheless, relatively large vacuoles were discernible in some specimens. Hepatic glycogen, glucose and lipid values were similar across all diets ($P > 0.05$; Table 5).

**Table 3:** Twelve-week growth response and feed conversion ratios (FCR) of largemouth bass to various experimental and a commercial feed. Data within a column with a different superscript were significantly different ($P < 0.05$). For dietary formulation details see Table 1.

| | wt (g) | wSGR | L (cm) | lSGR | *K* | RG (%) | FCR |
|---|---|---|---|---|---|---|---|
| **Treatment** | | | | | | | |
| Initial | 25.28±5.42$^{a}$ | - | 129.3±8.7$^{a}$ | - | 1.15±0.05$^{a}$ | - | - |
| COM | 58.67±11.21$^{b}$ | 0.79±0.16$^{a}$ | 162.8±9.2$^{b}$ | 0.27±0.05$^{a}$ | 1.15±0.06$^{a}$ | 130.6±7.8$^{a}$ | 1.06±0.19$^{a}$ |
| SPC | 50.91±17.04$^{b}$ | 0.64±0.26$^{a}$ | 153.7±16.2$^{b}$ | 0.21±0.09$^{a}$ | 1.16±0.09$^{a}$ | 101.7±10.6$^{ab}$ | 1.28±0.15$^{a}$ |
| FMC | 56.77±13.77$^{b}$ | 0.70±0.20$^{a}$ | 167.8±9.9$^{b}$ | 0.23±0.05$^{a}$ | 1.18±0.10$^{a}$ | 100.0±8.4$^{ab}$ | 1.25±0.06$^{a}$ |
| F2 | 54.56±13.58$^{b}$ | 0.71±0.22$^{a}$ | 156.9±12.6$^{b}$ | 0.24±0.07$^{a}$ | 1.18±0.06$^{a}$ | 108.1±6.2$^{ab}$ | 1.26±0.06$^{a}$ |
| F3 | 58.08±14.57$^{b}$ | 0.77±0.23$^{a}$ | 162.5±11.4$^{b}$ | 0.27±0.06$^{a}$ | 1.14±0.15$^{a}$ | 81.2±12.2$^{b}$ | 1.71±0.23$^{b}$ |

**Table 4:** Viscera, hematocrit and hemosiderin response of largemouth bass to different experimental, and a commercial diet, fed over a period of twelve weeks. Data within a column with a different superscript were significantly different ($P<0.05$). For dietary formulation details see Table 1.

| Treatment | VSI | VFI | SSI | hematocrit | MMC hemosiderin | Non-MMC hemosiderin |
|---|---|---|---|---|---|---|
| Initial | 2.08±0.51$^{a}$ | - | 0.09±0.04$^{a}$ | 40.25±4.44$^{a}$ | - | - |
| COM | 4.50±0.89$^{b}$ | 1.74±0.66$^{a,b}$ | 0.06±0.03$^{b}$ | 37.14±4.33$^{a}$ | 1.00±0.93$^{a}$ | 1.50±0.67$^{a}$ |
| SPC | 4.55±1.12$^{b}$ | 1.62±0.60$^{a,b}$ | 0.05±0.02$^{b}$ | 36.60±4.78$^{a}$ | 0.83±0.94$^{a}$ | 0.83±0.62$^{a}$ |
| FMC | 5.62±1.44$^{b}$ | 1.95±0.85$^{a,b}$ | 0.07±0.01$^{b}$ | 39.62±4.03$^{a}$ | 1.00±0.85$^{a}$ | 0.75±0.75$^{a}$ |
| F2 | 5.20±1.08$^{b}$ | 2.31±0.51$^{a}$ | 0.06±0.02$^{b}$ | 36.48±4.23$^{a}$ | 1.00±0.95$^{a}$ | 1.25±0.97$^{a}$ |
| F3 | 4.55±1.43$^{b}$ | 1.51±0.69$^{b}$ | 0.06±0.02$^{b}$ | 36.20±3.52$^{a}$ | 1.50±0.78$^{a}$ | 1.33±0.78$^{a}$ |

**Table 5:** Impact of experimental and commercial feeds on hepatosomatic ndex (HIS), hepatic vacuolization (VAC), glycogen (mg $g^{-1}$), lipids (%) and glucose (mg $g^{-1}$). Data within a column with a different superscript were significantly different ($P < 0.05$); initial HSI was 2.39±0.47[a]. For dietary formulation details see Table 1.

| Treatment | HSI | VAC liver | Hepatic glycogen | Hepatic lipid | Hepatic glucose |
|---|---|---|---|---|---|
| **COM** | 1.37±0.51[b] | 1.58±0.90[a] | 87.3±47.7[a] | 7.77±2.55[a] | 10.8±2.67[a] |
| **SPC** | 1.49±0.62[b,c] | 1.83±0.83[a] | 108.4±48.7[a] | 7.34±3.52[a] | 10.9±3.68[a] |
| **FMC** | 2.15±0.83[a,c] | 2.33±0.65[a] | 130.6±34.9[a] | 6.02±3.04[a] | 9.0±2.73[a] |
| **F2** | 1.61±0.34[b,c] | 2.17±0.22[a] | 111.2±37.3[a] | 7.33±4.22[a] | 10.7±2.47[a] |
| **F3** | 1.52±0.69[b,c] | 2.08±0.67[a] | 107.0±33.0[a] | 9.23±7.39[a] | 10.1±3.54[a] |

## 4. Discussion

Here, we describe the complete replacement of FM in LMB diets using blended, amino acid supplemented, terrestrial-based ingredients. Over 12 weeks feeding, the open formulæ described had no negative effects on fish growth and satisfactory FCRs for four of the five diets were recorded (Table 3). The ability of carnivorous fish to utilize PBM and SBM as substitutes for FM, either alone or in combination, has been assessed in many species [4]. In some fish, higher levels of PBM (50%+), with or without supplemental essential amino acids (EAA), has a negative impact on growth, feed intake and FCR [19]. However, in LMB [20], and other farmed fish [*e.g.,* 21], replacement of FM with ~70% or more PBM, with or without supplemental EAA, has only limited, or no effect on growth when compared against FMC diets. Negative growth effects have been observed for fish fed diets in which FM was substituted with SBM. SBM is a warehouse of antinutritional factors [22] and is commonly associated with inflammation of the distal intestine, causing loss in growth potential [23]. Histological examination of the distal intestine in the present trial, however, failed to reveal morphological changes and similar findings have been made with other species [23]. LMB are thus either insensitive to the level of SBM (11%) used in the current trial or experienced a transitory enteritis only, as previously described for carp [24]. Indeed, the latter possibility appears to have some warrant since He and colleagues [9] reported morphological changes in the height and width of mucosal folds in LMB fed SBM supplemented diets for 8 weeks. Similarly, Li and associates [8], discerned histopathologies to the distal intestine of LMB fed dehulled SBM-based diets for 9½ weeks. Differences between studies may thus have occurred due to varying trial duration, SBM type, sampling point, LMB strains employed [25], or some other, as yet undefined reason.

Another indication of the potential negative effect of dietary ingredients on fish health was afforded by examination of the spleen and hematocrit values of each group. Melanomacrophage centers (MMC) have functions in both immunity and the normal physiology of fishes [26]. In the present studies, hemosiderin was employed to isolate MMC and other splenic hemosiderin-positive cells. Our findings of equal intensity of staining for hemosiderin, equivalent SSI, and comparable hematocrit levels, provide a conglomerate of indicators to suggest that the health of experimental LMB went unaffected by diet. Nonetheless, others [7, 8], have reported that PBM and dehulled SBM induce negative influence on alternate complement and lysozyme activities in LMB.

The generally poor response of fish to alternative dietary proteins, and especially plant proteins, has often been attributed to imbalances in essential amino acids (EAA). In the current trial amino acid analysis of the diets did not indicate a severe EAA deficiency and all estimated requirements were realized (Table 2) [27]. Here, FCR were like those recounted for LMB of similar size, fed diets containing blends of FM, SBM and PBM [6]. The higher FCR of the F3 fish was akin to findings made previously with LMB fed alternative protein-based diets [28]. Differences were apparent with respect to HSI between groups which suggests differences in energy partitioning, but there were no differences in hepatic glycogen, lipid, glucose, or vacuolization. Analogous values for HSI for fish of similar size have been presented previously for LMB fed diets with blended proteins (FM, PBM, SBM) [6, 28]. All fish laid down visceral fat relative to initial levels, which were undetectable. Noted differences in VFI between F2 and F3 likely reflect the absence of fish oil from the F3 diet.

The characteristics of a feed and its processability is affected by the quality of raw materials and the apparent digestibility coefficients (ADC) of amino acids from various plant protein sources in LMB experimental diets has been shown to differ [29, 30]. Like plant proteins, the digestibility of PBM protein, and availability and digestibility of amino acids is highly variable, and several factors can influence ingredient quality and composition during production. These factors underlie the need to formulate fish diets based on an available, rather than gross nutrient basis. Differences in biological availability may represent one explanation for contradictions reported for alternative protein trials. The use of blends of different proteins to replace FM in aquafeeds is thus logical, since overall nutrient requirements of differing species will be more readily met and seasonal formulations more easily designed. Sourcing of different raw materials too will guard against problems associated with product quality variability, due to masking and dilution effects. Differences between ingredient batches can lead to problems in feed palatability and fish growth prospects, but this can be partly lessened with the use of phagostimulants [31]. It is perhaps not too surprising to find that diets comprising a blend of proteins have generally met with greater success than the use of single sources.

## 5. Conclusion

From the results presented here, together with accumulating evidence from elsewhere,[*e.g.,* 6,28] it is clear that when used as replacements of large parts of dietary protein, SPC, PBM, and hydrolyzed soy are suitable as cheaper, sustainable alternative proteins for LMB and other aquafeeds. The least costly experimental feed employed in the current study was ~25% less than commercial FM-based feeds of comparable protein content. Future studies should evaluate other protein blends and supplemental amino acids in efforts to further enhance fish performance on such aquafeeds.

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