# Enhancing Soybean Meal Utilization in Aquaculture Feeds: An Assessment of Nutritive Value and Optimization of Soybean Meal-Based Diets for Largemouth Bass

**FINAL REPORT**

June 25, 2018

Waldemar Rossi Jr., Ph.D.

James Tidwell, Ph.D.

Habte-Michael Habte-Tsion, Ph.D.

Aquaculture Research Center

Kentucky State University

Frankfort, KY 40601

## SUMMARY OF RESEARCH ACTIVITIES IN YEAR II

The objective two of the proposed research project has been completed. Left from the previous report were the chemical analyses of experimental diets used in the study, which are now included in this final report.

**OBJECTIVE 2:** Evaluate commercially available feed additives for increased phosphorus availability of SBM-based diets and largemouth bass (LMB) tolerance to dietary SBM.

**Objective 2.1:** Evaluate the effect of phytase supplementation on the phosphorus bioavailability of selected SBM (s) to LMB relative to calcium phosphate dibasic (Dical-P).

***Status:*** Concluded (May 2018) and fully reported.

## MATERIALS AND METHODS

## OBJECTIVE 2.1:

### Experimental fish

Feed-trained largemouth bass fingerlings were purchased from a commercial producer (F&L Anderson Hauling Inc., Lonoke, AR) and transported to the Aquatic Animal Nutrition Laboratory (AANL) of Kentucky State University, Frankfort, KY. Upon arrival at the facility, the fish were stocked into a 1500-L rectangular fiberglass tank operating as a recirculating system. In this system, fish were fed thrice daily to apparent satiation with a commercial diet containing 45% crude protein (CP) and 12% crude fat (AQUAXCEL®, Cargill® Inc. Minneapolis, MN, USA) until adequate size for the feeding trial was attained.

### Experimental Diets

Following a completely randomized experimental design, seven experimental diets (Table 1) were designed to evaluate the effects of supplemental microbial phytase (MP) enzyme on the production performance, health indicators, and expression of target genes in largemouth bass. All diets were formulated to contain 42% crude protein, 12% lipid and an estimated 3.6 Mcal of digestible energy (DE)/kg. A negative control diet (- Control) was formulated to contain 0.41% available phosphorus (P) and was deemed deficient in P. Four additional test diets were formulated identically to - Control except for the gradual supplementation of MP to obtain final levels of phytase ranging from 250 to 2000 units/kg. An additional diet was formulated identically to – Control and was supplemented with inorganic phosphorus (as calcium phosphate monobasic) to meet or exceed largemouth bass requirement for P (based on previous formulations in this laboratory) and was used as the positive control (+ Control). Finally, a test diet was formulated identically to the + Control and was supplemented with MP to obtain a phytase level of 1000 units/kg. This diet was designed to access any potential effects of phytase enzyme supplementation on fish performance that would not be attributed to effects on available P. In order to produce diets deficient in P while providing all other essential minerals, a new mineral premix was formulated in our laboratory containing only trace amounts of inorganic P.

The experimental diets were manufactured in the AANL. The ingredients were weighed using a digital scale and mixed for a total 30 min in a Hobart A200T 20-quart mixer (Hobart Corp., Troy OH). The oil (s) were added into the mixture at 20 minutes. Water and/or phytase (in liquid form) was gradually added to the mixture after 30 min while mixing until appropriate consistency for pelleting was attained. Each diet was then screw-pressed through a 3.2 mm die plate using a commercial food grinder (Hobart 4732A; Hobart Corp., Troy, OH). After dried to <10% moisture at room temperature and under forced air, diet strands were broken into adequate-size pellets, sieved to remove fines, and then kept under – 20 °C until fed.

Only small variations in protein and lipid content of the experimental diets was observed showing that diets were closely isonitrogenous and isolipidic (Table 2). Total phosphorus (P) in non-supplemented diets was near 1%, while in the positive control diets (+ Control, + Control + 1000 FTU) the element was found at a concentration of around 1.5%. Phytate-P was near 0.6%, resulting in a non-phytate P content of around 0.4% across diets. The amino acid composition of the – Control diet was found to be adequate and within target levels (Table 3).

**Experimental Conditions**

The feeding trial was carried out in the AANL using 28, 110-L aquaria operating as a recirculating system. Groups of 20 LMB juveniles (8.5 ± 0.3 g/fish) were stocked into each aquarium. Fish in each of four aquaria were fed with one of the randomly assigned test diets twice daily for 12 weeks.

All aquaria were supplied with continuous aeration through ceramic diffusers connected to a central regenerative air blower. Water quality was monitored routinely during the feeding trial following standard procedures and maintained within adequate ranges for LMB. Temperature, pH, salinity and dissolved oxygen will be monitored daily using a YSI-Pro Plus multiparameter instrument (Yellow Springs Incorporated, Yellow Springs, OH), while total ammonia nitrogen (TAN) and nitrite nitrogen were tested weekly using a spectrophotometer (HACH DR 2800, HACH, Loveland, CO, USA).

**Data Acquisition and Statistical Analysis**

At the commencement of the feeding trial, an initial sample of the stocked fish was obtained and stored at -20 °C pending composition analyses of initial fish. At both mid-way and conclusion of the feeding trial, fish in each aquarium were group-weighed and counted for the calculation of production performance parameters.

After twelve weeks of feeding, the experiment was ended. Fish from each tank were group weighed and counted for computing production performance parameters as follow:

- Weight gain, % = [(Final weight – initial weight)/(initial weight)] × 100;
- Feed efficiency ratio (FE) = [weight gain (g)/ dry feed consumed (g)];
- Thermal growth coefficient (TGC): [(final fish weight$^{1/3}$- initial fish weight $^{1/3}$) ÷ (# days on feed × culture water temperature (°C))] × 1000.

Resulting data from the feeding trial was validated for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively, then subjected to regression and one-way ANOVA analyses. For the latter, when significant differences ($P<0.05$) were detected, Duncan's multiple range test was used for the separation of treatment means. All statistical analyses were carried out using the SAS® software package (SAS Institute Inc., Cary, NC USA).

## Results

Largemouth bass accepted and fed readily on all experimental diets and the supplementation of microbial phytase up to 2000 phytase units (FTU)/kg did not affected the palatability of the diets. Survival was above 90% in all dietary treatments and no significant differences were found (Tables 4 and 5). As the level of phytase increased in the diets, a reduction in final weight and weight gain following a second order polynomial regression was observed ($P < 0.05$). Similar responses of largemouth bass to supplemental phytase was observed for thermal growth coefficient and feed efficiency (Fig. 1 and 2).

Regarding the Control treatments (Table 5), the lack of supplemental phytase in the phosphorus deficient diet (- Control) did not affect the growth nor the survival of the fish when compared to the phosphorus supplemented diets (+ Control and + Control 1000 FTU) ($P > 0.05$). The supplementation of 1000 FTU to the + Control in + Control 1000FTU treatment led to a significant reduction in feed efficiency.

## Brief Discussion

Phytase supplementation in aquaculture feeds has been shown to improve the availability of phytate phosphorus by increasing phosphorus digestibility, bone ash and overall phosphorus retention in the body (Gatlin and Li, 2008). However, the supplementation of phytase has been found to reduce protein digestibility in fish (Sajjadi and Carter 2004), which may affect growth performance. Although a reduction in growth performance and feed efficiency of largemouth bass in this study was unexpected, it is possible that the supplemental phytase negatively affected the digestibility of other dietary components despite any potential improvements in phosphorus digestion and retention. In addition, although the levels included in the diets were within those evaluated in other studies with fish and crustaceans (NRC 2011), it is possible that the levels evaluated were excessive for largemouth bass. Therefore, further studies to accurately define the potential of phytase supplementation in soybean-based diets for this species are warranted.

Table 1. Composition of the experimental diets.

| **Ingredients** | **- Control** | **250 FTU/kg** | **500 FTU/kg** | **1000 FTU/kg** | **2000 FTU/kg** | **+ Control** | **+ Control + 1000 FTU** |
|---|---|---|---|---|---|---|---|
| | | | | g/100 of dry matter | | | |
| Fish meal | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Poultry by-product meal | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Soybean meal | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 |
| Soy protein concentrate | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 |
| Corn protein concentrate | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 |
| Wheat gluten | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 | 3.40 |
| Wheat flour | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Dextrinized corn starch | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Others | energy, amino acid, mineral, vitamins and additives to complete formulation. | | | | | | |
| Phytase (mg, mixed) | 0.00 | 2.5 | 5.0 | 10.0 | 20.0 | 0.00 | 10.00 |

Table 2. Chemical composition of the experimental diets.

| Ingredients | - Control | 250 FTU/kg | 500 FTU/kg | 1000 FTU/kg | 2000 FTU/kg | + Control | + Control + 1000 FTU |
|---|---|---|---|---|---|---|---|
| | | | | g/100 of dry matter | | | |
| Protein | 40.73 | 40.47 | 39.52 | 37.90 | 37.79 | 40.95 | 37.94 |
| Lipid | 12.55 | 12.21 | 12.16 | 11.21 | 11.15 | 12.38 | 11.24 |
| Crude Fiber | 3.44 | 3.76 | 2.94 | 2.84 | 3.34 | 2.59 | 2.40 |
| Ash | 10.38 | 10.09 | 9.96 | 9.55 | 9.46 | 11.03 | 10.23 |
| Calcium | 2.61 | 2.48 | 2.40 | 2.46 | 2.30 | 2.81 | 2.39 |
| Phosphorus (P) | 1.00 | 0.93 | 0.64 | 0.97 | 0.90 | 1.52 | 1.40 |
| Phytate P | 0.60 | 0.55 | 0.53 | 0.57 | 0.56 | 0.58 | 0.62 |

Table 3. Amino acid composition of the negative control diet.

| Amino acid | g/100g of dry matter |
|---|---|
| Taurine | 0.16 |
| Hydroxyproline | 0.48 |
| Aspartic Acid | 3.39 |
| Threonine | 1.39 |
| Serine | 1.70 |
| Glutamic Acid | 7.14 |
| Proline | 2.71 |
| Lanthionine | 0.02 |
| Glycine | 3.24 |
| Alanine | 1.97 |
| Cysteine | 0.62 |
| Valine | 1.91 |
| Methionine | 0.90 |
| Isoleucine | 1.70 |
| Leucine | 3.04 |
| Tyrosine | 1.23 |
| Phenylalanine | 1.86 |
| Hydroxylysine | 0.08 |
| Ornithine | 0.03 |
| Lysine | 2.26 |
| Histidine | 0.88 |
| Arginine | 2.43 |
| Tryptophan | 0.34 |

Table 4. Production performance of largemouth bass fed gradual levels of supplemental phytase.

| Treatment FTU/Kg | IW (g) | FW (g) | WG % of initial | Survival |
|---|---|---|---|---|
| 0 | 9.6 ± 0.03 | 47.4 ± 3.0 | 380 ± 35 | 97 ± 2 |
| 250 | 9.5 ± 0.6 | 47.4 ± 2.8 | 327 ± 37 | 97 ± 3 |
| 500 | 9.4 ± 0.2 | 39.8 ± 1.3 | 316 ± 15 | 98 ± 3 |
| 1000 | 9.5 ± 0.03 | 35.9 ± 2.4 | 251 ± 20 | 93 ± 3 |
| 2000 | 9.5 ± 0.6 | 36.4 ± 3.2 | 275 ± 13 | 98 ± 3 |
| Regression | | | | |
| Pr > F | 0.729 | 0.003 | 0.006 | 0.451 |
| Model | NC | SOP | SOP | NC |
| $R^2$ | 0.11 | 0.63 | 0.57 | 0.12 |

FTU = phytase units; IW = initial weight; FW = final weight; WG= weight gain; SOP = second order polynomial; NC = no correlation.

Table 5. Production performance of largemouth bass fed the control treatments.

| Treatment | IW (g) | FW (g) | WG % of initial | TGC | FE | Survival |
|---|---|---|---|---|---|---|
| - Control | 9.6 ± 0.03 | 47.4 ± 3.0 | 380 ± 35 | 2.5 ± 0.2 | 0.6 ± 0.03[ab] | 97 ± 1.7 |
| + Control | 9.5 ± 0.03 | 48.8 ± 1.7 | 394 ± 18 | 2.6 ± 0.1 | 0.7 ± 0.02[a] | 97 ± 3.3 |
| + Control 1000 FTU | 9.6 ± 0.03 | 43.6 ± 1.2 | 327 ± 19 | 2.3 ± 0.1 | 0.6 ± 0.02[b] | 93 ± 1.7 |
| ANOVA (Pr > F) | 0.729 | 0.283 | 0.223 | 0.234 | 0.027 | 0.548 |

FTU = phytase units; IW = initial weight; FW = final weight; WG = weight gain; TGC = thermal unit growth coefficient; FE = feed efficiency. Means within the same column with different superscripts differ significantly (P<0.05).

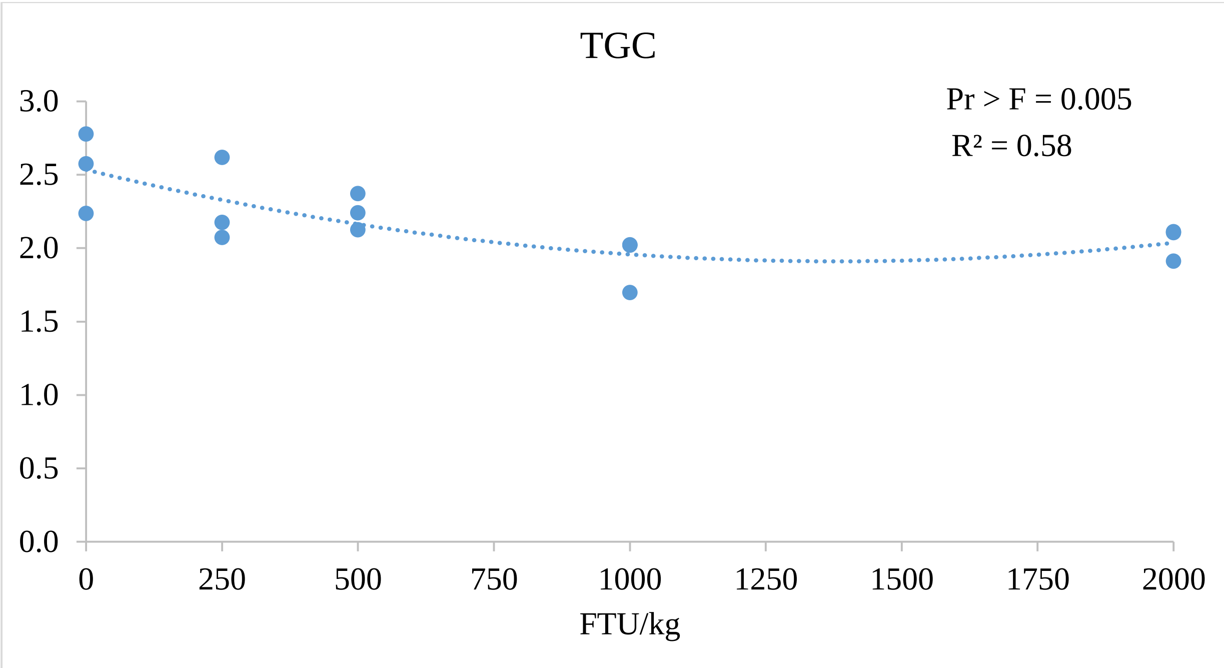


Figure 1. Thermal growth coefficient (TGC) of largemouth bass after twelve weeks of feeding diets supplemented with phytase.

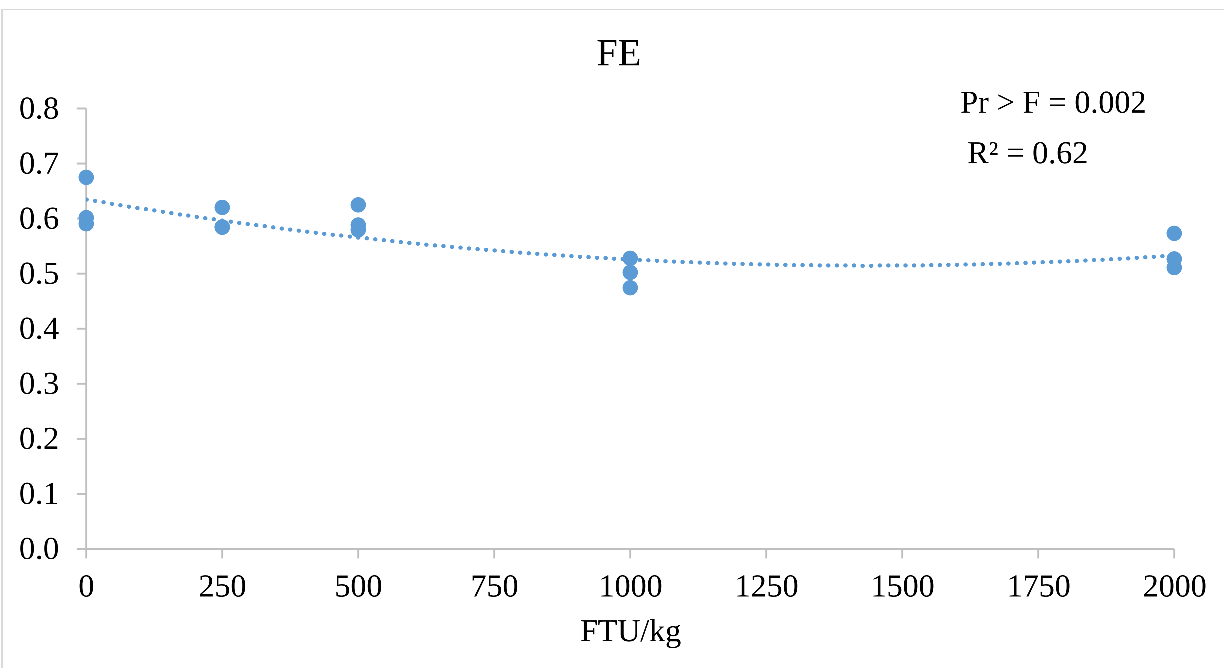


Figure 2. Feed efficiency (FE) of largemouth bass after twelve weeks of feeding diets supplemented with phytase.

**References**

Gatlin DM and Li P (2008). Use of diet additives to improve nutritional value of alternative protein sources. Pp, 501-522 in Alternative Protein Sources in Aquaculture Diets, C. Lim, C.D. Webster, and C-S. Lee, eds. New York: Haworth Press.

NRC (2011). Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, D.C., USA.

Sajjadi M and Carter CG (2004). Dietary phytase supplementation and the utilization of phosphorus by Atlantic salmon (Salmo salar L.) fed canola-meal-based diet. Aquaculture 240: 417-431.