

Purpose of study

Investigate Formimino-Glutamate (FIGLU) as a Potential Metabolic Marker of Nutritional Stress

NMR Metabolomics Investigation of FIGLU as a Biomarker of Nutritional Stress in Red Drum (Sciaenops Ocellatus) Fed Soy-Based Diets

South Carolina Department of Natural Resources, Dr. Fabio Casu, Dr. Aaron Watson, Justin Yost, Dr. T. Gibson Gaylord (USFWS), Dr. Daniel W. Bearden (NIST ret.) and Dr. Michael R. Denson

Purpose / Goals

The purpose of this study is to determine if a metabolite, FIGLU, is a marker of nutritional stress in fish diets. The overall goal is to improve the metabolic fingerprint of juvenile red drum based on comparison with the two best performing reference diets.

Study Pesign

A 12-week feeding trial on young red drum was conducted to investigate the metabolite

formimino-glutamate (FIGLU) as a potential metabolic marker of nutritional stress in fish fed diets of 60% or more soybean meal (SBM) as the protein source in fishmeal-free feeds.

Prior to the 12-week trial, a 7-week "pre-trial" was held in which fish were fed a complete fishmeal control diet.

Each fish, averaging 61.3 grams, was sorted into 24 experimental aquaculture system tanks with a temperature of 25° C at the Hollings Marine Laboratory, Charleston, SC. Each of the 24 tanks had 25 fish.



During the course of the 12-week study a total of eight different diets were evaluated:

- **1** ► Fishmeal control
- 2 Supplemented soybean meal (containing 2x methionine, but also supplemented with lysine, threonine and 0.5% (w/w) taurine
- **3** ► Unsupplemented soybean meal
- **4** ► Folate, 5x general folate requirements
- 5 Methionine, 2x general methionine requirements
- **6** Vitamin B12, 5x general vitamin B12 requirements
- 7 ► 5x B12, 5x folate & 2x methionine combination
- 8 Natural reference diet (cut squid, shrimp, fish)

Throughout the 12-week study, liver, intestine, heart and muscle tissue samples were taken, as well as plasma. Samples were collected pre-trial, at week zero, week six and at week twelve. While several tissues were sampled, nuclear magnetic resonance (NMR)-based metabolomic analysis focused on the liver tissue samples.

Results

- Over the course of a 12-week feed trial, experimental 60% SBM diets performed as well as, or better than, a fish meal control diet
- All diets feed conversion ratios are 1.0 or below, proving that all the diets performed well
- No significant differences detected in performance recorded among the soy-based diets, despite supplementation (See Table 1)
- Natural reference diet outperformed all experimental feeds
- FIGLU was not detected by NMR in fish fed natural diet
- FIGLU was detected in all soy-based diets and in the liver of fish fed the fishmealbased experimental diet which contained 41% of wheat flour
- FIGLU is not only a marker of soybean meal consumption, but appears in other diets as well

Table 1: Performance Metrics

Diet	Specific Growth Rate (SGR)	Weight Gain (% initial)	Feed Consumed (g/fish)	Feed Conversion Rate (FCR)
Diet #1	1.3 ± 0.08	201.04 ± 20.01	183.51 ± 23.82	0.94 ± 0.02
Diet #2	1.7 ± 0.07	327.71 ± 26.57	249.20 ± 1.92	0.77 ± 0.04
Diet #3	1.3 ± 0.12	203.37 ± 29.50	205.27 ± 20.14	1.00 ± 0.08
Diet #4	1.3 ± 0.16	197.85 ± 41.55	181.88 ± 25.59	0.93 ± 0.10
Diet #5	1.3 ± 0.04	207.91 ± 10.00	194.18 ± 6.31	0.96 ± 0.05
Diet #6	1.3 ± 0.15	205.73 ± 38.29	187.81 ± 11.73	0.94 ± 0.12
Diet #7	1.3 ± 0.07	204.92 ± 17.01	199.86 ± 14.41	0.99 ± 0.02
Natural Diet	2.2 ± 0.08	565.67 ± 33.80	1364.01 ± 44.77	2.43 ± 0.06
P (significant if <0.05)	0.0023	0.0006	0.0038	0.0243



Next Steps

- Overall, over the course of 12-weeks, lower FIGLU levels were found in the best performing diets (natural diet and 60% SBM diet #2), supporting hypothesis that higher FIGLU levels indicate a nutritional deficiency
- If proposed supplementations prove to significantly reduce FIGLU levels to improve the metabolic fingerprint, new supplementation protocols can be developed and adopted by fish nutritionists to produce alternative feeds
- Investigate this biomarker in other fish species to determine its general applicability

key takeaway

- Fish fed supplemented soybean meal diets of 60% had nearly the same growth, weight and feed conversion of fish fed natural reference (squid, shrimp and fish) diets
- Assessing this biological marker will allow nutritionists to develop feed alternatives within acceptable limits for fish species without causing nutritional stress

NMR Metabolomics Investigation of FIGLU as a Biomarker of Nutritional Stress in Red Drum (Sciaenops Ocellatus) Fed Soy-Based Diets, South Carolina Department of Natural Resources, Dr. Fabio Casu, Dr. Aaron Watson, Justin Yost, Dr. T. Gibson Gaylord (USFWS), Dr. Daniel W. Bearden (NIST ret.) and Dr. Michael R. Denson

