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Title of Project: REPLACEMENT OF FISH MEAL IN HYBRID STRIPED BASS DIETS WITH PROTEINACEOUS FERMENTATION BIOMASS

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Abstract: The objective of this study was to evaluate the potential use of ethanol yeast (EY), a co-product of bio-ethanol production, as a fish meal (FM) replacement in the diets of sunshine bass (white bass *Morone chrysops* x striped bass *M. saxatilis*). Fish meal is a finite resource, which has become economically and environmentally unsustainable for use as a primary protein source in aquafeeds. Ethanol yeast is becoming more available due to the increase in bio-ethanol production in the United States. Previous work has established that EY may replace some fraction of FM in practical sunshine bass diets. Complete replacement of FM with EY however, resulted in reduced palatability and ultimately reduced weight gain. Further study was conducted to firmly establish a suitable range of the amount of EY that can be used in a sunshine bass diet, as well as ensure that physiological robustness of sunshine bass was not negatively affected. A long term feeding trial concluded that FM may be reduced to as low as 7.5% to 15% of the total diet in an EY based feeds with negatively effecting production performance. However, fish did not readily consume FM-free, EY-based feeds; the addition of a palatant did not correct this effect. Although blood glucose and osmolality were not affected by dietary treatment further study will be needed to investigate the affects of EY on plasma cortisol and lysozyme, which exhibited some unexpected effects perhaps indicative of chronic stress.

Introduction: The demand for seafood, both marine and freshwater, is on the rise in the United States. While much of the seafood supply is still captured in the wild, aquaculture is contributing to a larger portion of the supply each year. In the United States, seafood demand is primarily focused on high value, carnivorous species. Accordingly, U.S. aquaculture emphasizes intensive culture of high value, carnivorous

species. An example of one such fish is hybrid striped bass, which are widely cultured as sportfish and foodfish (sunshine bass cross used as foodfish), particularly in Indiana and Illinois. Both states have undertaken extensive stocking programs of HSB as a sportfish because of their popularity with fishermen. In Illinois, sunshine bass have been the #1 cultured foodfish for the past 5 years (data from Illinois Aquaculture TechSERV records).

The caveat to intensive culture of carnivorous species, like HSB, is their need for nutrient-dense, protein-rich feeds. Protein is the most costly fraction of any aquafeed. Currently, the leading source of protein for aquafeeds used for culturing carnivorous fishes is fish meal (FM). Most FM is derived from small oceanic fishes like menhaden, mackerel, herring, etc. However, increasing demand and rising costs of FM, coupled with static landings of reduction fisheries have made continued use of FM in aquafeeds environmentally and economically unsustainable. While the goal of aquaculture is to reduce fishing pressure on wild fisheries by provide alternative sources of high quality seafood, by focusing on carnivorous species and using FM, aquaculture can indirectly increase fishing pressure on reduction fisheries. Alternative proteins in the form of animal and plant-derived proteins have been investigated in the past with variable success. However, observed limitations to these alternatives have led nutritionists to investigate new alternatives to FM. New alternatives must not only be suitable for proper fish nutrition but must also be readily available, sustainable and cost effective.

One potential new alternative to FM is yeast-based proteinaceous fermentation biomass (PFB). PFB is a co-product of bioethanol production. Yeast, used in the fermentation of corn, is collected along with unused corn protein and dried creating the aforementioned PFB. PFB is an excellent candidate for FM replacement due to its high protein content and increasing availability. Currently, there is no high-value use for PFB. Although it is not wasted, per se—generally, it is collected and incorporated with other low-value feedstuffs generated in corn processing—demonstrating a unique and high-value (comparable to fish meal) use for PFB would justify separate marketing of this potentially large-volume coproduct. This would benefit the biofuels industry as well as the aquaculture industry—by linking these industries through the use of a byproduct of one industry as a resource for another, the sustainability of both can be increased.

A previous study at SIUC (Gause and Trushenski 2010) has shown that PFB has potential as a partial FM replacement. HSB fed a feed in which PFB replaced 75% of dietary FM performed as well as HSB fed a FM-based feed (control). In fact, HSB reared on a 50% PFB feed performed better than those on the control feed. However, complete replacement of FM with PFB was not successful. Based on daily intake, specific growth rate, feed conversion ratio and visual observation of feeding behavior, it is believed that palatability was the primary issue preventing complete FM replacement with PFB. We believe the addition of a palatability/flavor enhancer like Finnstim, a

commercially made palatant/attractant commonly used in aquafeeds, will allow for 100% replacement of FM with PFB. Digestibility and utilization of PFB feeds appears to be quite good—if the fish can be encouraged to readily consume the PFB-based feeds, their growth performance may be equal to that of fish reared on an FM-based feed. The objective of this study, therefore, will be to determine if full replacement of FM with PFB is possible with the addition of palatability enhancers.

Narrative Report: Sunshine bass were reared on EY based diets containing decreasing levels of FM with and without a palatant for nine weeks (15%, 7.5% and 0% FM). After nine weeks of culture surviving fish were counted and group weighed to determine production performance including survival, weight gain (%), feed conversion ratio, feed intake, and specific growth rate. Whole body composition including moisture, crude protein, crude lipid, and ash was also evaluated. A stress challenge consisting of a two minute net chase was conducted to determine effects of diet on stress physiology specifically hematological variables including plasma glucose, osmolality, cortisol and lysozyme activity. Blood samples were collected pre-challenge to establish control levels and one hour post-stressor exposure to determine the influence of stress on these parameters.

Production results for the 15% and 7.5% FM treatments followed similar trends observed in the short term screening trial already conducted. Though results were not significant survival, weight gain, feed intake, feed conversion and specific growth rate are marginally better in sunshine bass fed the 15% FM treatment. The most significant difference between these two treatments was observed in whole body composition. The 7.5% FM treatment resulted in significantly lower levels of crude protein and significantly higher levels of crude lipid within the body when compared to the 15% FM treatment. This indicates that despite weight gain being similar fish in the 7.5% FM treatment were gaining weight via fat deposition rather than through lean muscle growth as would be ideal in food fish. This difference in whole body composition is most likely due to some level of amino acid imbalance that, while adequate for overall growth, is not adequate for lean muscle accumulation.

Complete replacement of FM with EY (0% FM treatment) proved to be problematic as it was in the screening trial. Sunshine bass often ate little or completely ignored this feed resulting in reduced weight gain and specific growth and a higher feed conversion ratio. Survival in this treatment was also significantly lower during the long term trial, a result not observed during the shorter screening trial. During this trial a few fish in each tank were able to take to the feed until they had grown larger than other fish that were not eating. These larger fish then resorted to cannibalism rather than continue consuming feed. Meanwhile, other smaller fish that were not consuming feed were eaten, died due to starvation, or remained very small. This resulted in a wide size range of fish observed in this treatment. It is likely that had cannibalism not been an issue many of the fish would have remained small resulting in more pronounced, significant differences in performance (lower weight gain, growth rate and higher feed conversion ratio). Additionally those fish that did survive had a less than ideal whole body composition

exhibiting significantly higher crude lipid and lower protein than both the 15% and 7.5% FM treatment.

The addition of a palatant did not affect overall intake or consumption. Studies with other yeast based products suggest that the yeast itself acts as an attractant/palatant, and that any additional palatant may have no effect. Much like in the first trial fish fed the 0% FM treatment consumed very little or completely ignored feed despite the addition of the palatant. Again, it has been suggested that beyond levels of 50% of the total diet, as is the case of the 0% FM treatment, yeast products simply become unpalatable to carnivorous species like the sunshine bass, and there may be little that can be done to correct this. However, there are a variety of attractants/palatants and methods of adding them to feed that would still need to be studied to be certain that intake could not be increased.

The stress challenge resulted in the expected results for glucose and osmolality. Glucose and osmolality both increased after the stressor but the increase was not related to dietary treatment. The release of glucose is a secondary stress response as the body needs extra energy for the “fight or flight” response. This influx of glucose and other solutes results in the increase in osmolality. Had osmolality decreased, this would have meant that there was an osmoregulatory failure within the fish. Cortisol was unexpectedly high in unstressed fish. Yeast products have been shown to increase the innate immune system within fish. The addition of EY to the diets may be resulting in an immunostimulation causing a chronic, low level stress within the fish and constant production of cortisol. Lysozyme activity, related to immune function, followed expected trends of decreasing after stress as immune function is typically reduced in priority during acute stress events. However, lysozyme may have already been at increased levels due to the presence of the yeast in the diet. In order to better understand the potential effects of EY on sunshine bass physiology and immune function, further study will be needed comparing EY based feeds to feeds with no EY.

The results of this study are nonetheless promising. The production performance and whole body composition results suggest that in EY based feeds, FM may be reduced to between 7.5% and 15%. This reduction in FM use could result in decreased costs for aquaculture, while increasing profits and efficiency. Additionally, the increased use of EY may result in increased profits and efficiency for the bio-fuels and support industries.

Potential Applications and Benefits: The use of EY in aquafeeds may help to reduce reliance on FM which is both economically and environmentally unsuitable for continued high level use. Using a product like EY can reduce feed costs for both food-fish producers as well as those that produce fish for sport-fish stocking and restoration. Feed costs one of the most limiting factors in many aquaculture operations. High feed costs have resulted in the contraction of both the catfish industry and the sunshine bass industry. A reduction in feed costs means the potential for greater output and higher profit margins. The growth of the bio-fuels industry means that EY may become increasingly available. Ethanol yeast, in combination with the various other FM alternatives, may allow for aquafeed costs to be maintained or reduced while

maintaining production performance of fishes. Furthermore, reducing the need for FM will result in the need for reduced input from marine capture fisheries that supply FM. This will undoubtedly benefit the aquaculture industry, as well as reduce our impact on the fragile marine food web.

Keywods: Fish meal replacement, bio-fuels, yeast, sunshine bass, *Morone spp.*

Lay Summary: With the world population growing and more people eating seafood, aquaculture production will have to increase to meet the growing food demands of the world. Creating low cost, efficient feeds may be the first step in creating sustainable aquaculture to feed the world. Decreasing the use of fish meal, which is both economically and environmentally unsustainable, in aquaculture feeds may result in higher profits and efficiency for aquaculture producers. Likewise the decreased use of fish meal will reduce our impact on fragile marine food webs. A number of alternatives to fish meal exist but more must be developed to continue to produce lost cost, high quality feeds. Ethanol yeast, a co-product of the growing bio-ethanol industry, may be on such alternative. Sunshine bass, a popular food fish raised in the United States, may be raised on diets containing reduced levels of fish meal with ethanol yeast used to replace the fish meal protein. These fish exhibited similar or better growth than fish raised on a feed without ethanol yeast. A fish raised on an ethanol yeast based diet containing as little as 15% fish meal produces a fish with similar lean muscle mass to a fish fed no ethanol yeast. This means that a fish producer may grow a fish using a less expensive feed, while still ensuring acceptable growth and survival of the fish.

International Implications: Much of the increase in aquaculture is taking place in Asia and other countries. Likewise, bio-fuels production is increasing around the world. A product like ethanol yeast may prove beneficial in feeds for not only the species raised in the United States but those in other countries. A cheaper to produce feeds means more efficient aquaculture and an increase in production resulting in more food for the world population.

Media Coverage: NA

Partnerships with other institutions/individuals initiated or continued by your project: This work was conducted as part of an ongoing research collaboration with Archer Daniels Midland Company, the supplier of ethanol yeast.

Publications: See attached publication draft that has been accepted for publication in the North American Journal of Aquaculture

Presentations: This data was presented at numerous professional conferences and workshops including:

Gause, B. and J. Trushenski. 2010. Replacement of fish meal in sunshine bass (*Morone chrysops* ♀ *x Morone saxatilis* ♂) diets with ethanol yeast. Cool Water Fish Culture Workshop. Rend Lake, IL

Gause, B. and J. Trushenski. 2010. Replacement of fish meal in sunshine bass (*Morone chrysops* ♀ *x Morone saxatilis* ♂) diets with ethanol yeast. Aqua 2010. San Diego, CA.

Gause, B. and J. Trushenski. 2010. Replacement of Fish Meal with Ethanol Yeast in the Diets of Sunshine Bass *Morone chrysops* x *M. saxatilis*. American Fisheries Society National Conference. Pittsburgh, PA.

Undergraduate/Graduate Names and degree: Brian Gause, M.S., 2010.

Related Projects: NA

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