Project Title: Asian Carp Muscle as an Initial Dietary Protein Source and Palatability Enhancer for Successful Production of Yellow Perch and Walleye Fingerlings [Progress Report]

Total Funds Committed: $198,614
Initial Project Schedule: September 1. 2021-August 31, 2022 [Extended to August 31, 2024]
Current Project Year: September 1, 2022-August 31, 2023
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Industry Liaison: Clarence Bischoff, CEO, Blue Water Farms

Project Objectives

1. To develop the optimal in vitro methodology for Asian carp muscle digestion using digestive enzymes obtained from adult yellow perch Perca flavescens and walleye Sander vitreus that can be used as a protein source and attractant in dietary formulations for larval and juvenile yellow perch and walleye.
2. To evaluate the effect of Asian carp muscle protein hydrolysate obtained using methodology in Objective 1 as a protein source in diets for yellow perch and walleye when used as first feed.
3. To evaluate the effect of Asian carp muscle protein hydrolysate obtained using methodology in Objective 1 as an additive/palatability enhancer in diets for yellow perch and walleye on successful weaning to formulated feeds.
4. To evaluate the effect of Asian carp muscle protein hydrolysate combined with soybean meal hydrolysate, both obtained using methodology in Objective 1, as additives in diets for yellow perch and walleye for successful weaning to formulated feeds and easier transition to plant-based feeds.
5. To provide the aquaculture community within the North Central Region (NCR) with guidelines on successful larval rearing protocols for both yellow perch and walleye in indoor systems.
6. To provide the feed/additive manufacturing industry with the knowledge and the tools required for production of high-quality well-digested dietary protein hydrolysate as a cost-effective source of protein and attractant for young fish feeds.

Project Summary

Limited knowledge of larval/juvenile nutritional requirements, the reliance on live food, poor weaning success to formulated diets, and inefficient utilization of soybean meal-based feeds have all limited expansion of Percid fingerling production. We propose an innovative dietary protein source and dietary attractant that will precisely match Percid larvae and juvenile requirements and induce high feed intake and positive growth responses when used as first feed and/or during weaning. This innovative dietary protein source will provide more control in production of Percid fingerlings by increasing dry diet acceptance and exposure to plant-based formulation at the earliest possible age. This innovative dietary ingredient and knowledge derived from the study will provide the aquaculture industry, particularly in the NCR, with the new approach for the development of high-quality starter feeds that will support sustainable expansion of the hatchery sector and consequently contribute to the development of competitive aquaculture market within the NCR.

Anticipated Benefits
We expect that the novel dietary ingredient originating from Asian carp muscle digested using YP and W digestive enzymes, characterized by the optimal molecular size of the protein fraction, will induce positive growth responses in YP and W larvae and juveniles, respectively. We also expect that Asian carp muscle hydrolysate combined with SBM hydrolysate both obtained using YP and W digestive enzymes will allow for successful weaning of the fish to formulated feeds without jeopardizing fish growth and survival. The Asian carp hydrolysate will likely support high feed intake and at the same time the exposure to pre-digested SBM will help adapt the fish to dietary plant protein earlier.

Project Progress
To date, objectives one and two have been undertaken. Approximately 400 Asian carp were repurposed from SIUC fisheries studies within the Mississippi River Basin into ground fishmeal and subsequently broken down (hydrolyzed) using adult Yellow Perch stomach and intestinal enzymes. The hydrolysis process that has been developed for objective 1 will be applied in exactly the same way to SBM to meet objectives 3 and 4, however the inclusion rates of hydrolyzed Asian carp-meal and/or SBM in the coming study will vary, as the hydrolysate is serving the purpose of an attractant and additive to enhance transition to plant-based feeds.

Study 1 was performed in the Spring of 2022 to address the larval response to hydrolysate at first-feeding. Larval fish were reared and sampled with aim to minimize all pain and stress. Rearing began from the egg stage.

Study 2 was conducted in spring of 2023 and aimed to assess the larval response in walleye (Sander vitreus) to hydrolysate when introduced at first feeding and when used as a weaning diet (hereafter first feeding trial and weaning trial respectively). Walleye larvae were obtained from eggs obtained from a captive broodstock held at UWSP NADF. At 4 days post-hatch (DPH) larvae were stocked into 240L tanks at a density of 5 larvae/L or 1200 larvae/tank. Tanks were provided with flow-through 20°C water containing clay (KT OM-4, L&R Specialties, MO) delivered via a peristaltic metering pump to increase turbidity and reduce maladaptive clinging behavior and cannibalism (Clayton et al., 2011; Rieger & Summerfelt, 1997). Flow rates began at 2 L/minute with tank stand pipes containing a small screen size. Both flow rates and screen size were increased at regular intervals as the larvae grew and more feed was required (Table 1). Water temperature, dissolved oxygen, turbidity were monitored daily and maintained at values optimal for walleye larviculture (Hauser et al., 2023; Summerfelt & Johnson, 2015). Dim lighting (2 lux) was provided 24h/day and a constant surface spray was maintained to disrupt the surface tension of the water and facilitate gas bladder inflation (Clayton et al., 2011). Tanks were inspected and cleaned daily via siphoning to ensure a small amount of uneaten feed was present each day. During daily cleaning, all mortalities were removed and enumerated (observed mortalities). A random sample of 15 fish per tank were taken every 10 days and examined to estimate gas bladder inflation, feed acceptance, deformity rate, length, and weight. Unobserved mortality was calculated as a percentage of the initial number of larvae that could not be accounted for (mortalities collected and enumerated during trial duration) and calculated using the formula: ((Pinitial-Pfinal-Psampled-Observedmortalities)/Pinitial)x100 where P is the tank population. Deformity rate and gas bladder inflation rates were calculated as a percentage of the final sample at day 30 or 40 (first feeding and weaning trials respectively). Samples were collected for histological analysis and qPCR, but have not been analyzed yet. All data were analyzed using R version 4.3.0 (R Core Team, 2021). Fish weight and survival were analyzed via Kruskal Wallace test and Wilcoxon Rank Sum post-hoc test with alpha = 0.05.

In the first feeding trial, three diets were tested in triplicate: Commercial starter (Otohime, Japan), Intact protein (Control), and Hydrolysate. Diets were fed following size and rate recommendations previously developed for walleye (Table 1; Hauser et al. 2023) and were delivered continuously over a 24-h period with rotary micro-diet feeders. In the weaning trial, all treatments were fed decapsulated artemia nauplii for the first 18 days before transitioning to one of 4 weaning diets (in triplicate): Commercial starter (Otohime), Intact protein (control), Hydrolysate, and CPSP . Artemia were fed 3 times daily at a rate of 1944 nauplii/fish/day based on the initial stocking density. On day 18, the C1 size of the respective diet was introduced following the same feed schedule from the first feeding trial.

In the first feeding trial, survival was highly variable at the end of the 30 day larviculture period. The hydrolyzed diet treatment resulted in both the highest (43.5%) and lowest (0%) survival rate. The commercial starter diet had more consistent survival rates, but there were ultimately no significant differences in survival among diet treatments (Kruskal-wallis, chi-squared = 0.62, df = 2, p-value = 0.73; Figure 1). Growth performance did differ significantly among diet treatments (Kruskal-Wallis, chi-squared = 84.244, df = 2, p-value < 0.0001). The commercial starter diet walleye reached the highest average weight at the end of the 30 day larviculture period (0.52g) followed by the hydrolyzed diet fish (0.33g) and intact diet fish (0.12g; Figure 2). Neither gas bladder non-inflation nor lack of feed in the gut were present in fish from any treatment at day 30. Deformities were present in 11.1% of intact treatment fish, but were absent in the commercial starter and hydrolyzed diet treatments.

In the weaning trial, survival was highest in the commercial starter diet treatment (Figure 3), but differences among treatments were not significant (Kruskal-Wallis, chi-squared = 6.5897, df = 3, p-value = 0.086). Average weight at the end of the weaning trial differed significantly among treatments (Kruskal-Wallis chi-squared = 131, df = 3, p-value < 0.0001). Larvae weaned onto the commercial starter diet were significantly larger (1.08g) than larvae weaned to the hydrolyzed (0.49g; p<0.0001), intact (0.55g; p<0.001), and CPSP (0.23g; p<0.001) diets (Figure 4). The larvae weaned onto the hydrolyzed and intact diets were not significantly different from one another (p=0.11), but were larger than those weaned onto the CPSP diet (Figure 4). Gas bladder non-inflation was not present in larvae from any treatment. Feed acceptance was also high in all treatments with lack of feed in the gut only present in 2% of larvae in the intact diet treatment and not present in the other treatments. Deformity rates were high (86.6%) in the CPSP treatment followed by the intact treatment (37.8%), and the hydrolyzed treatment (28.9%). No deformities were present in larvae in the commercial starter treatment.

This study suggests that the introduction of a hydrolyzed diet has considerable advantages for growth performance over intact proteins when fed as a first feeding diet for walleye. However, these advantages were not present when introduced as a weaning diet. Larval fish have physiological restraints on gut absorption capacity and digestion relative to fully developed adults (Kjørsvik et al., 2011), and during the first few weeks of larval development, peptide availability, amino acid availability, and protein solubility are critical for larval digestion (Hamre et al., 2013). These requirements are possibly more critical for walleye prior to the start of weaning to a dry diet in our study (18 days) than they are after that period. Regardless, our results highlight the critical importance of diet formulation in early larval development for walleye. Unsurprisingly, the commercial starter diet outperformed the hydrolyzed diet suggesting that it is adequately meeting the protein and solubility requirements for larval walleye and that a hydrolyzed protein is not the only component required for a successful larviculture feed. With further optimization, the hydrolyzed protein diet we tested may be able to compete with Otohime as a first feeding diet for walleye and at minimum demonstrates the importance of hydrolyzed protein sources in larviculture diet formulation.

Outreach Overview

Out of the two outreach and extension objectives (5 and 6), only objective 5 had work scheduled to be completed in Year 1 of the two-year project. Two trips were made to SIU at Carbondale's aquatic research lab to observe and document larval yellow perch culture practices once during live feeding and once during feed training. The research team documented egg incubation. Before scheduled filming dates, research and extension participants collaborated to develop a larval yellow perch culture procedures document. This document served as a guide for video shots and will be a foundational document for Year 2 outreach product development. The audio and visual assets recorded in Year 1 were used to develop a practical video guide for farmers in Year 2. A one-day hybrid workshop was provided to 63 farmers on larval feeds and intensive early life stage fish culture of commercial important NCR fish species—largemouth bass, yellow perch, and walleye. The total number of attendees at the workshop was 71—60 participants, 11 speakers (three speakers were farmers).

The following results were provided and published:

- Published recorded workshop talks on UWSP-NADF' YouTube channels and created a workshop playlist. Available online: https://www.youtube.com/playlist?list=PLP8KoWtbBLVy-Zpsxkp1cTQp81VLBP59Y

- Provided online access to PowerPoint Presentation after the workshop. Available online: https://uwspedu-my.sharepoint.com/personal/ehauser_uwsp_edu/_layouts/15/onedrive.aspx?id=%2Fpersonal%2Fehauser%5Fuwsp%5Fedu%2FDocuments%2FPROJECTS%2FWALLEYE%2FWalleye%202023%2FAsian%20Carp%20Hydrol%2FWorkshop%20SIU%2FIntensive%20Larval%20Culture%20Workshop%202023%2FFinal%20Presentations%2FIntensive%20Larval%20Culture%20Workshop%202023&ga=1

- Created a project page on the UWSP-NADF website. Available online: https://www.uwsp.edu/nadf/northern-aquaculture-demonstration-facility/initial-dietary-protein-source-and-palatability-enhancer-for-successful-production-of-fingerlings/

- Provided the opportunity for 11 farmers to tour SIU's aquatics research lab and see operational incubation, larval rearing, fingerling rearing, and live culture systems.

- Provide 108 workshop registries with links for workshop resources, recommended resources, and Q&A posted in the chat feature. Speakers were asked to register.

- Shot additional AV assets for a practical video guide on larval and fingerling yellow perch rearing.

- Created scripts for ten modules for the video guide. Topics covered by modules are cleaning and disinfection, setting up a incubation system, egg incubation, larval rearing tanks, stocking larval rearing tanks, first feeding, rotifer culture, artemia, cleaning and maintaining larval rearing tanks, and dry feed training.

Target Audiences

Further understanding the response of larval YP to significantly more sustainable ingredients such as Asian carp hydrolysate can benefit more than just further research into larval nutrition. The systems being used at SIUC almost objectively represent the sort of RAS system which real farmers can use to any degree. Creating a practical in vitro hydrolysis process and establishing guidelines for rearing larval YP and other percids has been stated as a high priority by multiple agencies across the NCR. As such, making progress into a more viable supply of fingerlings and larval rearing success can provide benefits that reach out to consumers and policymakers in the long run.

Outputs/Impacts

The innovative diet formulation and knowledge derived from the study will provide the US industry with new approach for obtaining a high-quality cost-effective protein source and development of successful high-quality feeds that will support sustainable expansion of the hatchery sector using RAS systems and consequently contribute to the development of competitive and intensive aquaculture market in the Midwest. These innovative feeds produced using SIUC commercial feed processing method (small scale) will allow for immediate implementation of the formulation by the aquafeed industry.

- Informational and instructional based media is currently being developed in conjunction with Purdue University. Media is, so far, planned to be distributed to farmers, public meetings, through Sea Grant, and at national/local aquaculture conferences at least through the year 2023.

- Increased new knowledge pertaining to rearing of largemouth bass, yellow perch and walleye to NCR residents and non-NCR. Evaluation results, indicated that a minimum of 23 NCR residents from seven NCR states (Illinois, Indiana, Kansas, Minnesota, Missouri, Ohio and Wisconsin) attended the workshop. Evaluation response rate was 66.6% based on participant registration (n=60). 39 out of 40 respondents answer the question that asked, "what state do live in". Non-NCR participants were from Poland, New Zealand, Texas, Canada, New York, Maryland, North Carolina, and Maine.

- Increased new knowledge pertaining to larval feeds to NCR residents and non-NCR.

- Provided new knowledge that fish culturist intended to implement. 22 attendees said that they were likely or highly to use information from the workshop to implement production methods. One commercial producer planned to implement new knowledge in 2024.

Impacts Summary

Relevance. — Fish feeds are a major bottleneck in aquaculture since they constitute up to 70% of total fish production costs and hence, their high quality is critical to achieve maximal growth. The proposed methodology for obtaining the optimal protein hydrolysate for YP and W larvae will become a practical way of attaining, in a controlled way, an innovative, natural, and cost-effective dietary ingredient for larval Percid diets that will meet both the nutritional requirements and functional capacity of the digestive system of larval YP and W. In addition, Asian carp hydrolysate used as a natural attractant for juvenile YP and W will help wean the fish to formulated plant-based diets by improving feed acceptance and its utilization. Finally, SBM hydrolysate will be better utilized by fish in their young stage due to improved digestibility and reduced content of anti-nutritional factors.

*Respons*e. — At the completion of the study we will be able to achieve larviculture of YP and W completely transitioned to formulated diets and presenting positive growth performances, low skeletal deformity rate, and high survival. More specifically, we will be able to observe acceptance of formulated feeds by larval Y and W right at the start of the feeding by providing well-utilized diets based on the right molecular weight and the optimal AA composition which will enhance dietary AA assimilation and utilization for tissue protein synthesis and hence, improve growth and survival of larval YP and W. The proposed study will deliver an innovative dietary formulation, which will replace live food by improving the growth and survival of fish characterized by a challenging and vulnerable larval stage as presented by Percids.

Results. — We also expect that Asian carp muscle hydrolysate combined with SBM hydrolysate both obtained using YP and W digestive enzymes will allow for successful weaning of the fish to formulated feeds without jeopardizing fish growth and survival. The Asian carp hydrolysate will likely support high feed intake and at the same time the exposure to pre-digested SBM will help adapt the fish to dietary plant protein earlier.

Recap. — This project will also deliver strong outreach component in a form of YP larval rearing fact sheet, larval rearing fact sheet, videos (mostly YP and W first feeding and larval rearing), dietary protein hydrolysate fact sheet (how to make it) for feed manufacturers, a webinar, and a workshop for all stakeholders. This project has strong support from many industry providers as shown by the attached letters of support.

Publications, Manuscripts, Workshops, and Conferences

Presentations-Oral

Boessen P. The use of in vitro hydrolysis towards utilization of invasive species as a source of protein for larval yellow perch diets. Aquaculture America, New Orleans, February 2023

Non-Peer-Reviewed-Popular Articles

Southern Illinois University News, St. Louis Post-Dispatch, SIUS-NRP