Project Title: Optimization of Practical Feed Formulation to Improve Fish Health and Production of Yellow Perch (*Perca flavescens*) [Termination Report]

Total Funds Committed: $169,467
Initial Project Schedule: July 1, 2019-June 30, 2021 [Extended to June 30, 2023]
Current Project Year: September 1, 2021-August 31, 2023
Participants: Dong-Fang Deng and R. Newton (University of Wisconsin-Milwaukee), K. Rosentrater (Iowa State University)
Extension Liaison: J. Poletto (University of Nebraska)
Industry Liaison: Rich Lackaff

Project Objectives

The ultimate goal of this proposal is to increase the profitability of yellow perch aquaculture by developing nutritionally balanced and cost-effective feed. To achieve this goal, the objectives of our two-year project are to:

1) Optimize practical feed formulation by determining the optimal dietary carbohydrate in feed for yellow perch based on growth performance and nutrient utilization;
2) Evaluate effects of different diets on gut microbial ecology and stress tolerance of yellow perch;
3) Determine production efficiency of the new feed at laboratory and commercial farms; and
4) Transfer technology and disseminate findings to industries to enhance the applications of findings.

Project Summary

Yellow perch (*Perca flavescens*), a freshwater fish, is an ecological, economical, and cultural staple of the Upper Midwest. Changes to the Laurentian Great Lakes ecosystem have disrupted the sustainable supply of yellow perch from fisheries, impacting consumer demand. To address this, aquaculture-raised yellow perch emerges as a potential solution to bridge the market gap and aid in the reintroduction of fish to depleted regions. However, existing commercial feeds fall short in providing optimal nutrients for yellow perch, leading to unhealthy fish and suboptimal growth. This project aimed to explore fish feeds with varying dietary carbohydrate sources and concentrations, along with lipid-to-carbohydrate ratios, to identify the optimal nutrient combination for enhancing yellow perch health and growth. The assessment of feed quality was based on criteria such as fish growth performance, health metrics, nutrient utilization, and fish tolerance to heat shock stress. Additionally, extension research and outreach activities were conducted to train farmers and raise community awareness about the significance of feed management, as revealed by this project. The study results offer new insights into the nutrient requirements of yellow perch, providing the feed industry with crucial information for tailoring feeds specifically for this species. By integrating these findings into the development of specialized feeds for yellow perch, aquaculture farms stand to increase their production profits through enhanced fish growth performance and health, thereby reducing operational costs.

Anticipated Benefits

The feed industry can produce specific feed for yellow perch farming based on findings from this project. Yellow perch farmers will have feed specific to perch grow-up. By using the new feed, we expect that yellow perch farms will increase their production profit because feed is one of the major costs in yellow perch production. We will help to train the next generation workforce by involving undergraduate and graduate students in hands-on research and extension activities.

Project Progress

Objective 1: **Estimate the optimal form and level of dietary carbohydrate in feed for yellow perch based on growth performance and nutrient utilization in practical feed formulations**.

Feeding Trial 1; The performance of yellow perch fed wheat or corn flour based diets processed using a cold extruding method.

Test Diet Preparation
In the initial feeding trial, we examined the impact of a lab cold-extruding method on yellow perch using feeds manufactured with wheat flour and corn flour. Six test diets, with varying proportions of wheat or corn flour (15%, 20%, or 25%), were prepared (see Table 1). A reference diet with no added carbohydrates was also included. The feed processing method followed protocols similar to those described by Jiang et al (2019). Dry feed ingredients were pulverized to particles less than 400 μm, mixed using a Hobart mixer (K5-SS, Hobart Corporation of Troy, USA), and combined with boiled water (50% w/w of the total dry mixture) and oils. This mixture was then extruded through a Hobart meat grinder. The resulting moist pellets were sealed with foil and baked at 80 ºC for 15 minutes to enhance carbohydrate gelatinization. Subsequently, diets were dried at 21 ºC until the moisture content was less than 10%. The dry pellets were crumbled and sieved to generate suitable pellet sizes (0.85 and 4 mm) for the feeding trials. All test diets were packed and stored at 4 ºC until use. The proximate composition and amino acid content of the test diets are presented in Table 1.

Fish Maintenance
Yellow perch from broodstock cultured at the Great Lakes Aquaculture Center, University of Wisconsin-Milwaukee, were used. Two weeks before the feeding trial, uniform-sized fish (50 fish per tank) were distributed into 21 tanks (350 L) for acclimation. The indoor culture system ran with dechlorinated municipal flow-through water (about 5 L/min) at a temperature of 21-22º C. During acclimation, fish were fed a mixture of the seven test diets, in equal proportions, to apparent satiation three times daily (09:00, 12:00, and 15:00). Before the feeding trial, fish fasted for 24 hours, then pooled into a larger tank. Fish of comparable size (average body weight: 14.8±0.2 g, n=21) were distributed into each tank with 35 fish Care per tank. Each test diet was randomly assigned to triplicate tanks. Fish were hand-fed three times daily (09:00, 12:00, and 15:00) at a daily feeding rate of 1.8 to 5% of body weight for 10 weeks. Fish were batch-weighed in water containing stress coat (1.5 ml/10L water; Fish care North America, Inc. Chalfont, PA, USA) every two weeks to obtain growth data, and feed rations were adjusted accordingly. The experiment followed the animal care protocols approved by the Animal Care and Use Committee, University of Wisconsin-Milwaukee.

Water Quality and Photoperiod Maintenance: During the feeding trial, water quality and photoperiod were maintained to meet optimal fish growth. Water temperature and dissolved oxygen were continuously monitored by automatic sensors. Other water quality parameters were monitored once a week in the morning. Throughout the growth study, the water temperature was maintained at 20-23 ºC, dissolved oxygen at >6.0 mg/L, total ammonia nitrogen at <0.08 mg/L, pH at 7.0-8.0. The photoperiod was maintained at a light: dark ratio of 12 h: 12 h.

Sample Collection and Analysis
At the end of the 10-week trial, all fish were fasted for 24 hours before batch-weighing and counting to obtain final values for survival and tank biomass. Four individuals were euthanized with an overdose of MS222 for whole-body proximate composition analysis. This included moisture, crude protein, lipid, and ash analyses, enabling the evaluation of protein efficiency ratio,

and protein and energy retention. Another four fish from each tank were euthanized for the measurement of individual body weight and body length values to calculate the condition factor (CF). Subsequently, these three fish were dissected to measure their liver, viscera, and viscera fat. Values of hepatosomatic index (HSI), viscerosomatic index (VSI), and viscera fat index (VFI) were calculated accordingly. Blood samples from four fish/tank were collected via caudal puncture of the hemal arch using a 1.5-ml non-heparinized syringe.

Proximate analysis of experimental diets and fish samples followed methods by AOAC and the methods described below. Fish moisture content was measured by drying samples in a vacuum freeze dryer for 48 hours to reduce moisture content to <95%. Subsamples from the freeze-dried samples were further dried in an oven at 105 °C for 24 hours. Protein content was determined by measuring nitrogen (N×6.25) levels using an elemental combustion system (ECS 4010 nitrogen/protein analyzer, Costech analytical technologies, INC, USA). Lipid content was determined by ether extraction using a Soxhlet Unit (Soxtec 8000 extraction unit, Foss, Denmark). Ash content was determined using a muffle furnace at 550 °C for 12 hours. Liver glycogen was analyzed using a glycogen assay kit (Cayman Chemical, Ann Arbor, Michigan, USA). Blood glucose was measured using MediSense Blood Glucose System (MediSense Inc., Waltham, MA). Metabolite extraction and NMR spectroscopy data acquisition followed a similar method described by Lu et al (2022).

Data Calculation and Statistical Analysis
Data were presented as mean ± SD of three replicates. All data underwent one-way or two-way analysis of variance (ANOVA). When overall differences were significant ($P<0.05$), LSD test was used to compare the mean values between the treatments. When the test of homogeneity of variances failed, the Games-Howell's test was used. Statistical analyses were performed using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA).

Results
Fish fed diets containing wheat flour exhibited better growth and a lower feed conversion ratio compared to those fed corn flour diets ($P < 0.05$; Table 2). The hepatosomatic index (HSI) was also higher for perch fed wheat flour than corn flour. This index increased with the rising carbohydrate level in the test diets. In contrast, fish fed a 20% carbohydrate-based diet showed a lower growth rate, higher feed conversion ratio, and lower condition factor than those fed diets containing 15% or 25% carbohydrate. The interaction between the carbohydrate level and sources was not significant ($P > 0.05$).

Fish on the wheat-based diet had higher lipid levels in both whole fish and liver tissues than those on the corn-based diet (Table 3). With increasing carbohydrate levels, the lipid content in both whole fish and liver tissues also increased. The 25% flour-based diets significantly induced glycogen accumulation and decreased protein content in the liver tissues compared to diets containing 15% or 20% flour (Fig 1). The glycogen content in liver tissues was not impacted by carbohydrate sources. Fish fed the diet with no added flour had the lowest glycogen accumulation in liver tissue. The postprandial glucose levels of fish fed wheat-based diets peaked at 3 hrs, while in fish fed the corn-based diet or the diet with no added carbohydrate, they peaked around 6 hrs post-feeding (Fig 2). Furthermore, the peaked glucose levels were higher in fish fed wheat-based diets than those fed corn-based diets. Overall glucose levels reflected the dietary carbohydrate levels and were the lowest in fish fed the diet with no added carbohydrate.

Results based on liver metabolomic profiles showed that levels of glycine, glycerol-3-P, and alanine correlated with the increase in carbohydrate levels in the experimental diets (Fig 3A). However, creatine levels decreased with increasing carbohydrate levels in the test diets. Myo-

inositol and glucose levels significantly decreased, while taurine and succinate levels increased in fish fed the diet with no added carbohydrate compared to those fed the other test diets (Fig 3B).

Under the current testing conditions, wheat flour promoted better growth in yellow perch juveniles than corn flour. Wheat flour was more lipogenic than corn flour in yellow perch. This may be attributed to the high digestibility of wheat flour compared to corn flour, supported by the higher level and earlier peak of blood glucose in fish fed a wheat flour-based diet. Furthermore, the elevated level of glycerol-3-P, a precursor molecule of lipid biosynthesis, indicated increased lipid biosynthesis in fish fed a 25% wheat-based diet. The current preliminary results also suggested that a high carbohydrate (25%) induced glycogenesis in yellow perch because a high glycogen level was observed in the liver tissues of those fish. The diets containing 25% wheat or corn flour did not depress growth compared to the diet containing 15% flour. It was unexpected that fish fed 20% flour had the lowest growth rate. The reason for this is not clear and warrants further investigation in subsequent studies.

Feeding Trial 2: The performance of yellow perch fed wheat or corn flour based diets processed using a cooking extruding method.

Methodology

Feed Preparation

An 11-week feeding trial was conducted to assess the impact on yellow perch when fed six test diets containing 15%, 20%, or 25% flour derived from either wheat or corn. Notably, these diets incorporated a reduced level of fish meal compared to experimental diet 1, and two commercial diets served as references. The feed underwent processing through cooking extrusion, with Dr. Kurt Rosentrater at Iowa State University overseeing the entire production process, from ingredient procurement to the creation of complete feeds for the feeding trials. This involved milling, mixing, conditioning, extruding, and drying of the feeds. The Viking hammermill was utilized for milling, Kobalt rotating mixer for mixing, and an InstaPro commercial extruder for the extrusion process. Following processing, the feeds were shipped to Dr. Deng for implementation in the feeding trials. The detailed feed formulation and nutrient composition are presented in Table 4A, 4B, and 4C.

Experimental Setup

The set up was similar to the method described in Trial 1. Each treatment involved three tanks of fish, with 35 fish per tank. The average initial body weight of the fish was 16.3 g. The fish were cultured in a flow-through water system with a temperature maintained between 22.5-24°C. They were fed three meals a day at a rate of 1.8-3% of their body weight.

Fish Maintenance and Sampling Protocol:

The overall protocol for fish maintenance and sampling followed the same methods as described previously. This included acclimation, fasting before the trial, distribution into tanks, and daily feeding routines. Growth data, feed ration adjustments, and adherence to animal care protocols were consistent with the prior feeding trial.

Stress Testing

Leftover fish from dissection underwent heat shock testing by gradually increasing the water temperature from 24°C to 32°C at a rate of 1°C every 15 minutes. Mortality was monitored for up to 24 hours post-stress period.

Sample Analysis and Statistics:

Sample analysis and statistical procedures mirrored the methods described in the earlier trial.

Proximate analysis, including moisture, crude protein, lipid, and ash content, followed AOAC methods. Statistical analyses involved mean ± SD of three replicates, and one-way or two-way ANOVA was conducted. The LSD test or Games-Howell's test was applied when appropriate. SPSS 19.0 for Windows facilitated statistical analyses, ensuring robust assessment and comparison of the different treatments.

Results

The two carbohydrate sources did not show different impacts on the growth performance of yellow perch, but growth tended to be lower in fish fed with corn flour (Table 5). This observation contrasts with the results of Experimental 1, suggesting that various feed processing methods may influence carbohydrate utilization, as seen in previous studies on other fish species. The feed conversion ratio (FCR) was lower in the current trial compared to Trial 1, suggesting that extruded feed may be better utilized by the fish. Additionally, differences in feed formulation between the studies may contribute to these varied observations.

The weight gain and condition factor of fish decreased, and the FCR increased with higher levels (20 or 25%) of wheat or corn flour in the diets. The growth of fish fed diets containing 15% flour was comparable to those fed with two commercially available feeds commonly used in yellow perch farming. It is noticed that the amino acid compositions of the test diets (Table 4C) were impaired due to the increasing levels of corn or wheat flour. Some indispensable amino acids, such as arginine, methionine, and lysine, decreased with the increase of corn or wheat flour. The cooking extrusion may have caused a Maillard reaction, which is a reaction between reducing sugars and amino acids.

The mortality of perch exposed to heat shock stress tended to increase with rising dietary carbohydrate levels, with wheat flour-based diets resulting in higher mortality. However, the differences were not significant among carbohydrate levels and sources. The values of the hepatosomatic index (HSI) were influenced by the level of dietary carbohydrates, with larger livers in fish fed 25% flour-based diets (Fig 4). Wheat flour-based diets also led to larger livers compared to corn flour-based diets, consistent with our observations in the first feeding trial. Furthermore, yellow perch fed 25% flour-based diets showed significantly lower hematocrit and carcass index than those observed in fish fed 15% flour-based diets.

The growth and FCR of yellow perch fed the two commercial diets were not significantly different (Fig 5). However, HSI was lower, and the visceral fat index (VFI) was higher in fish fed commercial diet 2 than those fed commercial diet 1. These differences may be attributed to varying lipid and carbohydrate contents in the two diets. Generally, lipids are more digestible and easier to absorb, explaining the high visceral fat observed in fish fed commercial diet 2, which contained 16% lipid compared to 9.8% lipid in commercial diet 1. In contrast, commercial diet 1, with high carbohydrate content, may induce glycogenesis, partially explaining the higher HSI in fish fed commercial diet 1.

In summary, under the current testing conditions, the findings suggest that adding 15% carbohydrate is optimal for feeding yellow perch. A combination of wheat and corn flour as carbohydrates may benefit the fish in terms of carbohydrate utilization and fish health concerns. The cooking extrusion method for feed preparation benefits the feed conversion ratio, but caution is needed to avoid nutrient loss. Optimizing the combination of carbohydrates and lipids is suggested to address concerns of fatty liver and excess accumulation of visceral fat. These preliminary findings provide critical information for defining a practical feed formulation for future studies on yellow perch.

Feeding Trial-3: The performance of yellow perch fed diets with different levels of starch and lipid.

Methodology

Test Diet Preparation

Five test diets were formulated (Table 6) to contain different added levels of starch (14%, 16%, 18%, 20%, and 22%) and lipid (10%, 12%, 14%, 16%, and 18%). The feed was processed using the protocol described in Tria-1 and stored at 20 ºC until used. Additionally, two commonly used commercial diets by fish farmers were included as references.

Fish Source and Maintenance

Yellow perch fingerlings were produced from broodstock housed at the School of Freshwater Sciences and raised to the sizes needed for this study. Uniform-sized fish were acclimated to the culture system for 2 weeks before the beginning of the feeding trial. Thirty fish per tank were distributed into 21 tanks (100 L water). The indoor culture system ran with dechlorinated municipal flow-through water at a rate of approximately 3 L min^{-1} $tank^{-1}$. The photoperiod was maintained at 12 h: 12 h = dark: light, and the water temperature was kept at 21-23 ºC. During the acclimation period, fish were fed a mixed diet with equal portions of the five test diets and the two commercial diets. The feeding rate was set at 4% of the total body weight, four times daily (09:00, 11:00, 14:00, and 16:00). Fish maintenance followed the protocol approved by the IACUC of the University of Wisconsin-Milwaukee.

At the end of the acclimation period, all fish were fasted for 24 h, pooled into a large tank for selection and redistribution. Fish that appeared normal and were similar in size (average body weight: 8.90 ± 0.27g, n=21) were randomly distributed into each tank with 20 fish per tank in the same system used for acclimation. Each experimental diet was randomly assigned to three tanks. Fish were fed using automatic feeders four times daily, as described above, at a daily feeding rate based on body weight of 2-5%. Fish were weighed every three weeks, and the feeding rate was adjusted accordingly. The feeding trial lasted for 12 weeks. All tanks were cleaned daily by siphoning to remove fecal matter before the first feeding. Mortality, water temperature, pH, and dissolved oxygen were monitored daily. Total ammonia nitrogen was monitored weekly. Water temperature was maintained at 21 - 23 ºC, dissolved oxygen > 6.0 mg/L, ammonia nitrogen < 0.08 mg/L, and pH around 7.2 - 8.0. The photoperiod followed the same cycle used during the acclimation period.

Sample Collection and Analysis

After stocking, three initial samples with 10 fish each were collected for proximate composition analysis. At the end of the 12-week feeding trial, all fish were fasted for 24 h before batch-weighing and counting to obtain the final survival and total weight of each tank. Four individuals from each tank were randomly collected and measured for body weight and total body length. They were euthanized by an overdose of MS 222 and kept at -80 ºC until used for proximate composition analysis (moisture, crude protein, crude lipid, ash). Another 4 fish per tank were euthanized and measured for individual body weight and total length, followed by cervical severing of the spinal cord before they were dissected. These 4 fish were dissected on ice to obtain the liver, intestine, carcass, and gonad for the calculations of hepatosomatic index (HSI), carcass index (CSI), visceral fat index (VFI), and gonad index (GSI), respectively.

Blood was collected via caudal puncturing of the tail and kept on ice for approximately 4 hours. Serum samples were collected after blood samples were centrifuged at 2000 g for 15 minutes at 4 C. All samples were stored in a -80 C freezer for further analysis.

After the initial sample, 8 fish (4 male and 4 female) were subjected to heat shock stress. Water temperature was increased gradually from 23 ºC by one degree every hour for eight hours until it reached 31 ºC. Temperature and dissolved oxygen (mg L-1) levels were recorded, and fish were

then held at ~31 C for 20 hours before being dissected for samples following similar protocols described above. Fish that experienced the same handling except for temperature shock were treated as control fish for each dietary treatment.

Sample analysis and data management followed the method described in Tria-1 and Tria-2

Results

The study demonstrated that fish on a diet with 20% starch and 13% lipid exhibited the highest growth, better than those on a test diet (14% starch, 18% lipid) and a commercial diet (16% lipid) ($P<0.05$; Table 7). While the feed conversion ratio (FCR) and protein efficiency ratio (PER) showed no significant differences among the five test diets, FCR tended to increase and PER decrease with higher lipid levels. The commercial diet with 12% lipid performed better than the one with 16% lipid. Growth performance was similar between fish on commercial and test diets containing 11-13% lipid. Hepatosomatic index (HSI) was significantly higher in fish fed the commercial diets than those fed the five test diets, with no significant impact on yellow perch morphology (Table 8). Total survival after heat shock stress did not differ significantly among dietary treatments. Regression analysis ($Y= -1.0045x^2 +28.571x-165.78$, $R^2 =0.6277$) indicated that a diet with 14.2% lipid and 18.8% starch provided the highest survival during heat shock stress. Females displayed higher visceral fat index and HSI, while males exhibited a higher gonadosomatic index (Table 9). Males were more sensitive to heat shock stress than females across all diets. No significant differences were found in the levels of moisture and protein of whole fish across treatments. Lipid levels increased with dietary lipid levels, while ash content was significantly different among treatments but did not correspond to dietary treatment (Table 10).

The study suggests that a diet with lipid and starch levels between 11-14% and 18-20%, respectively, is appropriate for yellow perch under the current conditions based on growth performance and heat shock tolerance. Diets exceeding 16% lipid may not be optimal for health and growth. Further investigation is warranted, particularly regarding nutrient requirements based on gender, crucial for broodstock feed development.

Objective 2: **Evaluate the effects of different diets on gut microbial ecology and stress tolerance of yellow perch.**

Results on stress tolerance are presented under objective 1.

Methodology

To further our understanding of how host-diet interactions influence the yellow perch gut microbiome, we collected fish intestines at the end of each feeding trial. Upon trial conclusion, fish were fasted for 12 hours and anesthetized before dissection. Then the entire intestine was removed, and the intestine was flash frozen and stored at −80 °C. To extract DNA for microbial community analysis, the intestine was thawed and opened longitudinally on a sterilized glass dish on ice. Any chyme or feces in the gut was then removed and cleared. Once clear, the intestinal mucosa layer was carefully scraped using a sterile blade and transferred to a microcentrifuge tube. DNA was then extracted from the intestinal mucosa using the QIAamp® Fast DNA Stool Mini Kit. After obtaining purified DNA, we used PCR to amplify the bacterial V5-V6 16S rRNA gene region. Triplicate PCR reactions were run for each sample and the products were pooled. An Illumina Nextera XT DNA library was created for each sample and DNA sequencing was conducted on an Illumina MiSeq with 2x250 chemistry.

All DNA sequence reads were trimmed of primers and adapters and processed to remove low-quality sequence information. Reads were then processed with the R package DADA2 (Callahan et al., 2016) to produce counts of unique amplicon sequence variants (ASVs). Taxonomy was assigned to ASVs using Silva v. 132 (Quast et al., 2013). A negative control reaction was also sequenced. Contaminant ASVs in the negative control were identified with the R package

Decontam (Davis et al., 2018) and removed prior to analyses. To assess feeding treatment effects on the microbial community, we compared beta diversity among treatments using ordinations, an analysis of similarity (ANOSIM), and PERMANOVA tests and alpha diversity differences among treatments with the Duncan and Wilcoxon tests.

Results

Yellow perch gut microbial communities with carbohydrate replacement in feed were dominated by the genus *Cetobacterium*. *Cetobacterium* spp. are common inhabitants of many freshwater fish intestines and are thought to provide beneficial properties for fish health. Although *Cetobacterium* spp. were the most abundant organisms overall, fish fed wheat carbohydrate tended to have a more variable gut microbial community (Wilcoxon W=616, p=0.0001), and this was most prominent at the lowest replacement level (15%, Fig 6). It appears that carbohydrate replacement of fishmeal drives the community toward a larger proportion of *Cetobacterium* spp. and that more variability occurs in the community when this replacement occurs at a lower level (15% vs. 25%) or with a source (wheat flour) that is less suitable for yellow perch health and growth (see other data sections). It is not yet understood if these differences interact with the host to have an impact on growth or what other control points can be instituted to direct gut microbes toward a desired outcome. Overall, the microbial data supports the fish health data that wheat carbohydrate replacement results in altered fish characteristics.

References

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 2013;41:590–6

Davis NM, Proctor DiM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 2018;6:1–14.

Objective 3: **Determine the production efficiency of the new feed at laboratory and commercial farms.**

Trial 1: Two test diets (a lab diet and a commercial diet) were evaluated at a commercial aquaponic farm. The test diet contained 18% poultry meal, 10% corn protein concentrate, 4% wheat gluten, 8.7% poultry blood meal, 12% menhaden meal, 12% oil mixture of corn oil, coconut oil and fish oil, 16% mixture of what flour corn flour and tapioca starch, and others including minerals, vitamin mixtures. There were three tanks per treatment with 40 fish per tank. The initial average body weight was 35 g. The test diet was processed using cold extruding method at Deng's lab. The test diet contains 45% protein and 14% lipid. A commercial diet contain 35% protein and 15% lipid was used to compare to the test diet. The feeding trial only lasted for one month and terminated due to facility damage caused by snowstorm. No conclusion can be drawn based on this feeding trial due to incomplete data collection.

Trial 2: The same two diets used in Trial 1 were evaluated at a commercial aquaponic farm (Fig 7). There were three tanks per dietary treatment with 26 fish per tank. The initial average body weight was 43.5 g. The water temperature ranged from 13.7°C to 19.5 °C during the feeding trial, which lasted from December 2, 2022, to Feb 11, 2023. Dissolved oxygen, pH, and alkalinity were

monitored weekly and maintained at >9 mg/L,7.5-7.6, and 220-260, respectively. Fish were fed to satiation and the average feeding rate was 1.5% during the feeding trial, which lasted for 70 days.

The results showed that yellow perch fed the two test diets were similar in growth performance and morphology (Table 11). The fish responded well and the overall FCR was 1.1. No mortality was observed during the feeding trial. The proximate composition of fish fed both diets we not significantly different.

During the feeding trial, farmers and students were trained to manage feeding to maintain good water quality for both fish and vegetables. Due to the impact of Covid, it has been difficult to get feed processed using commercial extruding methods regarding limited feed ingredients and workforce. The lab test diet can be modified if a cooking extruding method is used to produce the diet. We hypothesize that the cooking extruding method will improve the physical quality of the lab test diet, and thus will make it easier for the fish to utilize the nutrient. This warrants further investigation.

Objective 4: **Transfer technology and disseminate findings to enhance the applications generated from this project.**

Training

Through this funding, the PIs have established collaborate research and training with three local farms (Portfish, Ltd; Mulberry Aquaponics LLC. and Crystal-Clear Fish Farm). Activities include conducting extension research, training on feed selection and feed management for yellow perch, on-site visit to address questions from farmers, and assistance to prepare long term farm research by conditioning fish generated at lab conditions to the farm environment.

This project also involves training undergraduate, graduate students, and a postdoc. Two undergraduate students were trained in the Poletto Fish Physiology laboratory at the University of Nebraska Lincoln (UNL) and then placed in internships or jobs at local aquaculture facilities as already-skilled workers with knowledge of aquaculture practices. One graduate student was trained in feed processing at Iowa State University. One undergraduate student and one graduate student were trained in amicrobiology lab at the University of Wisconsin-Milwaukee. Three undergraduate students, three graduate students and one postdoc were trained through this project and were trained in feed management, yellow perch culture protocol, and fish farming in commercial farms.

Outreach Overview

Data dissemination

Research findings were presented through oral presentations at:

- NCRAC annual meetings (2020, 2022, & 2023)
- Wisconsin Aquaculture Association meeting (2023).
- Aquaculture American (one in 2020 & one in 2024)

Outreach activities at UWM

1) Education tours: hosted tours for students and teachers from high schools (e.g., True Skool aquaponic program, Slinger High School, Dominican High School, summer interns), international student summer exchange training (Germany) and the local community (e.g., Naulin foundation, community colleges).

2) Community engagement: Disseminate information to the local community: presentation at the events: Doors Open Milwaukee, Sturgeon Bowl, and Harbor Fest (2022 & 2023); Provide

training to local fish farmers on feed management (Portfish, Mulberry Aquaponics, and Crystal Clear Fish Farm).

3) Engage local communities through social media platforms. https://www.watermarksmke.org/dong-fang-deng

Target Audiences

The expected products include a new feed formulation, new knowledge on feed nutrition on perch, protocols on feed management, perch culture, and publication and presentations based on the results of this project. Yellow perch producers will benefit from an optimal feed for growing perch at a cost-effective approach. The feed industry will be able to adopt the new findings to make feed targeted on yellow perch; Students, researchers, and industrial partners or others interested in perch culture will be trained and gain new knowledge on fish feed nutrition and feed management and develop collaborations with feed industry and fish farmers.

Deliverables (Outputs)

Objective 1: There are three major findings from this objective. First, a combination of wheat and corn flour/starch serves as an optimal carbohydrate source for yellow perch, benefits growth optimization, and minimizes liver health concerns. Second, feeding perch a diet with 14% fat and 18-20% starch is recommended, provided sufficient protein is included; as higher fat levels, exceeding 16%, may compromise both growth and health. Third, different nutrient requirements should be considered for male and female fish. These findings provide crucial baseline information for the advancement of yellow perch practical feed development.

Objective 2: Methods were developed to study the microbial community associated with the intestines of yellow perch raised in aquaculture. The dominant gut microbes were consistent among fish fed with carbohydrate replacement of fishmeal and those raised on a fishmeal only diet. However, carbohydrate replacement in feed also consistently drove the community toward a more *Cetobacterium* spp. dominated community, except when lower amounts of wheat carbohydrate were supplemented.

Objective 3: The extension research provides hands-on experience for training students and farmers and improves communication between researchers and farmers. The results also showed that the lab feed formulation is compatible with commercial feed.

Objective 4: This project involved training farmers from three local farms, and 11 students and a postdoc. Six oral presentations were given in regional and national meetings. Partial outcomes were published in one video and a paper. Two papers are in preparation for publications. Active outreach activities including educational tours, research demonstrations, and engagement with farmers and the local community were organized by PIs and students to enhance the recognition of aquaculture by the public.

Outcomes/Impacts

- The findings from this project can be used by the feed industry to optimize feed for yellow perch or similar fish species. Fish farmers will benefit from new feed specific for perch to reduce production cost or produce healthy fish.

- The findings will help farmers to select the right feed for farming and improve feed management.

- The project provided training for the new generation (12 students from three universities), which will benefit the industry in the future through continued research or new workforce development.

- The engagement between researchers and the public is significantly improved and the awareness of local aquaculture is enhanced. Over three thousand visitors attended our research demonstration and education tours.

Impacts Summary

Relevance. — Yellow perch is a high demand seafood in the Great Lakes region. Feed is one of the major components accounting for yellow perch production cost. Aquaculture production and profitability of yellow perch are challenged by suboptimal feed, which is produced for Salmonid fish species. Current commercial feed used for yellow perch causes adverse impacts on fish health and production efficiency.

Response. — We determined the impact of different carbohydrate sources (wheat vs corn) and their inclusion levels (15, 20, and 25%) as well as interaction between carbohydrate levels an lipid levels in feed for perch based on growth performance, physiological responses, metabolism, and health. The optimal dietary starch and lipid levels were estimated based on growth performance and stress response. Students and farmers were trained with techniques related to perch culture.

Results. — The preliminary studies showed 18-20% starch and 14% lipid are optimal for feeding yellow perch and a higher level of carbohydrate (25%) and lipid (≥16%) depressed growth caused fatty liver and accumulated fat.

Recap. — The outcome of this project provides yellow perch producer with nutritionally balanced and cost effective feed, and help to train fish famers and the next generation workforce for the aquaculture industry.

Recommended Follow-Up Activities

- Further research connecting fish gut microbiomes to fish health performance could have a significant impact on fish mortality, health, and/or growth. Research in this space in humans has fundamentally altered the way human health and nutrition is viewed. Additional characterization of the gut community is needed to understand the primary gut inhabitants and their roles in fish nutrient uptake and/or the prevention of disease, especially as diets are formulated for individual fish species. Following this work, trials could be designed to manipulate (prebiotics) or supplement (probiotics) these communities toward desired fish health outcomes.

- It could be important as a follow-up activity to investigate modifications to the extrusion process in order to produce slow sinking feeds. These could include alterations in moisture content, screw speed, or barrel temperature. In addition, management of feed processing is critical for maintaining the feed quality such as by minimizing Maillard reactions, which caused reduction of amino acids as we found in our study.

- Longer farm testing and comprehensive evaluation are needed to verify the production of the new feed. Due to the impact of covid, the research was impaired because of limited manpower and budget shortage, which was not sufficient to support all activities.

- Additional research is essential to study gender-specific nutrient requirements and explore ways to enhance health and nutrient utilization through the incorporation of feed additives

Publications, Manuscripts, Workshops, and Conferences

1. Video on yellow perch: Fish of the people-A look at Yellow Perch Research (https://www.youtube.com/watch?v=Pp7ECRagn6g
2. Jiang, M., H. Zhao, S.-W. Zhai, R.J. Newton, B. Shepherd, J. Tian, A.G. Lofald, S.Teh, F.P. Binkowski, & D.-F. Deng. 2020. Nutritional quality of different starches in feed fed to juvenile yellow perch, Perca flavescens. Aquaculture Nutrition. doi: 10.1111/anu.13026.
3. John P. Conto*, Alexander Gregory, Patrick Blaufuss, Jacob Peterson, Ryan Newton, Kurt A. Rosentrater, Dong-Fang Deng. Impact of dietary lipid-to-carbohydrate ratios on growth performance, health, and heat shock tolerance in juvenile yellow perch (perca flavescens). (to be submitted to Aquaculture Nutrition, January 2024)
4. Fei Huang, Xin Lu, Jin Xu, Ryan, J. Newton, Kurt A. Rosentrater, Dong-Fang Deng. Utilization of wheat flour and corn flour in yellow perch juvenile (Perca flavescens). To be submitted to Animal Nutrition in Feb 2024.

Table 1. Test diet formulation prepared by cold-extruding and contained different levels of carbohydrates from wheat or corn flour(Feedingtrial-1)

Ingredients		Wheat flour (%)			Corn flour (%)		
	FM	15	20	25	15	20	25
Menhaden meal/wheat gluten	60.0	48.2	44.6	41.0	48.2	44.6	41.0
Wheat gluten	4.0						
Corn protein concentrate	20.0	21.0	19.3	17.6	21.0	19.3	17.6
Wheat flour		15.0	20.0	25.0			
Corn flour					15.0	20.0	25.0
Calcium phosphate dibasic	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix	1.6	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin premix	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Soy Lecithin	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Menhaden oil	3.0	4.0	4.3	4.6	4.0	4.3	4.6
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chromin oxide	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Carboxymethyl cellulose	3.6						
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Proximate composition (% as fed)							
Moisture	9.6	8.8	9.4	10.0	9.3	9.5	9.4
Ash	14.3	13.0	12.4	11.9	13.1	12.6	12.0
Protein	56.6	50.6	47.6	44.4	50.1	47.3	43.5
Lipid	12.2	12.2	12.5	12.1	12.8	13.0	12.6
Nitrogen free extract	7.3	16.9	20.0	24.0	16.3	19.5	24.6

Table 2. Growth performance and morphology measurements of yellow perch fed the cold-extruding diets containing different levels of wheat or corn flour for 10 weeks.

CHO source	CHO level (%)	Weight gain (%)	SGR (%.BW.d)	FCR	CF	CSI(%)	VSI(%)	HSI(%)
Wheat	15	233.3	1.72	1.36	1.21	88.3	7.38	1.03
Wheat	20	216.3	1.64	1.48	1.18	87.7	7.71	1.12
Wheat	25	235.1	1.73	1.37	1.22	87.6	8.13	1.44
Corn	15	206.0	1.60	1.50	1.19	88.3	7.43	0.84
Corn	20	171.8	1.43	1.74	1.16	88.1	7.67	0.96
Corn	25	218.6	1.66	1.38	1.22	87.1	8.25	1.23
Pooled SE		11.64	0.05	0.07	0.01	0.6	0.42	0.04
Source		**Means of main effect**						
Wheat		228.2a	1.69a	1.40b	1.21	87.8	7.74	1.2 0a
Corn		198.8b	1.56b	1.54a	1.19	87.8	7.79	1.01b
	Level (%)							
	15	219.7a	1.66a	1.43b	1.20a	88.3	7.41	0.93c
	20	194.1b	1.53b	1.61a	1.17b	87.9	7.69	1.04b
	25	226.8a	1.69a	1.38b	1.22a	87.3	8.19	1.33a
	ANOVA: P-values							
Source		0.009	0.008	0.033	0.001	0.968	0.898	0.000
Level		0.037	0.026	0.017	0.009	0.264	0.205	0.000
Source X Level		0.500	0.392	0.256	0.001	0.705	0.980	0.844

The initial body weight of fish was 14.8 ±0.13 (mean body weight ±SE, n=21).
Treatment means represents the average values of three tanks per treatment. Main effect means indicated by different letters are significantly different at $p \leq 0.05$ by Fisher LSD test.
WG (percentage of weight gain, %) = (FBW – IBW)/IBW × 100
SGR (specific growth rate, % body weight per day) = 100*Ln (final body weight/initial body weight)/feeding duration (days)
FCR（Feed conversion ratio） = (feed intake per tank, g) /(total final fish weight g –total initial fish weight g + dead fish g).
CF (condition factor, g/cm^3) = 100* (body weight, g)/ $(\text{body length, cm})^3$
CSI (carcass index,%) = 100* degutted fish weight (g)/fish body weight, g
VSI (viscerosomatic index, %) = 100× (viscera weight, g)/ (body weight, g)
HSI (hepatosomatic index, %) =100× (liver weight, g)/ (body weight, g)

Table 3. **Proximate composition of whole body and liver tissue of yellow perch fed the cold-extruding diets containing different levels of wheat or corn flour for 10 weeks (% wet weight).**

		Whole body				Liver		
CHO source	**CHO level (%)**	Moisture	Ash	Protein	Lipid	Moisture	Protein	Lipid
Wheat	15	66.00	4.72	18.05	11.80	59.45	12.35	16.73
Wheat	20	65.64	4.72	18.92	11.73	56.33	12.39	18.41
Wheat	25	65.14	4.63	18.02	12.59	58.38	10.79	18.46
Corn	15	66.37	4.75	18.21	11.02	62.15	13.03	14.56
Corn	20	66.48	4.87	18.15	10.50	60.60	13.06	15.38
Corn	25	65.43	4.70	17.89	11.96	61.17	11.60	15.87
Pooled SE		0.04	0.09	0.48	0.36	1.08	0.44	1.05
Source		**Means of main effect**						
Wheat		65.60	4.69	18.33	12.0a	58.1b	11.84	17.9a
Corn		66.09	4.77	18.08	11.2b	61.3a	12.56	15.3b
	Level (%)							
	15	66.2a	4.7	18.1	11.4b	60.8	12.7a	15.6b
	20	66.1ab	4.8	18.5	11.1b	58.5	12.7a	16.9ab
	25	65.3b	4.7	17.9	12.3a	59.8	11.1b	17.2a
	ANOVA: P-values							
Source		0.152	0.276	0.539	0.011	0.003	0.068	0.011
Level		0.089	0.383	0.488	0.019	0.139	0.006	0.034
Source X Level		0.500	0.761	0.813	0.623	0.687	0.723	0.985

Data is presented as mean ±SE (n=3). Treatment means represent the average values of three tank per treatment. Main effect means indicated by different letters are significantly different at $p \leq 0.05$ by Fisher LSD test.

Table 4A. Feed formulation of test diets prepared by cooking-extruding method and containing different levels of wheat or corn flour (Feeding trial-2).

Ingredients	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
			%			
Menhaden meal	35	32	29	35	32	29
Blood meal, poultry	8	7.3	6.6	8	7.3	6.6
Soy protein concentrate	16	14.6	13.3	16	14.6	13.3
Corn protein concentrate	10	9.14	8.3	10	9.14	8.3
Wheat flour	15	20	25			
Corn flour				15	20	25
Calcium phosphate dibasic dihydrate	1	1	1	1	1	1
Mineral premix	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix ARS 702	1	1	1	1	1	1
Soy Lecithin	1	1	1	1	1	1
Menhaden oil	4	4.3	4.6	4	4.3	4.6
Corn oil	3	3	3	3	3	3
Choline chloride	1	1	1	1	1	1
Yttrium oxide	0.1	0.1	0.1	0.1	0.1	0.1
Stay-C	0.4	0.4	0.4	0.4	0.4	0.4
Non- nutrition filler	4.4	5	5.6	4.4	5	5.6
Total	100	100	100	100	100	100
Estimated cost ($/kg)	1.19	1.13	1.07	1.11	1.05	0.99

Table 4B. Proximate composition of test diets prepared by a cooking extrusion method.

Nutrient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Commercial-1	Commercial-2
	% as fed							
Dry matter	98.1	98.6	98.5	98.6	98.4	98.9	93.1	92.8
Protein	47.8	44.8	41.5	46.7	42.5	39.2	41.9	42.5
Starch	11.6	14.6	17.5	12	15.4	19.1	13.5	13.6
Fat	12.4	12.1	12.6	12.2	12.2	12.6	9.82	16.2
Ash	10.8	10	9.1	10.4	10.2	9.7	9.9	7.1
Crude Fiber	1.23	1.23	1.3	1.72	1.34	1.45	3.22	2.32
NFE	29.7	32,1	35.4	29.2	32.8	36.3	31.45	27.2

Table 4C. Amino acids of the test diets prepared by a cooking extrusion method.

Amino acids	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Commercial-1	Commercial-2
Indispensable Amino Acids	% as fed (% in protein)							
Arginine	3.22 (5.73)	2.98 (5.6)	2.55 (5.11)	3.27 (5.92)	3.03 (5.88)	2.56 (5.37)	2.64 (6.3)	2.64 (6.21)
Isoleucine	2.1 (3.74)	2.04 (3.83)	1.86 (3.73)	2.22 (4.02)	2.11 (4.1)	1.84 (3.86)	1.61 (3.84)	1.79 (4.21)
Histidine	1.78 (3.17)	1.67 (3.14)	1.52 (3.05)	1.89 (3.42)	1.71 (3.32)	1.36 (2.85)	0.91 (2.17)	0.96 (2.26)
Leucine	5.04 (8.97)	5.02 (9.44)	4.33 (8.68)	5.48 (9.93)	5.09 (9.88)	4.48 (9.39)	2.95 (7.04)	3.82 (8.99)
Methionine	0.9 (1.6)	0.82 (1.54)	0.8 (1.6)	0.85 (1.54)	0.77 (1.5)	0.72 (1.51)	0.64 (1.53)	0.6 (1.41)
Lysine	2.91 (5.18)	2.79 (5.24)	2.25 (4.51)	2.94 (5.33)	2.74 (5.32)	2.02 (4.23)	2.35 (5.61)	2.63 (6.19)
Phenylalanine	2.9 (5.16)	2.56 (4.81)	2.2 (4.41)	2.83 (5.13)	2.62 (5.09)	2.35 (4.93)	1.66 (3.96)	1.97 (4.64)
Tryptophan	0.5 (0.89)	0.51 (0.96)	0.45 (0.9)	0.51 (0.92)	0.49 (0.95)	0.4 (0.84)	0.39 (0.93)	0.39 (0.92)
Threonine	2.09 (3.72)	2.53 (4.76)	1.96 (3.93)	2.68 (4.86)	2.02 (3.92)	1.53 (3.21)	1.74 (4.15)	1.92 (4.52)
Valine	3.03 (5.39)	2.86 (5.38)	2.54 (5.09)	3.2 (5.8)	2.93 (5.69)	2.55 (5.35)	1.99 (4.75)	2.15 (5.06)
Dispensable Amino Acids								
Alanine	3.43 (6.1)	3.41 (6.41)	3.05 (6.11)	3.49 (6.32)	3.42 (6.64)	3.09 (6.48)	2.31 (5.51)	2.55 (6.0)
Asparagine	4.89 (8.7)	4.66 (8.76)	3.99 (8)	4.88 (8.84)	4.69 (9.11)	4.07 (8.53)	3.14 (7.49)	3.4 (8.0)
Cystine	0.5 (0.89)	0.48 (0.9)	0.47 (0.94)	0.49 (0.89)	0.44 (0.85)	0.43 (0.9)	0.59 (1.41)	0.71 (1.67)
Glutamic acid	8.19 (14.57)	8.59 (16.15)	8.48 (16.99)	8.66 (15.69)	7.75 (15.05)	6.71 (14.07)	5.73 (13.68)	6.52 (15.34)
Glycine	2.98 (5.3)	3.02 (5.68)	2.63 (5.27)	3.3 (5.98)	3.12 (6.06)	2.6 (5.45)	2.71 (6.47)	2.52 (5.93)
Proline	3.16 (5.62)	3.5 (6.58)	2.99 (5.99)	3.41 (6.18)	3.26 (6.33)	2.8 (5.87)	2.68 (6.4)	3.01 (7.08)
Serine	2.43 (4.32)	2.55 (4.79)	2.04 (4.09)	2.57 (4.66)	2.38 (4.62)	2.09 (4.38)	2.05 (4.89)	2.39 (5.62)
Tyrosine	1.85 (3.29)	1.92 (3.61)	1.76 (3.53)	1.96 (3.55)	1.64 (3.18)	1.34 (2.81)	1.22 (2.91)	1.43 (3.36)

Table 4D. Mineral contents in test diets prepared by a cooking extrusion method.

Minerals	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Commercial-1	Commercial-2
Macro mineral	% as fed							
Sulfur	0.5	0.48	0.45	0.53	0.48	0.46	0.52	0.53
Phosphorus	2.01	1.9	1.8	1.98	1.99	1.82	1.42	1.14
Potassium	0.42	0.42	0.43	0.46	0.44	0.43	0.99	0.79
Magnesium	0.14	0.14	0.14	0.13	0.14	0.13	0.25	0.16
Calcium	2.89	2.66	2.44	2.68	2.84	2.48	2.48	1.41
Sodium	0.59	0.55	0.51	0.65	0.58	0.55	0.24	0.18
Trace mineral	mg/kg diet as fed							
Iron	465	419	405	443	411	417	244	314
Manganese	33.6	36.4	40.9	28.8	28.6	32.2	90	74.4
Copper	20.9	21	21.9	20.7	21.5	23.4	52.1	52
Zinc	112	110	108	117	113	117	109	89.8

Table 5. Growth performance and morphology measurements of yellow perch fed with cooked-extruding diets containing different levels of wheat or corn flour for 11 weeks.

CHO source	CHO level (%)	Weight gain (%)	SGR (%.BW.d)	FCR	CF	Heat shock mortality (%)
Wheat	15	282.7	1.73	0.86	1.20	87.7
Wheat	20	215.4	1.49	1.06	1.10	90.5
Wheat	25	193.3	1.40	1.13	1.05	100
Corn	15	274.4	1.71	0.87	1.22	86.1
Corn	20	209.9	1.46	1.05	1.08	92.6
Corn	25	168	1.28	1.23	0.99	91.1
Pooled SE		17.3	0.07	0.05	0.04	6.8
Source		**Means of the main effect**				
Wheat		230.5	1.54	1.02	1.11	92.7
Corn		217.5	1.48	1.05	1.10	89.9
	Level (%)					
	15	278.5a	1.72a	0.87c	1.21a	86.9
	20	212.6b	1.48b	1.05b	1.09b	91.5
	25	180.8b	1.34b	1.18a	1.02b	95.5
	ANOVA: P-values					
Source		0.379	0.008	0.391	0.632	0.623
Level		0.000	0.026	0.000	0.003	0.471
Source X Level		0.834	0.392	0.579	0.695	0.719

The initial body weight of fish was 16.3 ±0.08 (mean body weight ±SE, n=24).
Treatment means to represent the average values of three tanks per treatment. The main effect means indicated by different letters are significantly different at $p \leq 0.05$ by Fisher LSD test.
WG (percentage of weight gain, %) = (FBW – IBW)/IBW × 100
SGR (specific growth rate, % body weight per day) = 100*Ln (final body weight/initial body weight)/feeding duration (days)
FCR（Feed conversion ratio）= (feed intake per tank, g) /(total final fish weight g –total initial fish weight g + dead fish g).
CF (condition factor, g/cm^3) = 100* (body weight, g)/ $(\text{body length, cm})^3$

Table 6. Feed formulation and proximate composition of yellow perch test diets (Feeding trial 3)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Comm-1	Comm-2
	L-11/S-22	L-13/S-20	L-15/S-18	L-17/S-16	L-19/S-14		
			g/kg				
Mixed protein ingredients[1]	566	566	566	566	566		
Mixed starch[2]	220	200	180	160	140		
Menhaden oil:corn oil (2:1)	100	120	140	160	180		
Carboxymethyl cellulose	15	15	15	15	15		
Sodium alginate	15	15	15	15	15		
MP vitamin premix	30	30	30	30	30		
MP mineral premix	20	20	20	20	20		
Stay-C	3	3	3	3	3		
Calcium phosphate dibasic	10	10	10	10	10		
Choline chloride	10	10	10	10	10		
Soy lecithin	10	10	10	10	10		
Yttrium oxide	1	1	1	1	1		
Total	1000	1000	1000	1000	1000		
Proximate composition (% as fed)							
Dry matter	8.8	8.5	5.8	6.4	6.2	7.1	6.3
Protein						40.3	42.5
Lipid	11.4	13.8	15.6	16.5	18.1	10.4	16
Ash	5.6	5.8	5.3	5.8	5.4	8.9	9.2

[1]Protein ingredient mixture composition: 41.4% casein, 10.3% gelatin, 15.5% menhaden fishmeal, 31.0% soy protein isolate, 1.7% krill meal.

L:lipid; S, starch.

[2]Mixed starch, corn starch:tapioca starch=3:1

Table 7. Growth performance of yellow perch fed test diets for 12 weeks.

Dietary lipid/carbohydrate	Weight Gain (%)	SGR (%body weight. day^{-1})	Feed Conversion Ratio	Protein Efficiency Ratio	Protein Retention
L-11/S-22	311.9 ± 22.1ab	1.68 ± 0.06ab	1.22 ± 0.0	1.53 ± 0.07bc	31.4±1.9
L-13/S-20	335.6 ± 44.6a	1.75 ± 0.13a	1.14 ± 0.13	1.64 ± 0.19abc	33.3±1.7
L-15/S-18	304.2 ± 21.8ab	1.66 ± 0.0ab	1.22 ± 0.07	1.45 ± 0.09c	28.7±1.1
L-17/S-16	275.6 ± 5.8ab	158 ± 0.02ab	1.25 ± 0.02	1.43 ± 0.01c	29.8±1.6
L-19/S-14	255.0 ± 5.1b	1.51 ± 0.05b	1.29 ± 0.35	1.38 ± 0.03c	28.5±0.7
Comm-1	274.6 ± 14.6ab	1.57 ± 0.05^{ab}	1.25 ± 0.05	1.84 ± 0.07a	38.9±2.1
Comm-2	259.3 ± 18.9b	1.52 ± 0.06^{b}	1.24 ± 0.08	1.79 ± 0.11ab	38.3±1.5

The initial body weight of the fish was 8.9 ±0.27 (mean body weight ±SE, n=21).

Treatment means represents the average values of three tanks per treatment. The means within the same column indicated by different letters are significantly different at p≤0.05 by the Tukey test.

Table 8. Morphology and post heat shock survival of yellow perch fed test diets for 12 weeks.

Dietary lipid/carbohydrate	CF	HSI	VSI	VFI	GSI	Survival
		%	%	%	%	%
L-11/S-22	1.12±0.01	0.82±0.05a	7.58±0.48	2.51±0.24	2.25±0.51	31.3±12.0
L-13/S-20	1.16±0.02	0.83±0.06a	8.34±0.42	3.12±0.44	2.19±0.57	25.0±17.7
L-15/S-18	1.17±0.01	0.76±0.10a	8.20±0.41	3.08±0.32	2.24±0.60	43.8±12.0
L-17/S-16	1.14±0.02	0.82±0.04a	8.22±0.60	2.83±0.46	2.28±0.48	31.3±12.0
L-19/S-14	1.13±0.01	0.82±0.08a	7.32±0.41	2.55±0.21	2.01±0.57	12.5±7.2
Comm-1	1.10±0.01	1.50±0.10b	8.58±0.45	2.89±0.38	1.72±0.52	25.0±10.2
Comm-2	1.10±0.02	1.60±0.13b	8.81±0.50	2.95±0.46	1.86±0.61	25.0±17.7

The initial body weight of the fish was 8.9 ±0.27 (mean body weight ±SE, n=21).

Treatment means represents the average values of three tanks per treatment. The means within the same column indicated by different letters are significantly different at p≤0.05 by the Tukey test.

CF (condition factor, g/cm^3) = 100* (body weight, g)/ (body length, cm)^3
HSI (hepatosomatic index, %) =100× (liver weight, g)/ (body weight, g)
VSI (viscerosomatic index, %) = 100× (viscera weight, g)/ (body weight, g)
VFI (viscera fat index, %) = 100 x (viscera fat, g)/(body weight, g)
GSI (gonadosomatic index, %) =100× (gonad weight, g)/ (body weight, g)

Table 9. Impact of different genders on the morphology and post heat shock survival of yellow perch fed test diets for 12 weeks.

Gender	CF	HSI %	VSI %	VFI %	GSI %	Survival %
Female	1.16±0.02a	0.89±0.04b	7.43±0.04a	3.34±0.29b	0.86±0.18a	42.9±6.1b
Male	1.14±0.01a	0.74±0.04a	8.43±0.24b	2.26±0.14a	3.52±0.25b	12.5±4.3a

Data is presented as mean ±SE (n=3). A significant difference between means was indicated by different letters and determined at p≤0.05 by the Tukey test.

Table 10. Proximate composition of yellow perch fed test diets for 12 weeks.

Dietary lipid/carbohydrate	Moisture	Protein	Lipid	Ash
L-11/S-22	69.3±0.4	19.4±0.2	5.3±0.2a	4.3±0.1c
L-13/S-20	68.1±0.5	19.6±0.3	6.2±0.5ab	4.1±0.1ab
L-15/S-18	69.0±0.8	19.1±0.4	6.5±0.6abc	3.8±0.1a
L-17/S-16	66.7±0.8	20.3±0.3	7.1±0.3bc	4.7±0.1d
L-19/S-14	66.4±1.0	19.2±0.4	8.2±0.5bc	4.2±0.1ab
Comm-1	66.5±0.9	19.7±0.5	7.5±0.2c	4.7±0.1d
Comm-2	66.9±0.6	19.9±0.2	7.8±0.2c	4.7±0.1d

Data is presented as mean ±SE (n=3). A significant difference between means was indicated by different letters and determined at p≤0.05 by the Tukey test.

Table 11. Growth performance and proximate composition of yellow perch fed two different diets for 70 days at a commercial aquaponic farm.

Test Diet	Weigh gain %	FCR	HSI%	VFI%	CF	CSI%
Lab diet	85.3±3.3	1.16±0.12	1.62.3±0.12	6.94.3±0.42	1.24±0.03	85.7±0.4
Commercial diet	85.6±6.4	1.18±0.10	1.48±0.11	6.96±0.31	1.22±0.03	86.0±0.3

	Proximate composition % of wet fish			
Test Diet	Moisture	Protein	Lipid	Ash
Lab diet	64.7±0.5	19.6±0.8	8.1±0.7	4.9±0.4
Commercial diet	66.5±0.5	18.9±0.4	7.6±0.2	5.1±0.1

Data is presented as mean ±SE (n=3). A significant difference between means was determined at p≤0.05 by the Tukey test.

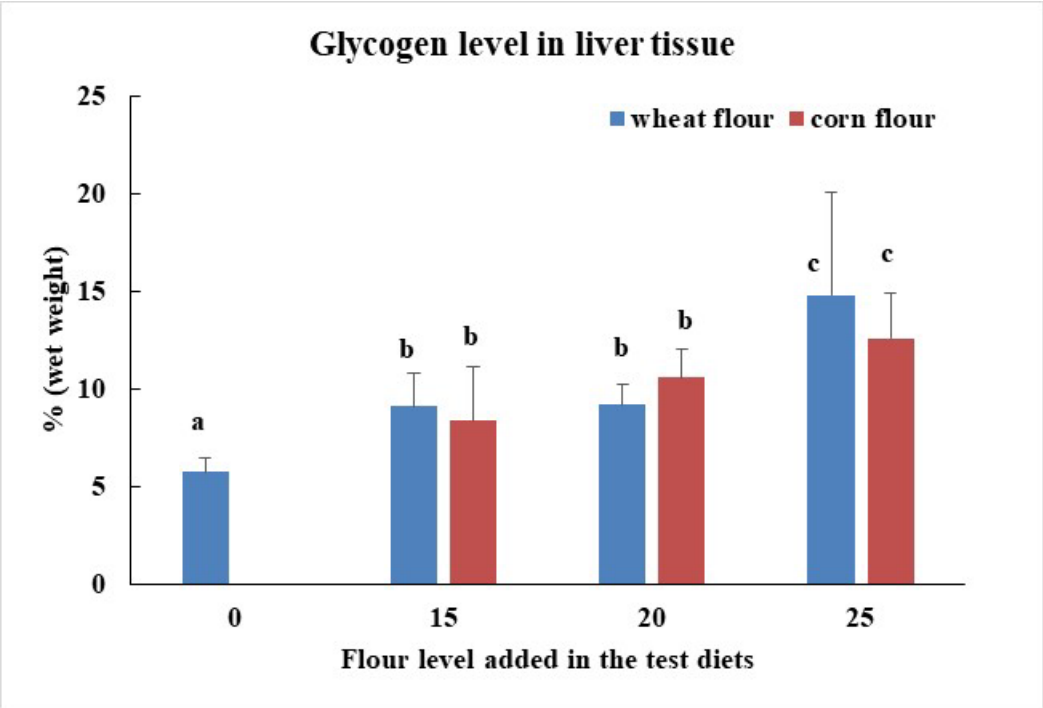

Figure 1. Glycogen levels in liver tissue of yellow perch after fasting for 24hrs. Data is presented as mean ±SE (n=5). Main effect means indicated by different letters are significantly different at $p \leq 0.05$ by Tukey test. (Feeding trial -1)

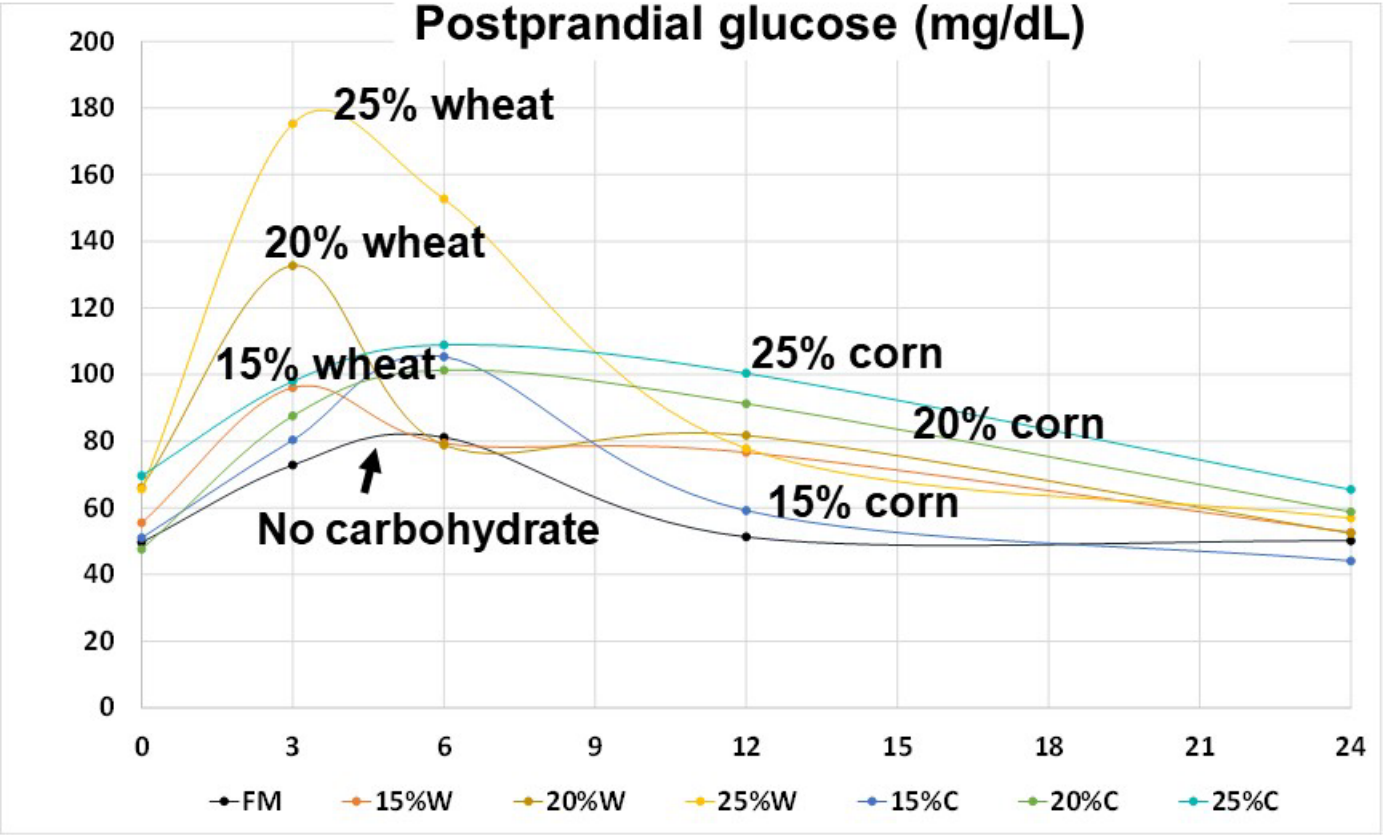

Figure 2. Blood glucose levels in yellow perch after feeding with different diets added with 15%, 20% or 25% wheat or corn flour. FM: no carbohydrate added; W, wheat; C, corn; Data is presented as mean ±SE (n=5). (Feeding trial-1).

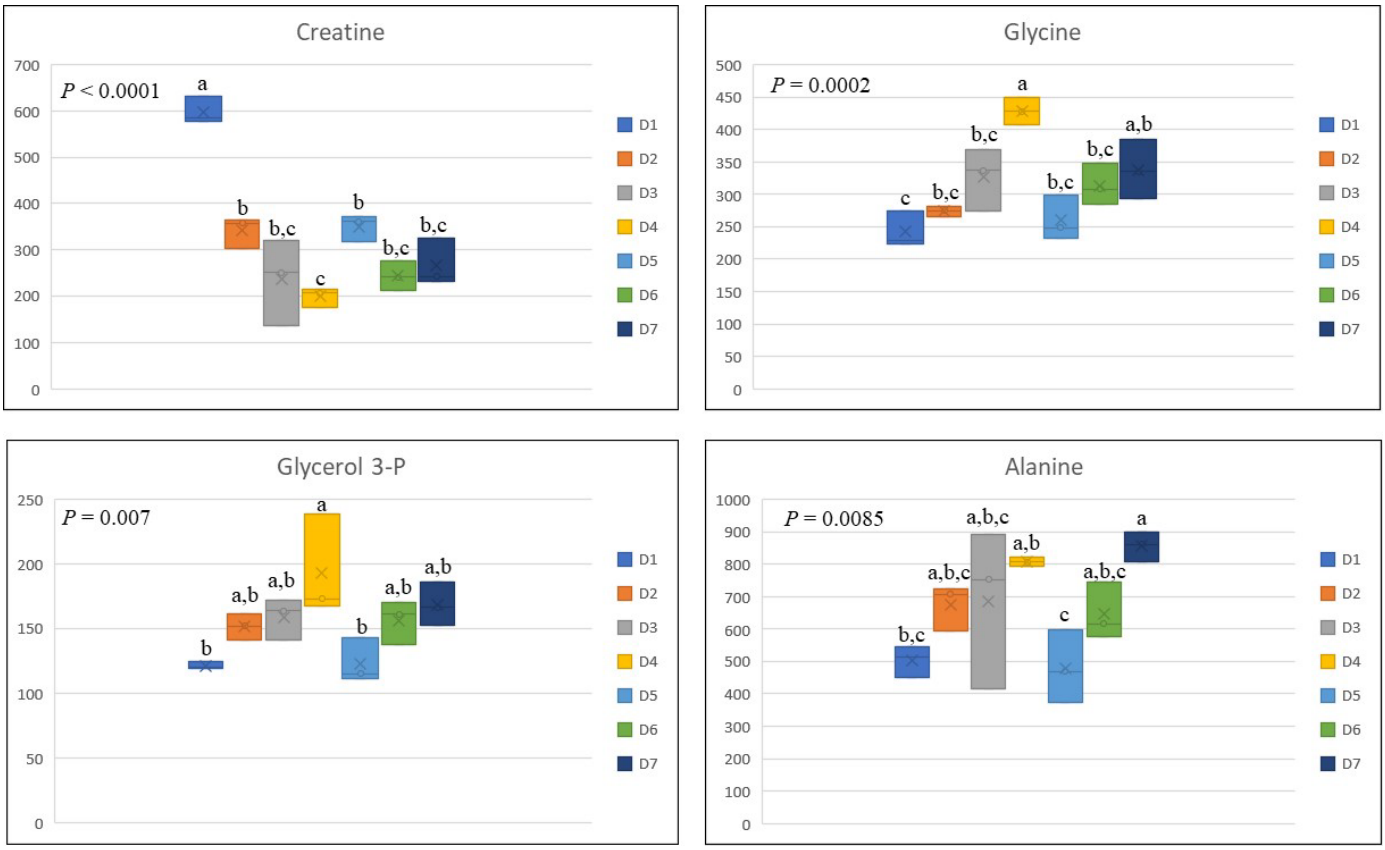

Figure 3A. Metabolite levels of liver tissues from yellow perch after feeding the test diets for 10 weeks. Data is presented as mean ±SE (n=3 tanks per treatment; Feeding trial-1)

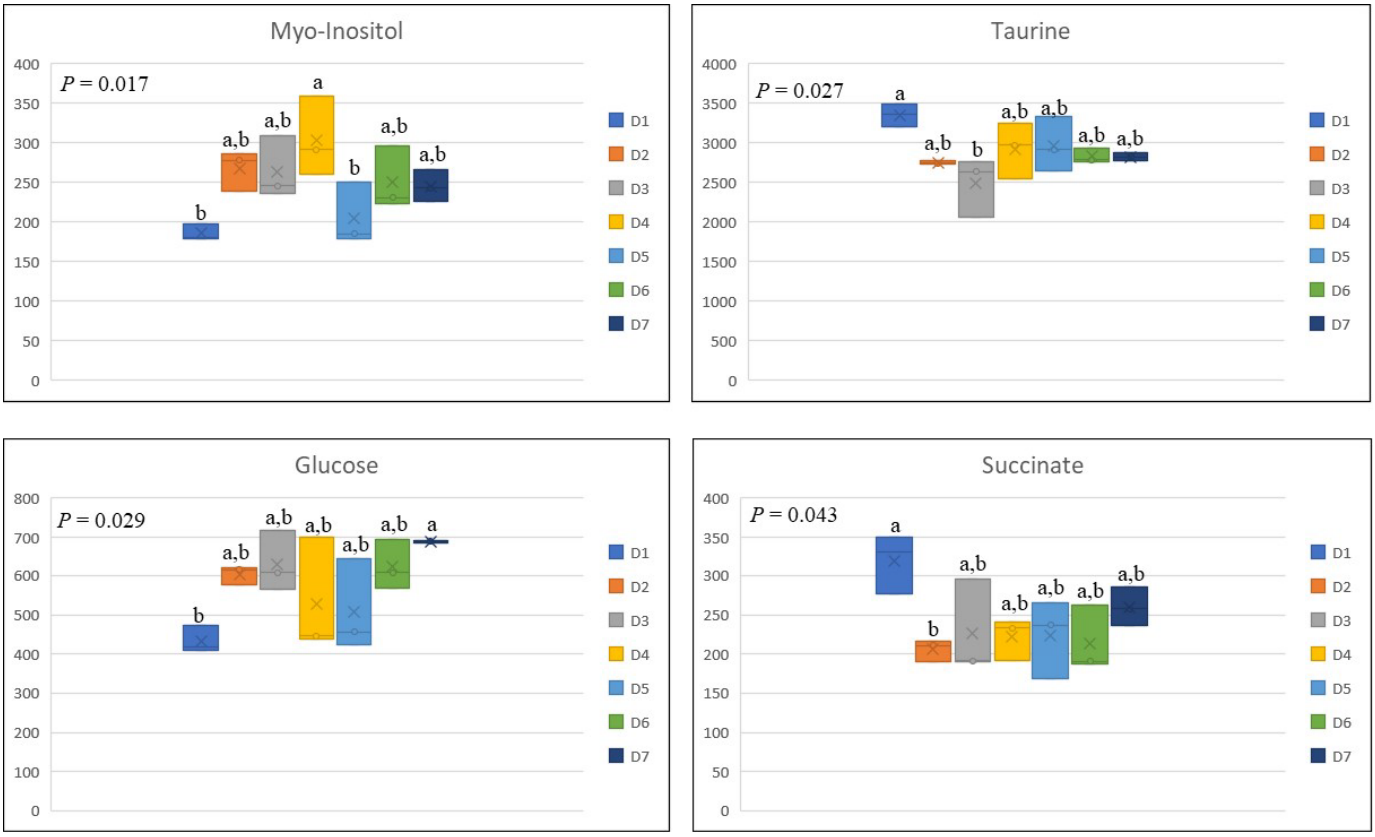

Figure 3B. Metabolite levels of liver tissues from yellow perch after feeding the test diets for 10 weeks. Data is presented as mean ±SE (n=3 tanks per treatment; Feeding trail-1).

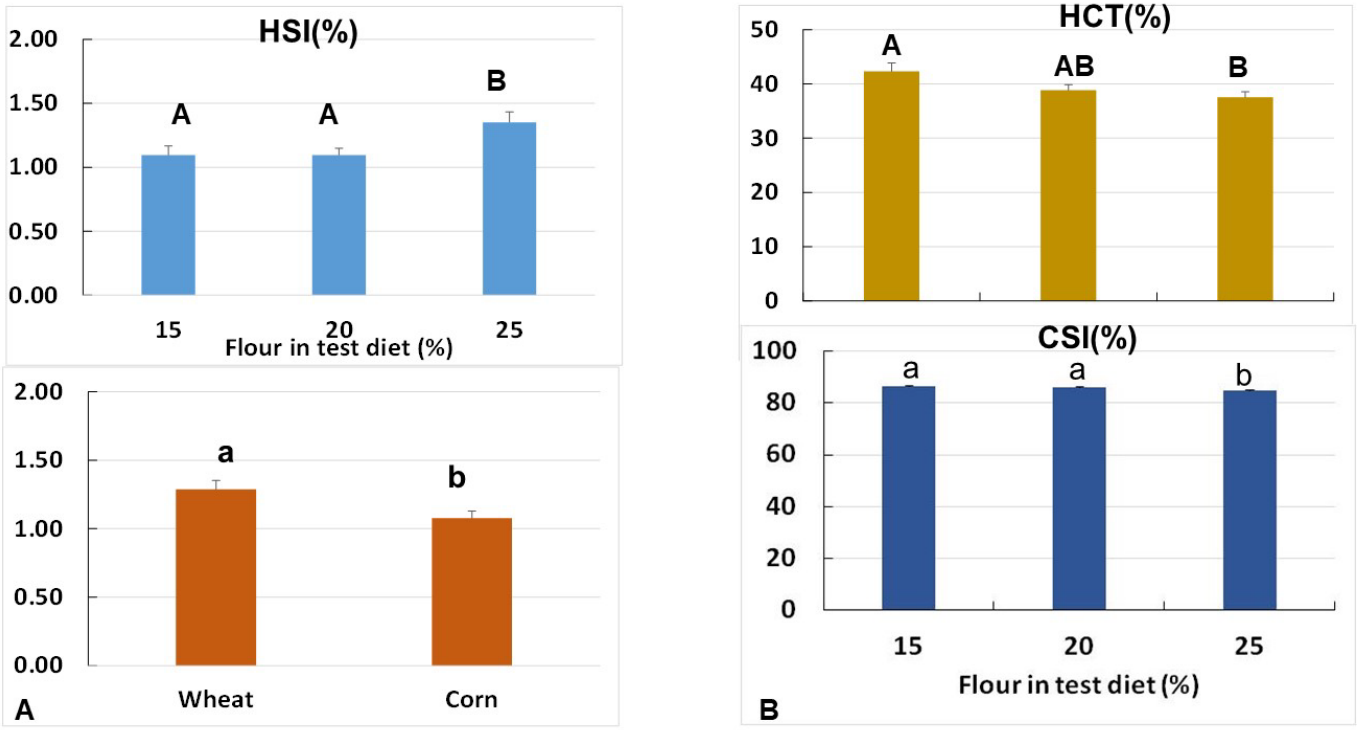

Figure 4. Weight gain, feed conversion ratio (FCR), and condition factor (CF) of yellow perch fed the test diets for 11 weeks. The means indicated by different letters are significantly different at p≤0.05 by Tukey test (feeding- trial-1)

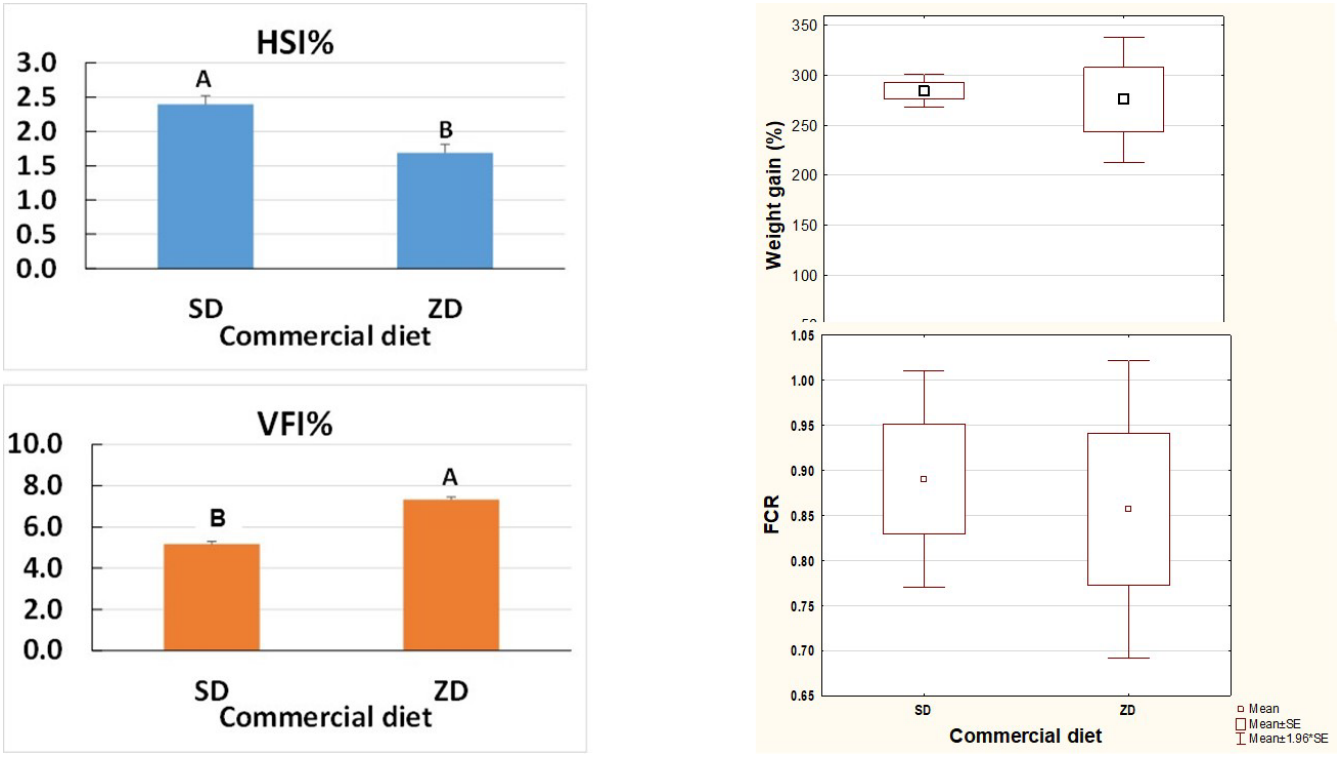

Figure 5. Weight gain, feed conversion ratio (FCR), hepatosomatic index (HSI), and visceral fat (VFI) of yellow perch fed the test diets for 11 weeks. Data was presented as mean ± SE, n=3. Different letters indicate significant differences at p≤0.05 by Tukey test. SD: commercial diet 1; ZD, commercial diet 2.(Feeding-trial-2)

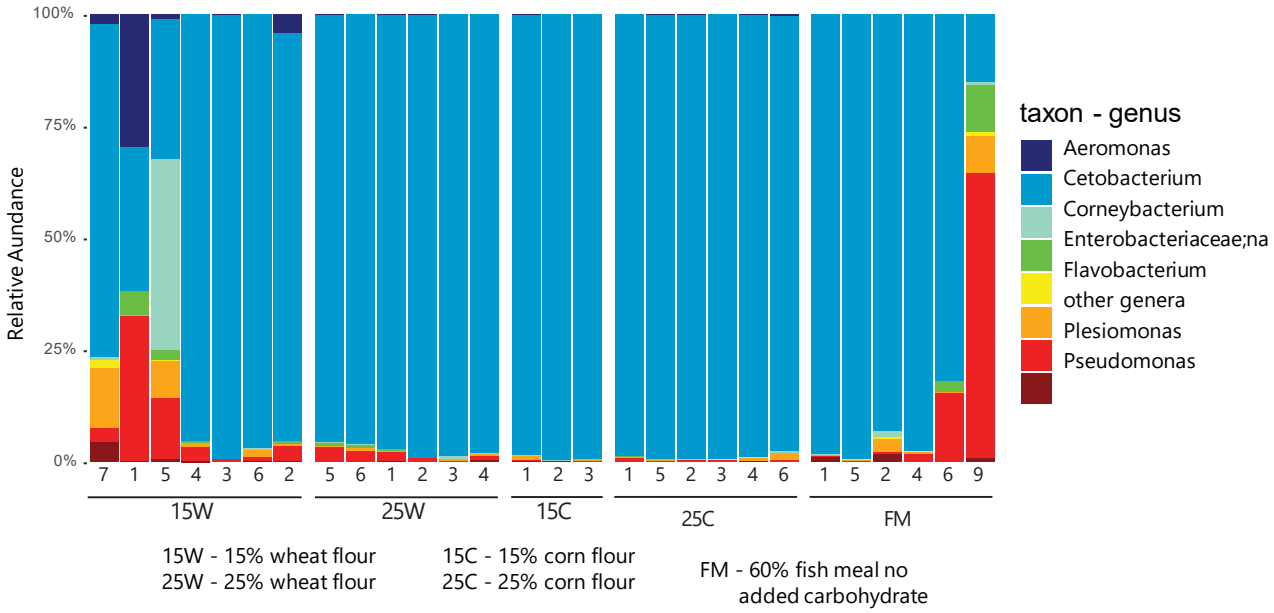

Figure 6. The microbial community of yellow perch fed different test diets for 10 weeks. Full guts were collected for microbial analysis (Feeding trial-1)

Figure 7. A 70-day farm testing at an aquaponic farm to evaluate a lab diet vs a commercial diet.