ATTACHMENT C

OPTIMIZATION OF PRACTICAL FEED FORMULATION TO IMPROVE FISH HEALTH AND PRODUCTION OF YELLOW PERCH (*PERCA FLAVESCENS*) PROJECT

DETAILED PROJECT OUTLINE FOR THE AMENDMENT TO THE PLAN OF WORK FOR GRANT #2016-38500-25753

Optimization of Practical Feed Formulation to Improve Fish Health and Production Performance of Yellow Perch *(Perca flavescens*)

Chairperson: Dong-Fang Deng, University of Wisconsin-Milwaukee

Industry Advisory Council Liaison(s): Rich Lackaff

Extension Liaison(s): Dr. Jamilynn Poletto University of Nebraska-Lincoln

Funding Request: $ 169,467

Duration: 2 Years (July 1, 2019 –June 30, 2021)

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;
4) Transfer technology and disseminate findings to industries to enhance the applications of findings.

Deliverables:

1. An optimal practical feed formulation and its production protocol for yellow perch grow out.
2. A graduate student and a part-time undergraduate student to be trained on aquaculture, feed nutrition, microbiology, and feed processing.
3. Present findings at a professional conference and publish peer-reviewed papers
4. Four students' internship training at UN and UWM; Meet with feed industry to establish collaboration of feed production.

Proposed Budgets (adjust the number of years accordingly if different than the example below):

Institution/Company	Principal Investigator(s)	Objective(s)	Year 1	Year 2	Total
University of Wisconsin-Milwaukee	Dong-Fang Deng	1-4	$49,075	$44,617	$93,692
University of Wisconsin-Milwaukee	Ryan Newton	1, 2,4	$15,153	$18,357	$33,510
Iowa State University	Kurt Rosentrater	2, 4	$15,000	$6,500	$21,500
University of Nebraska-Lincoln	Jamilynn Poletto	3, 4	$10,253	$10,512	$20,765
		Totals	**$89,481**	**$79,986**	**$169,467**

Non-funded Collaborators:

Facility	Collaborator(s)
Fingerling-YEP, Minnesota	Topher Jacobson
PortFish Ltd, Wisconsin	Pat Wilborn

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Project Summary

Fatty liver and extra viscera lipid are often seen in yellow perch *(Perca flavescens)* reared on commercial diets, suggesting that the current commercial feeds do not provide optimal nutrients for this fish. Our previous research shows that wheat starch (20% in a diet), but not corn and potato starch, induce significant fatty liver and viscera lipid accumulation in perch compared to those fed on a diet with no carbohydrate. Thus, we hypothesize that optimization of dietary carbohydrate used in fish feed will help to increase fish health and growth performance as well as reduce feed cost for yellow perch. Specifically, we will investigate how different carbohydrates influence 1) growth performance and nutrient utilization in laboratory and farm conditions; 2) fish tolerance in response to acute temperature shocks or hypoxia challenge; and 3) bacterial community composition and active community fraction in the host gut across diet regimes. This work will integrate lab studies and farm testing to evaluate production efficiency of the new practical feed compared with a commercial feed in selected farms. This proposal combines expertise in nutrition, feed processing, microbial ecology and extension research, to generate a comprehensive evaluation of feed quality. It is our goal that the outcome of this project will decrease feed cost by 20% without jeopardizing growth performance. We will use the outcome to train students and skilled workforces to support the aquaculture industry. Therefore, results of this study will benefit the research community, feed industry and aquaculture of yellow perch or other regional species of fish.

Justification

The production of yellow perch from fisheries is decreasing and harvest and recreational capture is suspended, and thus local seafood production is hindered. Yellow perch (*Perca flavescens*) are an important food fish and ecological species in the Midwestern United States. Perch are the mainstay of the regional Friday night fish fry in many Great Lakes communities. A major reason for the high demand for perch is due to its firm flesh and low fat content. Perch fillets have a long shelf life, resist damage due to freezing, and have minimal problems with off-flavor. Yellow perch are sold to retailers and restaurants primarily as skin-on fillets with a retail value $15/lb (http://www.walleyedirect.com/category/perch.html). Historically in the U.S. and Canada, perch were supplied to this market by commercial fisheries. Peak harvests of >33 million lb/year occurred in the 1950s and 60s, but by the 1990s, wild harvests declined to 11-18 million lb/yr (Malison 2003), with substantial decreases occurring in Lake Michigan (Marsden and Robillard 2004; Wilberg et al. 2005) with the exception of Lake Erie (and Green Bay), commercial fishing for perch has been terminated in the Great Lakes and quotas for sport fishing have been greatly reduce. Even if wild perch populations rebound, changes that have occurred in the Great Lakes ecosystem (invasive species and predation) and the concern over the effects of netting on the sport fisheries make commercial perch fishing a difficult proposition (e.g., Lake Michigan) (Tacon and Metian 2008; Committee 2014). Although there is renewed interest in establishing yellow perch restocking programs, for Lake Michigan, fisheries managers continue to express resistance to such efforts: “Stocking yellow perch in an attempt to bring about lake wide population recovery is not recommended at this time given recent changes in the lake ecosystem, the fact that natural yellow perch recruitment is occurring, numbers of stocked perch necessary to enhance year classes will be substantial and costly, success of stocking is unknown and the risk of introducing harmful disease or detrimental genetics may be high” (Committee 2014). Thus, aquaculture has become an important approach to produce yellow perch desired for human consumption in the Laurentian Great Lakes region.

Aquaculture production and profitability of yellow perch are challenged by suboptimal feed. Current commercial feed causes adverse impact on yellow perch health and production efficiency. Despite declines in wild populations, consumer demand for yellow perch remains high, and the reduced supply of wild fish has driven the development of commercial perch aquaculture. Perch aquaculture technology has improved steadily over the past few decades, with some major obstacles, such as larval production, having been partially addressed. Several bottlenecks remain, including the slow growth rate and lack of commercial grow-out diets that are optimized for improved performance and nutrient utilization of intensively-farmed perch. Perch producers continue to use a range of commercially-available grow-out diets formulated based on the requirements of rainbow trout or based on the results of a small number of laboratory studies. These formulations are suboptimal for perch grow-out, leading to poor nutrient utilization and physiological maladies that decrease animal performance, collectively adding to production costs and nutrient waste (Brown et al. 1996). A high level of visceral lipid and a fatty liver are commonly observed in yellow perch fed with trout diets (Fig. 1). Visceral lipid in yellow perch accumulates up to 10% of body weight versus the

level of < 5% of body weight in a wild harvested fish of the same age (not published data) from our lab study). The high visceral lipid accumulation and fatty livers indicate that the feed energy/nutrients are not utilized for a maximum growth and necessary physiological functions. Similar symptoms have been documented in other species of fish due to either lacking nutrients or overloaded nutrient/energy (Tacon 1996; Caballero et al. 2004). The long lasting impact of a nutritionally unbalanced diet affects fish growth and likely other physiological functions, such as stress response and reproduction.

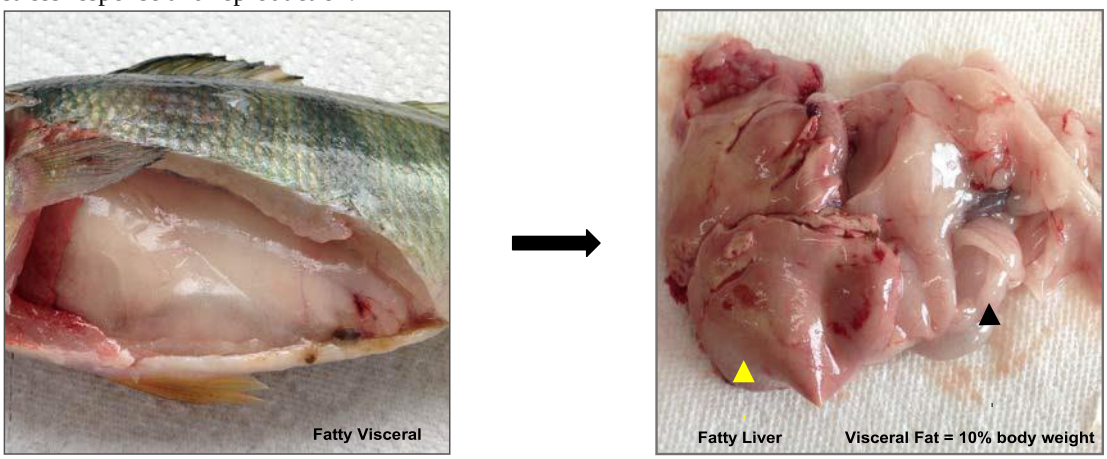

Figure.1. Fatty liver and accumulated viscera fat in yellow perch fed a commercial feed for 6 months.

Current information is insufficient to bridge results from lab-based diet studies to practical commercially-relevant application. While previous studies shed some light on the necessity of specific nutrient requirements of yellow perch, there is limited applicability to practical grow-out diets in a commercial setting. First, most laboratory studies were based on the use of semi-purified feed. These formulated feeds were developed typically with highly digestible feed ingredients, such as casein and gelatin as a protein source instead of fishmeal or soybean meal, and dextrin or hydrolyzed starch as carbohydrate sources instead of wheat or corn flour (Brown et al. 1996; Twibell and Brown 1997; Twibell and Brown 2000; Twibell et al. 2000). It has been shown in many studies that purified or semi purified ingredients illicit fish responses, such as in digestibility, palatability, and nutritional quality, which do not actually represent the actual responses in fish fed a practical feed commercially. In addition, some of the nutrient requirement results are dated and will need further investigation due to the improvements in culture conditions, understanding fish genetics and feed ingredients/formulations, and feed processing technology.

Depending on the production purpose, available equipment, and economic input, aquatic feed is processed in different ways, which mainly include: 1) cold forming by meat grinder; 2) steam pelleting; and 3) extrusion. Most semi-purified diets used in lab studies were processed via a cold-extruded method using a meat grinder. This method does not involve high temperature and pressure pelleting or extruding, which are used normally in the feed industry. Of these feed processing methods, extruding technologies are also best for species targeted feed formulations because they allow for control over nutritional quality (such as digestibility and nutrient contents) and physical quality (water stability, hardness and density) of feed pellets. For example, the cold forming and steam pelleting methods are only capable of producing sinking pellets. The difference in pellet buoyancy can affect fish feeding efficiency and growth performance. A previous study by Creswell (2005) found that extruding floating pellets led to higher growth than sinking pellets fed to hybrid tilapia. Catfish, and carp are also known to prefer floating feed. The extrusion method can produce feed pellets with different buoyancies, which allows for optimization to species preferences. For this study, we know yellow perch prefer slowly sinking feed. Pellet water stability and durability are also influenced by the processing method including feed formulations (Kraugerud et al. 2011; Sorensen 2012). Typically, extruded pellets have better water stability and durability than cold forming or steam produced pellets. Beside the effects on physical quality, different processing methods also change the nutritional quality of pellets and thus influence the utilization of dietary nutrients. A high cooking temperature during extrusion can lead to destruction of anti-nutritional factors, pathogenic organisms and viruses in the feed (NRC 2011; Sorensen2012). Gelatinization of carbohydrate due to high temperature cooking can also increase the binding capacity of ingredients

and alter feed intake or nutrient digestibility (Barrows et al. 2007; Lundblad et al. 2011; Morken et al. 2012). Therefore, results generated from semi-purified diets made by cold extruding method do not readily transfer to practical formulations.

Finally, it is critical to test feed formulations under both lab and farm conditions. Often feed management or formulations need to be modified to scale appropriately to farm production. Modifications can result from farm conditions with different production temperatures, and dissolved oxygen, photoperiod, non-feed nutrient availability, and stocking densities. Each change in culture condition can affect the outcome of fish production. Thus, information generated from a farm testing is necessary component of establishing feed formulation and feeding management protocols.

Overall, the primary challenge for feed optimization is to increase the feed supply to meet industry growth while decreasing costs and environmental impacts from feed components. Fishmeal is the traditional protein source for carnivorous fish feed, like that used for yellow perch. Fishmeal provides dietary requirements for protein synthesis, energy needs, and other physiological functions (NRC 2011). Increased fishmeal use requires large capture fish harvests, which are expensive and not sustainable long-term (9). The aquatic feed industry is shifting to plant-based ingredients to replace fishmeal or minimizing dietary protein levels by increasing lipids or carbohydrates, even for carnivores (Tacon et al. 2011). Many advances have been made in this area, so that in some cases fish production performance on plant-based diets is comparable to that from fishmeal diets (Carter and Hauler 2000; Davidson et al. 2013). However, this strategy unavoidably results in the inclusion of carbohydrate sources from plant meals (such as corn meal) that are not found in fishmeal; and the amounts and types of carbohydrates vary widely depending on the plant used. Carbohydrates are the cheapest energy source and play an important role in the physical properties of feeds (i.e., binding, stability, prevention of nutrient leakage) (Hemre and Deng 2015; NRC 2011). Utilization of carbohydrates to spare protein can reduce protein catabolism and decrease the amount of ammonia released to the environment from protein oxidation. It is critical to optimize dietary carbohydrate in commercial feeds to address the issues of feed cost and environmental pollution related to aquaculture.

Feed cost accounts for 40-60% of aquaculture production. This proposal's focus is relevant to aquaculture of the Laurentian Great Lakes Region because the results will lead to a targeted feed for yellow perch production. In current yellow perch aquaculture, expensive commercial feeds containing fishmeal as the major protein source are standard, leading to a feed cost of $1.8-2.2/kg feed (Purina Animal Nutrition, LLC). Some farms have used feed formulated with decreased fishmeal use by increasing soybean meal, which decreased the feed cost to $1.1/kg. However, a slow growth and poor fish health were observed for yellow perch with this formulation (communication with farmers). With this project, we intend to optimize a feed formulation based on nutrient requirements determined for yellow perch to decrease feed costs but maintain fish production performance. Developing yellow perch-specific diet formulations will make this fish a more sustainable aquaculture product. The PIs of this proposal are combining their expertise in nutrition, feed processing and life cycle analysis, microbial ecology, and aquaculture extension education, to provide a wealth of baseline data from which to build an understanding of host-diet-microbiota interactions specific to aquaculture management. The information gained from these studies can be used by the feed industry to produce feed targeted for yellow perch. The outcome will also help fish farmers alter aquaculture best practices to enhance fish health and production. In the ongoing development of feed processing technology, the cooking extruding method is becoming a popular approach for feed manufacture. This technology requires a certain level of carbohydrate to achieve accepted physical feed pellet quality. Thus, it is important to understand the impact of different carbohydrates on fish health, especially for carnivorous fish, which typically have poor capacity in carbohydrates utilization. The knowledge gained in the current proposal will be applicable to other candidate aquaculture species of fish cultured in the region, such largemouth, hybrid striped bass, and walleye. By integrating lab research and farm testing, the results will be ready for the feed industry to establish production protocols. The project will also provide a platform for training skilled workers, offering students with hands-on experience in rearing fish, and bridging the knowledge gap between lab research and industrial applications. Thus, the research outcomes will benefit scientific research, the feed industry, and aquaculture stakeholders.

Related Current and Previous Work

Many attempts have been made to study the nutrient requirements of juvenile yellow perch (Twibell and Brown 1997; Twibell and Brown 2000; Twibell et al. 2000; Hart et al. 2010), but available information is still relatively limited compared to other common aquaculture fish species, such as channel catfish (*Ictalurus punctatus*), Atlantic salmon (*Salmo salar*), Nile tilapia (*Oreochromis niloticus*) and rainbow trout (*Oncorhynchus gorbuscha*). For yellow perch, a minimum of 36% crude protein was recommended in practical diets by Brown et al. (1996). A lower protein level, 21-27% in a semi-purified diet, was recommended by Ramseyer and Garling (1998), but the feeding trial was not long enough for the fish to obtain weight gain >300%. Thus, the conclusion needs confirmation. A series of studies determined the requirements of some indispensable amino acids including lysine (Twibell et al. 1998), arginine (Twibell and Brown 1997), and methionine (Twibell et al. 2000; Hart et al. 2010) using semi-purified diets. A lower dietary lipid of <10% has been found to promote the good growth of juvenile and growth-out yellow perch under laboratory conditions (Brown et al. 1996; Cartwright 1998; Ramseyer and Garling 1998; NRC 2011; Mjoun et al. 2012). Dietary lipid in practical diets for growing yellow perch ranges from 6 -15%. No study has investigated the optimal level of dietary carbohydrate for yellow perch.

It is generally accepted that fish do not have a dietary requirement for carbohydrates, because glucose can be synthesized via gluconeogenesis from amino acids and other non-glucose substrates. However, numerous studies with fish have shown that digestible carbohydrates (principally starch) included in formulated feeds serves an energy substrate, sparing protein and lipid for essential metabolic functions and growth, although the ability to do so varies greatly among fish species (Hemre and Deng, 2015). NRC (2011) recommends maximum dietary inclusion levels of 10-25% digestible starch for carnivorous species such as salmonids and marine fish. To date, no published studies with yellow perch have specifically addressed this question. A highly digestible starch derivative, dextrin, has been included at levels of 30-40%, where it serves as an energy source to determine the optimal protein and protein/energy ratio in semi-purified diets for this species (Ramseyer and Garling 1998). However, fish livers in this study were documented to have increased weight and discoloration (Ramseyer and Garling 1998). Also, the feeding trial was conducted to only ~100% weight gain, which is relatively low for typical determination of nutrient requirements. It is likely the study under estimated the impact of test diets on the fish. A few studies have examined sustainable protein sources (soy meal, wheat gluten meal, dried distillers grains solids and carp meal), as replacements for fishmeal in yellow perch grow-out diets (Kasper et al. 2007; Schaeffer et al. 2011; Kwasek et al. 2012; Schaeffer et al. 2012). Replacement of up to 50% of fishmeal protein with these alternatives could be suitable for yellow perch grow-out, but may result in reduced growth (Brown et al. 1996; Kwasek et al. 2012), high visceral fat (10%-20%) and high liver somatic indices (fatty liver) (Brown et al. 1996; Ramseyer and Garling 1998; Kasper et al. 2007).

Corn, potato, and wheat starches are the most common carbohydrate sources for fish feeds (Hemre and Deng 2015; NRC 2011). Their characteristics vary in many ways, but the amylose to amylopectin ratio is one of the major differentiating factors. A starch's molecular characteristics also impact the physical and nutritional properties of feed, such as hardness of pellets, water stability, and nutrient digestibility (Stone 2003). Rainbow trout have a wide range of digestibility on different starches when they were added to experimental diets (Bergot 1993). To our knowledge, no study has investigated the utilization of different carbohydrate sources in yellow perch or how type or level impacts growth/health. Our recent study showed that the growth of yellow perch was not affected by different carbohydrates (corn, potato, wheat), which was used to replace 20% fishmeal in the non-starch diet (Figure 2A). However, the yellow perch fed the wheat starch diet developed a fatty liver as indicated by the increased size

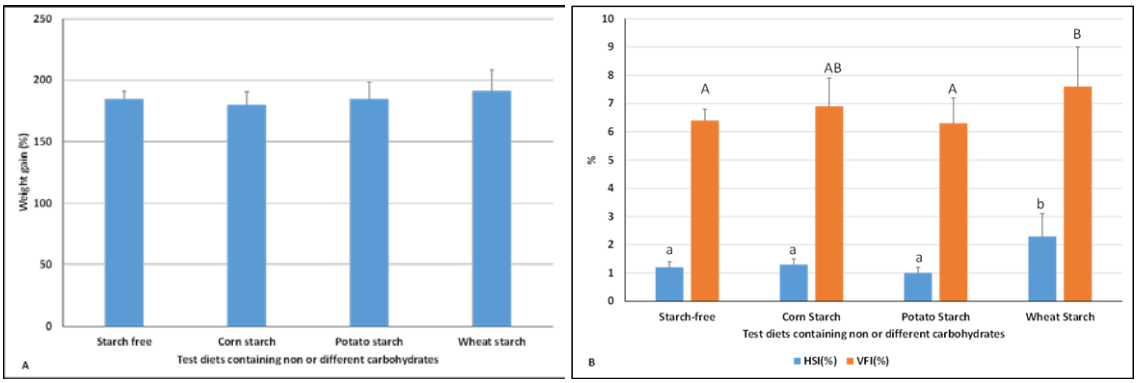

Figure 2. Effect of test diets containing different carbohydrate (20% in the diet) on the performance of yellow perch juveniles after 8 weeks of feeding. HSI (hepatosomatic index):100* liver weigh/ fish weight; VFI (visceral fat index): 100*visceral fat/fish weight.

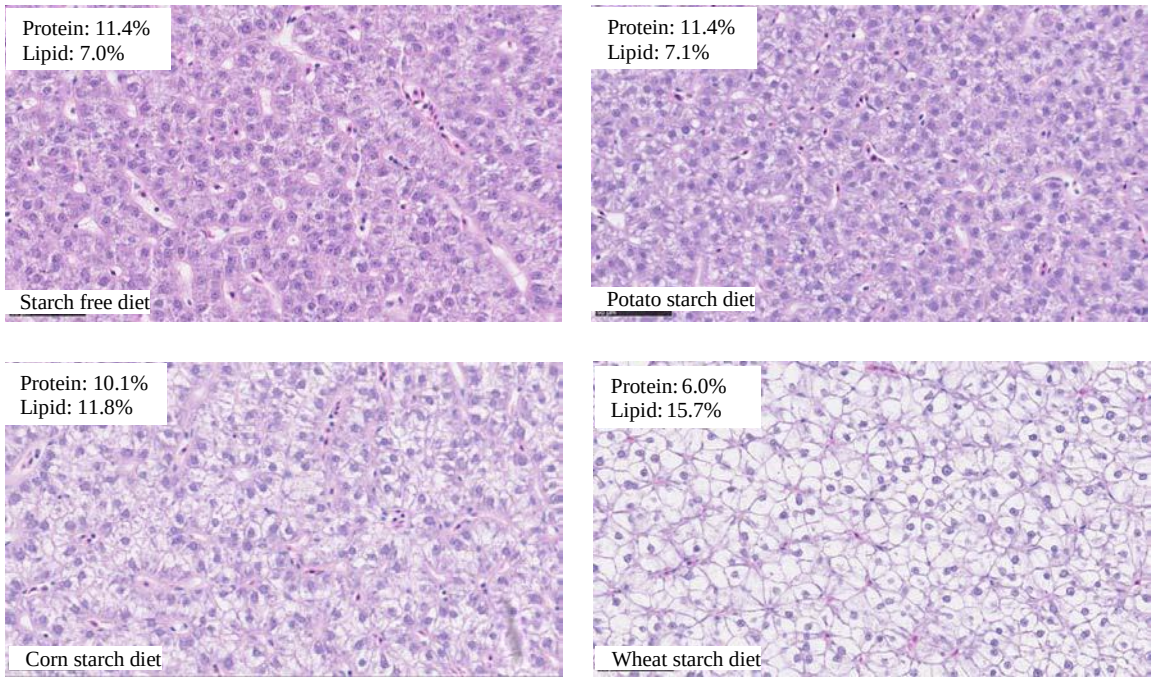

Figure 3. Effect of different carbohydrate on the histology and nutritional composition of liver tissue from yellow perch fed the test diets for 8 weeks.

and lipid content of liver tissue, enlarged liver cells, and increased lesion scores based on histological evaluation (Figure 2B, Figure 3, Table 1). Furthermore, PCA score of liver metabolites based on metabolomics analysis was significantly separated between the fish fed the non-starch diet and the wheat starch diet (Figure 4). Fish fed the corn starch diet and the potato starch diet had overlapping scores with the fish fed the no-starch diet. These results indicated that potato or corn starch may have less negative impact on yellow perch health than wheat starch, and thus could lead to better production efficiency in a long-term study. It seems suggesting that carbohydrate selection in a practice feed is critical for practical feed production. Further studies will be needed to test this hypothesis on commercially practical feed formulations and testing conditions. The information is essential to understand carbohydrate utilization mechanisms and the development of a cost-effective feed.

Table 1. Histological evaluation of liver tissue from yellow perch fed test diets for 8 weeks

Group	No-starch	Corn starch	Potato starch	Wheat starch	*P value*

Liver cell diameter(μm)	14.7±1.5[a]	15.5±0.4[ab]	15.7±0.5[ab]	17.7±1.3[b]	0.039
Kupffer cell number	2.9±0.3[a]	2.0±0.4[b]	1.5±0.2[b]	0.6±0.1[c]	0.000
Classification score	1.7±0.6[c]	2.9±0.2[b]	2.4±0.3[bc]	4.0±0.0[a]	0.000

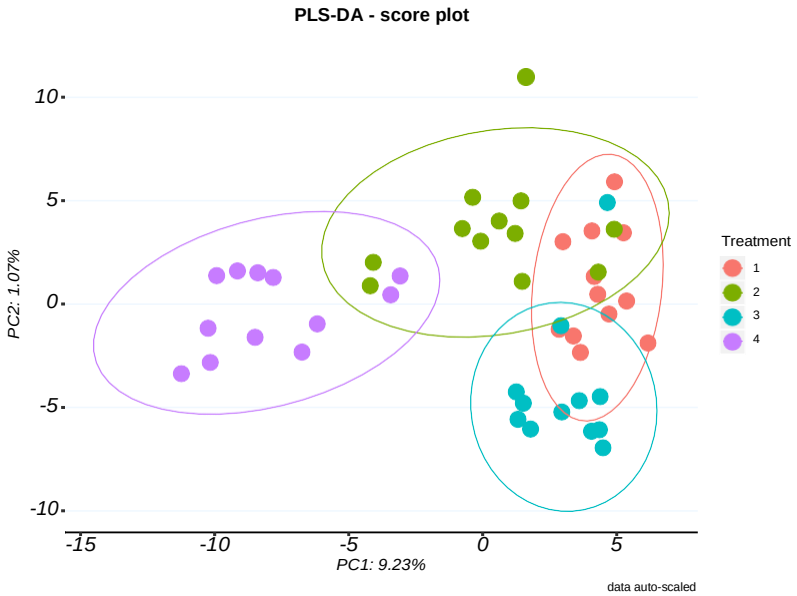

Figure 4. PCA scores measured based on major significant compounds in liver of fish fed different carbohydrates. (Diet 1, starch free; Diet 2, corn starch; Diet3, potato starch; Diet 4, wheat starch).

Traditionally, feed formulation quality is evaluated based on fish growth performance, physiological response or biochemical and molecular marker measurements. Recent interest in the microbiota associated with farm-raised fish has advanced the understanding of healthy aquaculture-associated bacteria (Wong et al. 2013) and the potential for probiotic or other microbial technologies (Defroirdt et al. 2011, De Schryver and Vadstein 2014). It is now known that both temporary and permanent disruption of the native microbial communities that inhabit hosts can result in disease or illness (Round and Mazmannian 2009; Lozupone et al. 2012). Microorganisms in the gut are extremely diverse and have an immense but highly partitioned (among taxa) capacity to metabolize the spectra of compounds found in vertebrate host diets (Scott et al. 2013). Diet therefore represents a specific selective pressure that influences community interactions. Alterations to diet can lead to decreased host energy utilization from food products or synthesis, via shifts in the microbial community or by lost functional capability (Lozupone et al. 2012). It is now thought that environment rather than host genetics is the primary determinant of intestinal microbiota in humans (Rothschild et al. 2018). In fish, where the environment is more intimately interacting with host surfaces, and especially in aquaculture where diet is controlled, the interactions between host, diet, and host microbiota may have an even larger role in host health (Llewellyn et al. 2014), but remain largely unknown. Previous studies involving fish and diet manipulation have shown that the inclusion of soya protein (Ringø et al. 2006; Green et al. 2013) mixed grains (Wong et al. 2013), and chitin (Zhou et al. 2012) can result in gut microbial composition shifts. For example, different carbohydrate sources had diet-specific effects on bacterial profiles of intestinal mucosa of sea bass (Gatesoupe et al. 2014). Yellow perch fed commercial diet with 40% protein including soybean meal as major protein source, and 10% lipid, have been shown to damage intestines within 6 weeks after feeding (communication with farmers). From these early studies, research is now moving toward identifying whether fish gut microbiota alter fish development or health as well as feeding efficiency. Recent studies have shown a correlation between fish growth and intestinal microbiota composition (Forberg et al. 2016; Trinh et al. 2017), suggesting that intestinal microbial communities play a role in fish growth. This also suggests that manipulation of fish microbiota could be used to improve aquaculture production. In our own preliminary work, we found both feeding rate and feed formulations alter intestinal microbial communities, but in different ways. Low (by body mass) feeding rates

delayed microbial community succession observed for fish provided higher rates, while diet formulation altered the dominant taxa present in the intestines (Figure 4). These preliminary results indicate the importance of feed quality/quantity on intestinal microbial communities, which may serve as a new approach for evaluation on fish feed or feeding management. Thus, it is imperative to incorporate microbial data as a criterion in feed optimization schema including ingredient selection and feed formulation evaluation if we are to truly optimize fish feed formulation. No study has been investigated the interaction between dietary nutrients and intestinal microbial community in yellow perch.

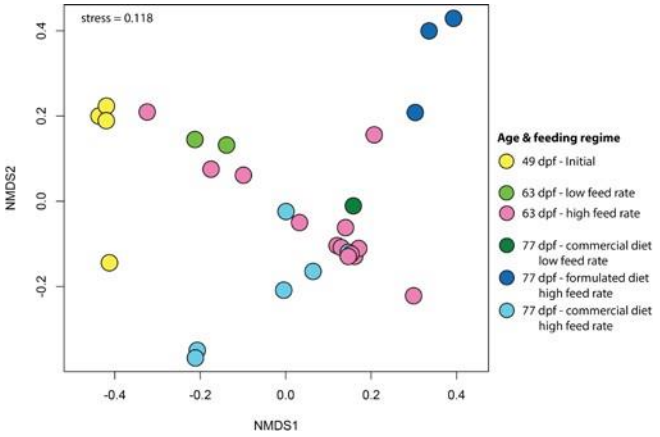

Figure 4. Nonmetric multidimensional scaling plot of bacterial community composition from *Acinpenser fulvescens* fingerling intestines grown under different feeding regimes. dpf = days post fertilization, Initial: first day of the feeding trial, Low feed rate: fed at < 5% of body weigh per day, High feed rate: fed at ≥5% of body weigh per day. Ordination stress is indicated on the plot.

Furthermore, feed quality or quantity can change the fish nutritional stuatus, which could result in different tolerances to stress. A previous study on cod (*Gadus morhua* L.) had found that in response to handling, a significnatlly higher stress level was dectedted in fish fed a carbohydrate diet than those fed a carbohydrate free diet (Hemre et al. 1991). Different feeding rates or starvation has been reported to cause poor nutritional conditions and less tolerance to temperature shock based on the response of stress protein expression in white sturgeon, *Acipenser transmontanus* (Deng et al. 2009; Han et al. 2012). Fluctuations of water temperatures and dissolved oxygen levels are commly observed in aquaculture systems. These two factors play critical roles in supporting fish performance. Thus, it is important to integrate stress challenges of temperature or dissolved oxygen into a nutritional study for feed evaluation.

Statement of Duplication of Research

The research activities proposed in this project are an original research and do not duplicate any previously funded projects based on records by USDA Current Research Information System and NOAA database. The following keywords have been used to search for funded project and publications: yellow perch, carbohydrate, feed nutrition and microbial community. The outcome of this project will generate innovative information for practical feed production to support yellow perch aquaculture.

Anticipated Benefits

First, with the high demand for yellow perch in the aquaculture and aquaponic industries, the supply of cost-effective feed is a critical hindrance preventing the expansion of these industries. The success of this project will benefit fish farming by providing a protocol for stable and cost-effective yellow perch feed. Second, the feed industry can make use of our findings to produce specific feed for yellow perch. Third, the microbiota component of this project has the potential to change a wide range of aquaculture practices in the long-term. Currently, the majority of fish-microbiota studies remain relatively small in the number of samples and depth of microbial coverage. The data collection proposed would expand greatly the scope of this research to include more biological replication and a measure of microbial activity for fish-microbiota interactions. The data generated also will provide baseline information to assess community inter-individual variability, identify dominant host bacterial community members, and relate diet-induced changes to changes in host metabolism and growth and host microbiota. From these data, we will be able to identify more refined questions that could lead to significant improvements in common indoor aquaculture rearing/feeding practices akin to insights that are now developing around host-microbiota relationships and human health. Food microbiology is an active area of research across all aspects of life, and our work will contribute to a growing understanding of the importance of diet and host microbiota for host health.

Furthermore, the development of a fatty liver is not unusual for aquaculture-raised fish. It can be caused by different factors such as imbalanced dietary nutrients, suboptimal culture conditions, and feeding management. Even with the same nutrient levels (such as carbohydrate), different nutrient sources could lead to a fatty liver, as we observed in yellow perch in our preliminary study. In the ongoing development of feed processing technology, the cooking extruding method is becoming a popular approach for feed manufacture. This technology requires a certain level of carbohydrate to achieve accepted physical feed pellet quality. Thus, it is important to understand the impact of different carbohydrates on fish health, especially for carnivorous fish, which typically have poor capacity in carbohydrate utilization (NRC 2011). The knowledge gained in the current proposal will be applicable to other candidate aquaculture species of fish cultured in the region, such largemouth, hybrid striped bass, and walleye. Research on the interaction of carbohydrate/nutrients, microbial communities, and fish health/growth is still in its infancy. If we discover correlations between gut microbial communities and feed formulations and fish health/growth, then these findings will provide a baseline for exploring hypotheses regarding these interactions in both yellow perch and among other aquaculture species. In addition, all microbial community data provides a value toward identifying the microorganisms that associate with fish intestines. So that future studies can determine those that are ubiquitous among and specialized to particular fish species, thereby initiating the creation of databases that can be used to develop new methods for monitoring or predicting fish health. This project will also provide integrative training including nutrition, microbiology, feed processing and extension experience graduate students. NCRAC or other related agents, who can also make use of the compiling result for extension education.

Objectives

The ultimate goal of this proposal is to increase the profitability of yellow perch aquaculture by developing nutritionally balanced and cost-effective feed. As a part of this goal, we will decrease fishmeal protein reliance by optimizing carbohydrate utilization. We hypothesize that corn flour will be better utilized as an energy source than wheat flour by yellow perch. The effect of different carbohydrates on yellow perch will vary depend on their levels.

To achieve this goal, the specific objectives of our 2-year project is to:

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.

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;

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

5. This proposal will be an integrative project between extension education and research, with collaborations among scientists with specialties in nutrition, feed processing, and microbial ecology and experts in aquaculture extension as well as industry stakeholders.

Deliverables

1. An optimal practical feed formulation and its production protocol for yellow perch grow out.
2. Trained graduate students and undergraduate students on aquaculture, aquatic feed nutrition, microbiology, and feed processing.
3. Published papers on research findings on interaction of dietary nutrition and gut health as well as stress tolerance of yellow perch. Annuals reports to NCRAC
4. Presentations on professional conference and extension workshop. Meet with feed industry to establish collaboration of feed production.

Procedures

Task 1 (Year-1 &2)). Evaluate the effects of carbohydrate sources and levels on the growth performance and nutrient utilization of juvenile yellow perch

This task is to address the objectives 1&2 proposed in this study. A two-way factorial design will be used to test nine experimental diets including two carbohydrate sources (wheat starch and corn starch) at three levels each (14, 20 and 26%). Our previous study showed that corn starch and potato starch had similar effect on yellow perch. In this proposal, corn flour is selected instead of potato flour because it is more commonly used than potato starch. Six diets will contain different levels of protein/carbohydrate but will contain the same level of lipid (12%). The quality of protein will remain the same for all test diets, but the protein levels will vary between 37-42%, which is in the range suggested for yellow perch (Brown et al. 1996). Two commercial diets (Zeigler Bro. Inc and AquaMax) commonly used by regional farmers will be used as reference diets. All feed ingredients will be pulverized to less than 400 μm particles before they are extruded using the method described in Task-2. Yttrium oxide will be added into test diets as an inert marker to measure the apparent digestibility coefficient of different test diets.

Yellow perch diet number	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Estimated protein level (%)	**43%**	**40%**	**37%**	**43%**	**40%**	**37%**
Ingredients	g/100 g					
Menhaden meal (63%CP)	36.0	33.1	30.2	36.0	33.1	30.2
Soybean meal (47%CP)	6	5.5	5.0	6	5.5	5.0
Soy protein concentrate (65% CP)	6	5.5	5.0	6	5.5	5.0
Corn protein concentrate (63% CP)	18	16.6	15.2	18	16.6	15.2
Wheat flour	14.0	20.0	26.0			
Corn flour				14.0	20.0	26.0
Menhaden oil	3.0	3.3	3.6	3.0	3.3	3.6
Soybean Oil	4.0	4.0	4.0	4.0	4.0	4.0
Others	13	12	11	13	12	11
Total	100.0	100.0	100.0	100.0	100.0	100.0
Estimated cost of feed ingredients ($/kg)	0.97	0.92	0.88	0.96	0.90	0.85

Others: includes premix of vitamins, minerals, Y_2O_3, and cellulose and non-nutritional filler.

Palatability: Yellow perch fingerlings (3-6 g) will cultured in an indoor system with flow through water supplied at 3 L/min to provide water quality meeting the optimal growth of this fish following established protocols: water temperature, 21-23 ºC; dissolved oxygen, >6.0 mg/L; NO_2 <0.05 mg/L; total ammonia nitrogen, <0.08 mg/L; pH, 7.0-8.0. The photoperiod will be maintained at light:dark = 12 h:12 h. Three tanks will be assigned randomly to each test diet with 30 fish per tank (300 L water in a tank). After one week of acclimation to a mixture of the above 8 diets in equal proportion, fish will be fasted for 24 hours and then weighed for initial bodyweight. Fish will be fed the assigned diets twice daily (09:00 and 15:00) at a feeding rate of 4% per day for one week. During the first feeding, fish will be allowed to feed for 15 minutes before any leftover feed is collected. The left-over feed then

will be dried at 105° C for 24 h to obtain dry matter. The leftover feed will be collected from each tank for five days and data will be pooled for each tank.

Growth trial: Following the protocol above for conditioning fish. Yellow perch with an initial weight of 3-6 g will be fed the 8 test diets for eight to ten weeks or longer depending on growth performance. Fish will be fed three times (09:00, 12:00, and 15:00) daily with three replications randomly assigned to each treatment. The targeted growth will be ~300% weight gain for the fish fed commercial feed. Fish will be batch-weighed in water at the beginning of the feeding trial and every two weeks thereafter. Feed amount will be adjusted according to the weight change every two weeks. Fish will be fed three times daily at 3-5% of body weight based on the fish growth. At the end of the feeding trial, all fish will be batch-weighed and counted to obtain final evaluations for survival and total biomass

Nutritional analysis: Four fish from each tank will be sampled for proximate composition analysis (dry matter, ash, protein, and lipid) following methods by AOAC (2000). Minerals will be analyzed using inductively coupled plasma atomic emission spectroscopy (Thermo Jarrel Ash Corporation, Franklin, Massachusetts, USA)

Fish health: Another four fish from each tank will be anesthetized to collect blood, and then euthanized to obtain liver weight, visceral lipids, and the small intestine. Blood will be centrifuged to collect serum for analysis using the method described by Zhai et al. (2018). The following parameters will be determined on each serum sample: albumin, alkaline phosphatase, alanine amino transferase, amylase, calcium, globulin, glucose, total bilirubin, inorganic phosphorus, and total protein. Liver tissue will be used for nutrient analysis (liver, glycogen, protein, and lipid). Liver glycogen will be measured by a glycogen assay kit (#700480, Cayman Chemical, Michigan, USA). Liver lipid will be measured by the Folch method (Folch et al., 1956), and liver protein will be measured by the Bradford assay.

The small intestinal samples will be collected according to the protocols described by Stephens and coauthors (Stephens et al. 2015); except that mid-gut intestinal samples (50-100 mg) will be dissected. Samples will be collected from three fish of each replicate tank. DNA extraction will be conducted with the Qiagen PowerSoil extraction kit, following the manufacturer's instructions, except that an additional 1-minute bead-beating step will be added. Following DNA extraction, bacterial 16S rRNA genes (v5v6 region) will be amplified from each sample. Following amplification, we will carry out community sequencing procedures and post-sequencing quality controls as we previously described (Fisher et al. 2015; Newton et al. 2015). All community amplification and sequencing procedures will be performed at the Great Lakes Genomics Center at the School of Freshwater Sciences (UWM). Bacterial community composition similarity will be assessed (e.g. Bray-Curtis similarity) for inter-fish, intra-treatment, and inter-treatment variability and dominant taxa across each treatment category. Multivariate statistics such as MANOVA and multivariate ordinations will be conducted in the R statistical language (Core Team 2013) to relate treatment effects and fish nutritional and metabolic outcomes to shifts in bacterial community composition.

Digestibility test: At the end of the feeding trial, ten fish will be fed for two weeks with the same test diets containing 1% Cr_2O_3 as an inert marker to measure apparent digestibility (NRC 2011). One hour after the first feeding in the morning, all tanks will be cleaned by siphoning, and a fecal sample will be collected through siphoning five hours after feeding. Fecal samples will be collected daily for 10 days. Feces from each tank will be freeze dried and then stored at -20°C until analysis. Methods used for analysis will be the same as described above.

Environmental stress challenge tests: Three fish from each tank (22°C) will be exposed to acute temperature shock at 30°C by increasing water temperature at 1°C/ 15 minutes (Deng et al., 2009) and then maintaining it at 30°C for 18 hours before samples are collected. Fish survival and heat shock protein 70 in liver and gill tissues will be measured to evaluate the tolerance of fish to temperature shock following the method described by Deng et al. (2009). Another six fish from each tank will be challenged with hypoxia (25% of normoxia or less than 3 mg/L) in a static water system. The DO levels will be maintained by aeration of nitrogen and/or air into each tank. Fish mortality will be monitored for 24 hours post challenge. At the end of 48 hours, liver and gill tissues will be collected from three fish. Tissues will be frozen in liquid nitrogen and then stored at -80C until needed for analysis of hypoxia-inducible factor-1α (HIF-1α). Analysis of HIF-1α will follow a similar method described by Li et al.

(2017) and Rimoldi et al. (2012). The care, handling, and sampling of fish will be performed following animal care protocols approved by the Animal Care and Use Committee, UWM.

Data calculation

Specific growth rate (SGR) (% body weigh.day^{-1}) = 100×Ln (Final body weight (g)/initial body weight (g)) /feeding period (day)

Fish weight gain (g)/day

Feed conversion ratio (FCR) = Feed weight as dry (g)/weight gain (g)

Protein efficiency ratio (PER) = Fish weight gain (g)/protein fed (g)

Protein Retention (PR, %) = 100* (final body protein (%)*final fish weight (g)-initial body protein (%)*initial body weight (g))/protein fed (g)

Energy Retention (ER, %) = 100* (final body energy (%)*final fish weight (g)-initial body energy (%)*initial body weight (g))/energy fed (g)

Apparent digestibility coefficient (ADC) of a diet=1- Cr in feed/Cr in feces

ADC of a dietary nutrient= 100*(1-(Cr in feed* nutrient in feces)/ (Cr in feces* nutrient in feed). ADC of a nutrient in a test ingredient (%) =ADC_{test} + ((ADC_{test} – ADC_{ref})* ($Nutr_{ref}$ * 0.7)/ (0.3 * $Nutr_{ingredient}$)).

ADC_{test} is the apparent digestibility of the test diet. ADC_{ref} is the apparent digestibility of the reference diet. $Nutr_{ingredient}$, $Nutr_{test}$ and $Nutr_{ref}$ are the level of the targeted Nutrient in the ingredient, test diet and reference diet

All data obtained will be subjected to two-way ANOVA to determine if there is significant difference among dietary treatments and interaction between carbohydrate sources and levels. Data will be subjected to transformations if they do not meet the ANOVA assumptions. Differences among means will be determined using the LSD multiple test. Treatment means will be considered significantly different when *P*-values were < 0.05.

Drs. Deng and Newton will supervise a graduate student and an hourly assistant to prepare feed, conduct diet tests and analyze the samples. This task will be conducted at the School of Freshwater Sciences, UWM.

Task 2 (Year-1&2). Manufacture test diets following a protocol used in a commercial pilot feed mill.

Task 2 is to develop practical feed processing protocols and test the physical quality of feed, which will be used for tests to address objectives 1 to 3. With this project, selected test diets (100 kg of each diet) will be processed in a commercial pilot scale feed mill with optimized protocols to the feed formulation. Based on the results generated from Task 1, corn starch will be supplied from corn meal and wheat starch will be replaced by wheat flour or middling. Thus, the over feed cost from ingredients will be decreased accordingly. For example, if previous results show that 20% corn starch-based feed is optimal for the fish, we will modify the feed formulation of diet 6 in Table 1 by using 25% corn meal instead of 20% corn starch. The fishmeal level will be decreased to 30% in the diet to reduce the overall protein from 40% to 37%. Thus, the feed ingredient cost will be decreased from $0.88/kg to $0.71/kg. The Co-PI Rosentrater will be responsible for ingredient sourcing and feed manufacturing according to standard Iowa State University (ISU) protocols. Prior to extrusion, all ingredients will be blended and ground to a uniform particle size less than 200 um. All feeds will be manufactured in a commercial-scale InstaPro extruder. During processing, water will be added, and various screw speeds and processing temperatures will be used in order to achieve optimal pellet production. Process settings will depend upon the protein contents of the blends as well as the nature of the other ingredients used and will be adjusted as necessary to achieve high quality, slow sinking, water stable feeds. Typically, extrusion temperatures range from about 80^{o}C to 150^{o}C, and moisture contents will range from about 25% to 40%. Processing conditions, including feed and die temperatures, drive torque, specific mechanical energy consumption, and product and feed material throughput rates, will be monitored during processing. The resulting pelleted products will then be subjected to extensive physical and chemical characterization following the procedures described by Ayadi et al. (2012), which will include pellet moisture content, water activity, protein content, fat content, ash content, carbohydrate content, product diameter, expansion ratios, unit and true densities, color, water absorption and solubility, and durability. Once appropriate quantities of high-quality feed have been manufactured, they will be sent to the other PIs for use in the fish feeding trials at UWM. The nutritional compositions of test diet and a commercial diet will be analyzed at UWM.

Task 3 (Year 1&2). Evaluate production efficiency at laboratory and fish farms.
Task 2 is conducted to address objective 3 proposed in this study. We intend to collaborate with fish farms (PortFish, Wisconsin and Fingerlings-YEP, Minnesota) to test the new diet versus a commercial feed. For the Portfish farm, we can either test the diet in an aquaponic system or a traditional tank system. PIs and Students or technician from UWM will be on site for starting the experiment, weighing fish and collect samples. Supplies for water quality monitoring and sample collection will be provided by UWM. The fish farms will collaborate with this project at no cost. At the end of the feeding trial, the fish will be left for the farms after sufficient samples are collected for evaluation.
Protocols will be reviewed before the tests are implanted. We will provide feed produced from Task 2 and fish generated from University of Wisconsin-Milwaukee at a no cost for these tests. The initial fish body weight will be 20-30 g. There will be 2-3 replications per diet and the feeding trial will last for 10-12 weeks depending on the growth rate. Stocking density will follow the identified farm protocols and the fish will be fed 2-3% body weight using automatic feeder. Fish growth will be monitored by weighing subsamples monthly from each replicate system. During the trial, water temperature will be monitored daily. Dissolved oxygen, ammonia and pH will be measured weekly. At the end of the feeding trial, growth, feed efficiency, fillet yield, nutritional composition of fish, liver and visceral lipid will be evaluated for subsamples (10-20 fish from each replicate). Drs. Deng and Newton will supervise a graduate student and hourly assistant for data collection and sample analysis. Production efficiency will be evaluated based on feed cost and growth performance of fish.

Task 4 (Year 1&2). Extension activities and results dissemination.
Task 4 is to address objective 4 proposed in the study. Findings of this project will be disseminated through presentations at local and national meetings, publication in professional journals or magazines, public Medias, and training workshops. We plan to present our findings at workshops/meetings organized by NCRAC and the World Aquaculture Society. At least one publication will be published in a peer-reviewed journal. We will meet stakeholders including feed industries (Zeigler Bros., Inc) and farmers, who are interested in the outcomes of this seed project, for further collaborations to explore long term research funding. We will also plan to train four students through internship or summer certificate program at the University of Nebraska-Lincoln and University of Wisconsin-Milwaukee (two undergraduate student internships). University of Wisconsin-Milwaukee has established an aquaculture certificate program providing courses covering topics aquaculture system, fish health, water chemistry, feed nutrition and freshwater technology etc. This project will help to provide hand-on experience with both research and aquaculture industry. Aquaculture industry partners throughout the Midwest have stressed that one problem limiting successful aquaculture practices is the lack of available and qualified staff. For Fish and Wildlife majors in the School of Natural Resources at the University of Nebraska-Lincoln (UNL), undergraduate students must complete an internship, job, or volunteer position outside the university that is related to their degree. We will use this program as a way for students to get hands-on experience at aquaculture facilities, which may lead to aquaculture-related jobs in the future. We will train students in the Fish Conservation, Physiology, and Behavior Laboratory at UNL to learn basic fish husbandry, rearing and handling methods, and then facilitate the placement of these students at an aquaculture facility in Nebraska for an internship. All PIs will be responsible for the collaborative presentation, publications and extension activities related to this project.

Outreach and Evaluation Plan

The main goals of our outreach plan are to promote local food production and consumption through public education, and to collaborate with local industry partners to improve the efficacy and reach of our research. These outreach activities will be diverse and dynamic, so as to reach as large of a community as possible. Importantly, several outreach activities will directly benefit industry partners and those new to the field:

1. The University of Wisconsin-Milwaukee will host training workshops, in which topics such as Nutrition of Fish Feed, Feed Management, and Fish Health will be discussed. Advances in the development of feed with be presented, and the benefits of using the new diet will be clearly explained. We also hope to attract farmers interested in using this diet or this approach with other fish species that are cultured in the area and strengthen these collaborations. We will gauge the success of these programs by quantifying not only how well attended they are, but how many participants return or request additional information.

2. The Fish Conservation, Behavior, and Physiology Lab at UNL will train at least two undergraduate students to become proficient working in an aquaculture setting. Before graduation, students must meet the qualification of working in an internship, volunteer position, or job position related to their area of study. This outreach program would allow undergraduate students to gain the knowledge they need to work in the aquaculture industry prior to working with local farmers. This would also allow farmers and industry partners to use skilled workers without having to pay financially for training (as they will have already been trained through the program at UNL) or for a stipend (as the students will need the internship to graduate). Hopefully this will allow for greater productivity for industry partners, more hands-on education for students, and a stronger collaborative relationship between the aquaculture community and the university. We will gauge the success of this program by quantifying the number of students that apply for this opportunity, the number of farmers requesting students to work for them, and the number of attendees at an informal meeting between students and aquaculture farmers at the end of the internship.
3. Researchers (co PIs and students) will have a presence at the annual Water for Food Global Conference in Lincoln, NE, to discuss food security, innovation aquaculture and aquaponics practices, and disseminate research findings. These international events also allow for innovative collaborations and opportunities for future research projects.

Outreach activities will also focus on engaging local communities through technology – these may be social media platforms, local TV and radio programming, and opportunities for students and adults through university classes and local events. Ultimately, we seek to not only disseminate information about diet development, and secure a partner in the feed industry, but also to help local farmers enhance their practices in a way that promotes more, better, and more pervasive use of aquaculture at the local and regional level.

LOGIC MODEL

Situation	Inputs	Outputs		Outcomes		
		Activities	Participation	Short term	Medium term	Long term
1) High market demand of yellow perch. 2) Decreased landings of yellow perch from fisheries due to declined natural stocks. 3) No commercial feed specific for yellow perch production. 4)Current commercial feeds are either expensive or do not meet the nutrient requirements of this fish	**Expertise/funding** Faculty Research staff Students Industrial partners Volunteers NCRAC **Resources** Fish culture facility Feed processing equipment Analytical lab Office spaces **Materials** Yellow perch broodstocks Research protocols Office software Training for students and partners	1) Cost effective feed formulation with different carbohydrate sources 2) Feed processing based on industrial protocol 3) Feed evaluation results based on laboratory and field tests based on growth performance, fish health and economics analysis 4) Technology dissemination through training or professional activities. Compile research findings into reports and presentations.	1) Students, research staff and university research community 2) Local fish farmers and feed companies 3) State Agency staff and policy makers	1) Identify practical feed formulations suited for yellow perch grow out. 2) Gain knowledge related to interaction of feed nutrition and fish health for cool water/yellow perch. 3) Students and farmers receive training on feeding and feed management of yellow perch.	1) Aquaculture farmers will have additional feed that is specific to yellow perch. 2).Extension program will adapt the new technology and help to make change commercial aquaculture industry. 3).Build up networks for collaborations between researchers and industry players to produce and use the new feed. 4) the region will have more trained staff involved in aquaculture industry	1) Expand yellow perch or other regional species of fish aquaculture and increase in the number of fish farms 2) Grower will have new diet in the region. 3) Satisfy the market demand of yellow and increase local seafood security and safety 4) Create more jobs in feed and fish production

Assumptions

- Local fish farmers are interested in a new diet for yellow perch production.
- Optimization of feed formulation will increase profitability of yellow perch culture.
- Lacking skilled labor is one of the factors impacting regional aquaculture development.

External Factors

- Acceptance and increased demand of the new feed and interest in yellow perch culture.
- Overall production cost of feed is affordable.
- The interest of public for local seafood.

Facilities

University of Wisconsin-Milwaukee (Aquaculture, Nutrition, Genomic and Microbiology)

Broodstocks and aquaculture facilities: The UW-Milwaukee Great Lakes Aquaculture Center (GLAC) has a 1,394 m^2 aquaculture workspace with both flow-through and recirculating systems. An automated system supplies dechlorinated water at ambient cold water, hot water, and refrigerated water to the fish rearing tanks at a capacity of 4,542 L/min. Water temperature can be controlled to meet the requirement of a study. SFS also has analytical laboratories and shop facilities to support a wide variety of aquatic research investigations, including the expertise and ability to modify the specialized feeding trial tanks, if needed. The wet labs have well-established protocols for biosecurity control, zooplankton and artemia culture, as well as fish maintenance at different life stages. The proposed project will take advantage of the selective and out-of-lifecycle broodstock for fingerling production. Specific systems available for this project include the following system: 30 x 100 L tanks, and 21 x 350 L tanks, two large 2.44-m (8-ft) diameter tank used for stocking, and 18 X600L (5-ft) diameter circular for backup used for Task-3. The feed lab has specific workspace for feed preparation and equipment for sample preparation and nutritional analysis such as Labconco freeze dryer, automatic Soxhlet extraction, nitrogen analyzer, HPLC, liquid nitrogen pulverizer, oven dryer, dehydrator, muffle furnace oven, microscopes, microPhazir-AG, spectrophotometer, analytical balances, centrifuge, homogenizer, freezers (-20C and -80C), water quality kits, and automatic feeders. The feed processing lab has equipment for grinding, mixing, cold extruding and drying as well as freezers and refrigerators.

Genomics Core Facility (UWM)*:* The School of Freshwater Sciences (SFS) maintains a genomics core sequencing facility that has a PacBio RS II sequencing system for deep sequencing and epigenomic analyses, an Illumina MiSeq for short fragment analysis, and an ABI 3730S sequencer and ancillary equipment (e.g., centrifuges and thermal cyclers) for in-house, high-throughput plasmid preparation, nucleic acid sequencing and genotyping by microsatellites

Microbial Analysis Lab: The Newton lab is equipped with all the molecular lab equipment needed to extract, purify, and quantify nucleic acids. Four automated thermocyclers for PCR are available for use through the Great Lakes Genomics Center (GLGC), which is located on the same floor as the Newton laboratory. Additionally, the equipment needed for illumina-based DNA library preparation and subsequent paired-end read sequencing are available through the GLGC. Post-DNA sequence generation, the Newton lab is equipped with a desktop iMac and several laptops to provide access to the high-performance computing cluster on UW-Milwaukee's main campus. We routinely use the bioinformatics software Minimum Entropy Decomposition, DADA2, Anvio, Mothur, ARB, and R, and basic word processing, computational, and graphic editing software to analyze and report on microbial community data, and have 4-node RAID array for backup and long-term storage of data..

*Shared use BSL2 Pathology Lab**: SFS-UWM*** has a shared use facility for working with the VHSv virus. The Aquatic BL2 facility has 4 (500 sq.ft.) quarantine bays that connect, via common corridor, to a high containment research suite (~800 sq.ft.) and a separate high containment aquatic challenge facility (~1,000 sq.ft.) necropsy room. This facility also contains a separate *in vivo* suite, for pathogen challenges and other studies that have high infective risk. All effluents and liquid waste generated within the facility is chlorine/acid treated. The in vivo suite has adjacent animal receiving areas, a necropsy room, and a wash-down/ autoclave room with a pass-through autoclave. The lab is equipped with all necessary equipment for disease challenge tests.

Feed processing and analysis facility (Iowa State University)

A broad range of facilities and equipment are available at Center for Crops Utilization Research (CCUR) at Iowa State University with over 35,000 ft^2 of pilot plant processing and support space available for wet processing, dry processing, fermentation and product recovery, hazardous solvents extraction, industrial product development, and food processing operations. There are different types of mills and mixers such as cutting roller mill disc mill, hammer mill, impact mill (Entoleter), impact mills (comminuting, Fitzpatrick), microcut grinder, turbo/pin mill ultra-centrifugal mill, mixer and lifting cart (Lightning Mixer), ribbon mixer (Cedar Rapids Machinery), variable-speed gear batch mixer. For feed pelleting, CCUR has a small-scale pellet mill (California Pellet Mill), single and

twin-screw ¾-inch laboratory extruders, pilot-scale cooker extruder/expander with oil cage, twin-screw pilot-scale extruder, commercial-scale autogenous single-screw extruder with cutter, and a tray dryer. Research facilities also include one laboratory space for feed ingredient preparation, and one for chemical and physical property analyses of both ingredients and finished feed products. Feed ingredient sorting, test diet processing, pellet physical property measurement will be conducted at ISU and be responsible by Dr. Kurt Rosentrater.

University of Nebraska-Lincoln (Aquaculture Methods Training Facility)

The Fish Conservation, Behavior, and Physiology Laboratory (FCBP) is overseen by Dr. Poletto at the University of Nebraska-Lincoln (UNL) East Campus. This facility consists fish rearing and holding systems as well as physiological and behavioral experimental space. The primary fish holding system consists of three independent recirculating aquaculture systems (RAS) with 4 in-line 1000L circular fiberglass. Water is delivered to each RAS independently from a 3785L water reservoir that has passed through 2 312L Vantage PTC Carson Filters. Each RAs is also outfitted with a Pentair Arias 8000 Fiberglass sand filter, a Jandy VS FloPro variable speed pump, Aqualogic Delta Star in-line water chiller controlled by an Aqualogic digital temperature controller, an Aquatic Eco-Systems Clearwater low space bioreactor. Beneficial bacteria within the bioreactor will reduce ammonia and nitrite levels before the water will be gravity fed through a UV-light before water is delivered back into the system. This RAS set up allows for precise temperature and water velocity control within the tanks, reduces diseases or infections from wild fish, and prevents cross contamination. In addition to the RASs the facility is equipped with 3 flow-through 1000L circular fiberglass tanks, 2 Minn-o-cool living streams, and multiple static aquaria (5.5-15 gal). This diversity allows for a variety of different sizes and species to be used.

The FCBP is also outfitted with supplies used to diagnose disease, take tissue samples and process samples, monitor water quality data, and perform both physiological and behavioral experiments, in part due to an extensive camera monitoring system. The laboratory also has a mini bomb calorimeter to quantify energy densities, and is in the process of acquiring a spectrophotometer for in-depth mechanistic investigations. Ultimately the accessibility to the laboratory and the diversity of skills required to work proficiently in the laboratory make it ideally suited to train students in creating and maintaining a functional aquaculture system, with an exceptional level of hands-on training and involvement in the day-today operations.

References

AOAC, 2000. Official methods of analysis, 18th edition. Association of Official Analytical Chemists, Gaithersburg.

Ayadi, F., K. A. Rosentrater, K. Muthukumarappan, and M. L. Brown. 2012. Twin screw extrusion processing of distillers dried grains with solubles (DDGS)-based yellow perch *Perca flavenscens* feeds. Food and Bioprocess Technology 5:1963–1978.

Barrows, F. T., D. A. J. Davis, and R. W. Hardy. 2007. The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout *Oncorhynchus mykiss*. Aquaculture 265:244–252.

Bergot, F. 1993. Digestibility of native starches of various botanic origins by rainbow trout *Oncorhynchus mykiss*. Pages 857–865 in S. J. Kaushik and P. Luquet, editors. Fish Nutrition in Practices. INRA, Paris, France.

Brown, P. B., K. Dabrowski, and D. L. Garling. 1996. Nutrition and feeding of yellow perch *Perca flavescens*. Journal of Applied Ichthyology 12:171–174.

Caballero, M. J., M. S. Izquierdo, E. Kjørsvik, A. J. Fernandez, and G. Rosenlund. 2004. Histological alterations in the liver of sea bream, Sparus aurata L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. Journal of Fish Diseases 27:531–541.

Carter, C. G., and R. C. Hauler. 2000. Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. Aquaculture 185:299–311.

Cartwright, D. D. 1998. Dietary lipid studies with juvenile yellow perch. Master's thesis. Purdue University, Lafayette, Indiana.

Committee, L. M. 2014. Lake Michigan yellow perch summit. Great Lakes Fishery Commission, Ann Arbor, MI.

Creswell, D. 2005. The feeding and nutrition of the tilapia, Part 2. Feed management. AQUA Culture Asia Pacific Magazine Press, Singapore.

Davidson, J., C. Good, F. T. Barrows, C. Welsh, P. B. Kenney, and S. T. Summerfelt. 2013. Comparing the effects of feeding a grain- or a fish meal-based diet on water quality, waste production, and rainbow trout *Oncorhynchus mykiss* performance within low exchange water recirculating aquaculture systems. Aquaculture Engineering 52:45–57.

De Schryver, P., and O. Vadstein. 2014. Ecological theory as a foundation to control pathogenic invasion in aquaculture. ISME Journal 8:2360–2368.

Defroirdt, T., P. Sorgeloos, and P. Bossier. 2011. Alternatives to antibiotics for the control of bacterial disease in aquaculture. Current Opinion in Microbiology 14:251–258.

Deng, D. F., S. F. Wang, S. H. Lee, S. C. Bai, and S. S. O. Hung. 2009. Feeding rates affect heat shock protein levels in liver of larval white sturgeon *Acipenser transmontanus*. Aquaculture 287:223–226.

Fisher, J. C., R. J. Newton, D. K. Dila, and S. L. McLellan. 2015. Urban microbial ecology of a freshwater estuary of Lake Michigan. Elementa, Washington, D.C., USA.

Folch, J., M. Lees, and G. H. Sloan-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry 226:497–509.

Forberg, T., E. B. Sjulstad, I. Bakke, Y. Olsen, A. Hagiwara, Y. Sakakura, and O. Vadstein. 2016. Correlation between microbiota and growth in Mangrove Killifish *Kryptolebias marmoratus* and Atlantic cod *Gadus morhua*. Scientific Reports 6:21192.

Gatesoupe, F. J., C. Huelvan, N. L. Bayon, A. Severe, I. M. Aasen, K. F. Degnes, D. Mazurais, S. Panserat, J. L. Zambonino-Infante, and S. J. Kaushik. 2014. The effects of dietary carbohydrate sources and forms on metabolic response and intestinal microbiota in sea bass juveniles, *Dicentrarchus labrax*. Aquaculture 422:47–53.

Green, T. J., R. Smullen, and A. C. Barnes. 2013. Dietary soybean protein concentrate-induced intestinal disorder in marine farmed Atlantic salmon, *Salmo salar* is associated with alterations in gut microbiota. Veterinary Microbiology 166:286–292.

Han, D., S. S. Hung, W. F. Wang, D. F. Deng, and S. S. O. Hung. 2012. Starvation reduces the heat shock protein response in white sturgeon larvae. Environmental Biology of Fishes 93:333–342.

Hart, S. D., B. J. Brown, N. L. Gould, M. L. Robar, E. M. Witt, and P. B. Brown. 2010. Predicting the optimal dietary essential amino acid profile for growth of juvenile yellow perch with whole body amino acid concentrations. Aquaculture Nutrition 16:248–253.

Hemre G. I., and D. F. Deng. 2015. Carbohydrates. Pages 95–110 in C. S. Lee, C. Lim, C. Weber, and D. Gatlin, editors. Dietary Nutrients, Additives and Fish Health. John Wiley & Sons, Hoboken, New Jersey.

Hemre G. I., G. Lambertsen, and O. Lie. 1991. The effect of dietary carbohydrate on the stress response in cod (Gadusmorhua). Aquaculture 95:319–328

Kasper, C. S., B. A. Watkins, and P. B. Brown. 2007. Evaluation of two soybean meals fed to yellow perch *Perca flavescens*. Aquaculture Nutrition 13:431–438.

Kraugerud, O. F., H. Y. Jorgensen, and B. Svihus. 2011. Physical properties of extruded fish feed. Animal Feed Science and Technology 168:250–256.

Kwasek, K., K. Dabrowski, K. Ware, J. M. Reddish, and M. Wick. 2012. The effect of lysine-supplemented wheat gluten-based diet on yellow perch *Perca flavescens* (Mitchill) performance. Aquaculture Research 43:1384–1391.

Li, H. L., X. H. Gu, B. J. Li, X. Chen, H. R. Lin, and J. Xia. 2017. Characterization and functional analysis of hypoxia-inducible factor HIF1α and its inhibitor HIF1αn in tilapia. PLoS ONE 12:173478.

Llewellyn, M. S., S. Boutin, S. H. Hoseinifar, and N. Derome. 2014. Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Frontiers in Microbiology 5:207

Lozupone, C. A., J. I. Stombaugh, J. I. Gordon, J. K. Jansson, and R. Knight. 2012. Diversity, stability, and resilience of the human gut microbiota. Nature 489:220–230.

Lundblad, K. K., S. Issa, J. D. Hancock, K. C. Behnke, L. J. McKinney, S. Alavi, E. Prestlokken, J. Fledderus, and M. Sorensen. 2011. Effects of steam conditioning at low and high temperature, expander conditioning and extruder processing prior to pelleting on growth performance and nutrient digestibility in nursery pigs and broiler chickens. Anim. Animal Feed Science and Technology 169:208–217

Malison, J. A. 2003. A white paper on the status and needs of yellow perch aquaculture in the North Central Region. Special publication of the North Central Regional Aquaculture Center, East Lansing, MI.

Marsden, J. E., and S. R. Robillard. 2004. Decline of yellow perch in southwestern Lake Michigan, 1987–1997. North American Journal of Fisheries Management 24:952–966.

Mjoun, K., K. A. Rosentrater, and M. L. Brown. 2012. Culture performance and tissue fatty acid compositions of yellow perch *Perca flavescens* fed different dietary lipids. Aquaculture 360:17–24.

Morken, T., O. F. Krauferud, M. Sorensen, T. Storebakken, M. Hillestad, R. Christiansen, and M. Overland. 2012. Effects of feed processing conditions and acid salts on nutrient digestibility and physical quality of soy-based diets for Atlantic Salmon *Salmo salar*. Aquaculture Nutrition 18:21–34.

Nayak, S. K. 2010. Role of gastrointestinal microbiota in fish. Aquaculture Research 41:1553–1573.

Newton, R. J., S. L. McLellan, D. K. Dila, J. H. Vineis, H. G. Morrison, A. M. Eren, and M. L. Sogin. 2015. Sewage reflects the microbiomes of human populations. mBio 6:02574–14.

NRC. 2011. Nutrient requirements of fish and shrimp. Animal Nutrition Series. National Research Council of the National Academies. The National Academies Press, Washington, D.C., USA.

Core Team, R. C. T. R. 2013. R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna.

Ramseyer, L. J., and D. L. Garling. 1998. Effects of dietary protein to metabolizable energy ratios and total protein concentrations on the performance of yellow perch *Perca flavescens*. Aquaculture Nutrition 4:217–223.

Rimoldi, S., G. Terova, P. Ceccuzzi, S. Marelli, M. Antonini, and M. Saroglia. 2012. HIF-1a mRNA levels in Eurasian perch *Perca fluviatilis* exposed to acute and chronic hypoxia. Molecular Biology Reports 39:4009–4015.

Ringø, E., S. Sperstad, R. Myklebust, S. Refstie, and Å. Krogdahl. 2006. Characterisation of the microbiota associated with intestine of Atlantic cod *Gadus morhus*: The effect of fish meal, standard soybean meal and a bioprocessed soybean meal. Aquaculture 261:829–841.

Rothschild, D., O. Weissbrod, E. Barkan, A. Kurilshikov, T. Korem, D. Zeevi, P. I. Costea, A. Godneva, I. N. Kalka, N. Bar, and S. Shilo. 2018. Environment dominates over host genetics in shaping human gut microbiota. Nature 555(7695):210.

Round, J. L, and S. K. Mazmanian. 2009. The gut microbiota shapes intestinal immune response during health and disease. Nature Reviews Immunology 9:313–323.

Schaeffer, T. W., M. J. Hennen, M. L. Brown, and K. A. Rosentrater. 2012. Nutritional composition and use of common carp muscle in yellow perch diets. North American Journal of Aquaculture 74:297–305.

Schaeffer, T. W., M. J. Hennen, M. L. Brown, and K. A. Rosentrater. 2011. Effects of dietary distillers dried grains with solubles and soybean meal on extruded pellet characteristics and growth responses of juvenile yellow perch. North American Journal of Aquaculture 73:270–278.

Scott, K. P., S. W. Gratz, P. O. Sheridan, H. J. Flint, and S. H. Duncan. 2013. The influence of diet on the gut microbiota. Pharmacological Research 69:52–60.

Sorensen, M. 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquaculture Nutrition 18:233–248.

Stephens, W. Z., A. R. Burns, K. Stagaman, S. Wong, J. F. Rawls, K. Guillemin, and B. J. M. Bohannan. 2015. The composition of the zebrafish intestinal microbial community varies across development. ISME Journal 10:644–654.

Stone, D.A. 2003. Dietary carbohydrate utilization by fish. Reviews in Fisheries Science 11:337–369.

Tacon, A. G. J. 1996. Feed formulation and evaluation for semi-intensive culture of fishes and shrimps in the tropics. Pages 29–43 in C. B. Santiago, R. M. Coloso, O. M. Millamena, and I. G. Borlongan, editors. Proceedings of the National Seminar-Workshop on Fish Nutrition and Feeds. Aquaculture Department, Southeast Asian Fisheries Development Centre, Iloilo, Philippines.

Tacon, A. G. J., M. R. Hasan, and M. Metian. 2011. Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects. FAO Fisheries and Aquaculture Technical Paper 564:71-87.

Tacon, A. G. J., and M. Metian. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285:146–158.

Trinh, L. T., I. Bakke, and O. Vadstein. 2017. Correlations of age and growth rate with microbiota composition in Atlantic cod *Gadus morhua* larvae. Scientific Reports 7:8611.

Twibell, R. G., and P. B. Brown. 1997. Dietary arginine requirement of juvenile yellow perch. Journal of Nutrition 127:1838–1841.

Twibell, R. G., and P. B. Brown. 2000. Dietary choline requirement of juvenile yellow perch *Perca flavescens*. Journal of Nutrition 130:95–99.

Twibell, R. G., D. Cartwright, K. A. Wilson, and P. B. Brown. 1998. Dietary lysine requirement of juvenile yellow perch. World Aquaculture 98:553.

Twibell, R. G., K. A. Wilson, and P. B. Brown. 2000. Dietary sulfur amino acid requirement of juvenile yellow perch fed the maximum cystine replacement value for methionine. Journal of Nutrition 130:612–616.

Wilberg, M. J., J. R. Bence, B. T. Eggold, D. Makauskas, and D. F. Clapp. 2005. Yellow perch dynamics in southwestern Lake Michigan during 1986–2002. North American Journal of Fisheries Management 25:1130–1152.

Wong, S., T. Waldrop, S. Summerfelt, J. Davidson, F. Barrows, P. B. Kenney, T. Welch, G. D. Wiens, K. Snekvik, J. F. Rawls, and C. Good. 2013. Aquacultured rainbow trout *Oncorhynchus mykiss* possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. Applied and Environmental Microbiology 79:4974–4984.

Zhai, S.W., B.S. Shepherd, S. Yang, H.H. Zhao, M. Jiang, F.P.B. Binowski, and D.F. Deng. 2018. Efficacy of tricaine methanesulfonate (MS-222) as an anesthetic agent for short-term anesthesia in juvenile yellow perch *Perca flavescens*. The Israeli Journal of Aquaculture 70: 1515-1525

Zhou, Z., Ø. Arlsen, S. He, R. E. Olsen, B. Yao, and E. Ringø. 2012. The effect of dietary chitin on the autochthonous gut bacteria of atlantic cod *Gadus morhua* L. Aquaculture Research 44:1889–1900.

Project Leaders

State	Name/Institution	Area of Specialization
Wisconsin	Dong-Fang Deng/University of Wisconsin-Milwaukee	Nutrition and Aquaculture
Wisconsin	Ryan Newton/ University of Wisconsin-Milwaukee	Microbiologist/fish health
Iowa	Kurt A. Rosentrater/Iowa State University	Feed processing and Life Cycle Analysis
Nebraska	Jamilynn Poletto/ University of Nebraska-Lincoln	Aquaculture and Fish Physiologist

University of Wisconsin 600 E. Greenfield Ave. Milwaukee, Wisconsin, 53204				**USDA AWARD NO.** Year: 1 Objective: 1, 2,3,4			
PROJECT DIRECTOR(S) PI Name Dong-Fang Deng				Duration Proposed Months: _12_ **Funds Requested by Proposer**	Duration Proposed Months:_ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer	1,056			
a. _1 (Co)-PD(s)			0.1				
b.___Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c.___Paraprofessionals							
d. _1 Graduate Students.....				19,968			
e._1_ Prebaccalaureate Students.....				6,240			
f.___Secretarial-Clerical							
g.___Technical, Shop and Other							
Total Salaries and Wages.....ϒ				27,264			
B. Fringe Benefits (If charged as Direct Costs)				2,311			
C. Total Salaries, Wages, and Fringe Benefits (A plus B).....ϒ				29,575			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				18,500			
F. Travel				1,000			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I).....ϒ				49,075			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) .....ϒ				49,075			
N. Other .....ϒ							
O. Total Amount of This Request.....ϒ				49,075			
P. Carryover -- (If Applicable).....Federal Funds: $Non-Federal funds: $Total $							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)						Leave Blank	
Cash (both Applicant and Third Party) .....ϒ							
Non-Cash Contributions (both Applicant and Third Party) .....ϒ							

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

University of Wisconsin 600 E. Greenfield Ave. Milwaukee, Wisconsin, 53204				**USDA AWARD NO.** Year: 2 Objective: ,2,3,4			
PROJECT DIRECTOR(S) PI Name Dong-Fang Deng				Duration Proposed Months: _12_ **Funds Requested by Proposer**	Duration Proposed Months:_ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer	2,174			
a. _1 (Co)-PD(s)			0.2				
b.___Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. _1 Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c.___Paraprofessionals							
d. _1 Graduate Students.....				19,968			
e._1_ Prebaccalaureate Students.....				6,240			
f.___Secretarial-Clerical							
g.___Technical, Shop and Other							
Total Salaries and Wages.....ϒ				28,382			
B. Fringe Benefits (If charged as Direct Costs)				2,735			
C. Total Salaries, Wages, and Fringe Benefits (A plus B).....ϒ				31,117			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				10,500			
F. Travel				3,000			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I).....ϒ				44,617			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) .....ϒ				44,617			
N. Other .....ϒ							
O. Total Amount of This Request.....ϒ				44,617			
P. Carryover -- (If Applicable).....Federal Funds: $Non-Federal funds: $Total $							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)						Leave Blank	
Cash (both Applicant and Third Party) .....ϒ							
Non-Cash Contributions (both Applicant and Third Party) .....ϒ							

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Budget Explanation – Univ. of Wisconsin-Milwaukee
(Deng)

Objective #1, 2, 3, 4
Years 1 & 2:

Total budget = $ 93,692

C. **Salary, Wages and Fringe Benefits ($60,692):** Total amount is $29,575 and $31,117 required for Year 1 and Year 2, respectively. Budget for Year-1 is needed to cover a part time graduate student stipend ($21,705=$19,968, salary+ $1,737, fringe), 3 months' salary of an trainee on the extension activity ($6,440=$6,240+$200, fringe), and 0.1 month salary of the PI ($1,430=$1056, salary+$374);

Budget for Year-2 is required to cover stipend of a part-time graduate student ($21,725 = $19,968, salary + $1,757, fringe), 3 months' salary of a trainee ($6,440=$6,240+$200, and 0.2 month salary of the PI ($2,952= $2,174, salary+$778, fringe). The PI will supervise a graduate student to perform feeding trials, collect samples and carry out analysis. The PI will collaborate with Co-PIs to train students, writing reports, presenting data and publishing papers.

E. Materials and Supplies ($29,000): A budget of $18,500 for Year-1 is required to cover cost for feed ingredients, nutritional analysis, analytical lab supplies and stress protein assay, HIF-1a assay, wet lab supplies and office supplies; a budget of $10,500 is required for Year-2 to cover nutritional analysis of samples collected from field testing, lab chemicals and supplies, office supplies for report and publication.

F. Travel ($4,000): The cost for travel for Year-1 ($1,000) is to cover domestic travel of the PI to workshops, visit identified farms for discussion and preparation of farm tests proposed in year-2. A budget of $3,000 is requested in Year-2 to cover visits to local farms for monitoring the proposed feeding trials and collect data ($1,000). Cost is also needed for the PI to World aquaculture society or other aquaculture nutrition meeting to present data ($2,000) including registration, flight tickets and hotel.

UNIVERSITY of WISCONSIN
UWMILWAUKEE

Office of Sponsored Programs

3203 N. Downer Ave.
Mitchell Hall 273
P.O. Box 340
Milwaukee, WI
53201-0340
414 229-3332 phone
414 229-5000 fax
http://uwm.edu/officeofresearch/osp.

April 5, 2019

Dr. Joseph E. Morris, Director
North Central Regional Aquaculture Center
Iowa State University
339 Science II
Ames, Iowa 50011-3221

RE: UWM MIL114717: "Optimization of Dietary Carbohydrate to Improve Fish Health and Production Performance of Yellow Perch (Perca flavescens)"

Dear Dr. Morris:

This serves as confirmation that the University of Wisconsin-Milwaukee (UWM) will collaborate with Iowa State University, in a joint proposal entitled, "Optimization of Dietary Carbohydrate to Improve Fish Health and Production Performance of Yellow Perch (Perca flavescens)" that will be submitted to the North Central Regional Aquaculture Center. Dr. Dong Fang Deng, UWM School of Freshwater Sciences (SFS), will serve as Principal Investigator at UWM. Dr. Ryan Newton, UWM SFS, will serve as Co-Principal Investigator.

The proposed project sub-award budget totals $169,467, for the period July 1, 2019 – June 30, 2021.

The University of Wisconsin-Milwaukee's participation is hereby endorsed on behalf of the Board of Regents of the University of Wisconsin System. The appropriate programmatic and administrative personnel of UWM involved in this grant application have reviewed our participation and are prepared to establish the necessary inter-institutional agreement(s) consistent with our status as a public university of the State of Wisconsin.

If you have any technical questions, please contact Dr. Deng at (414) 382-1700 or dengd@uwm.edu. Any contractual questions may be addressed to Vince Bauer at (414) 229-2487 or vrbauer@uwm.edu.

Sincerely,

Thomas R. Marcussen

Thomas R. Marcussen
Director, Office of Sponsored Programs

Cc: Dr. Dong Fang Deng

DUNS: 627906399 EIN: 39-1805963 WI-004

ORGANIZATION AND ADDRESS Iowa State University 3327 Elings Hall Ames, IA 50011City, State, ZIP PROJECT DIRECTOR(S) PI Name Dr. Kurt Rosentrater				**USDA AWARD NO.** Year: 1 Objective: 2			
				Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a.___(Co)-PD(s)							
b.___Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c.___Paraprofessionals							
d.___Graduate Students.....							
e.___Prebaccalaureate Students.....				10,934			
f.___Secretarial-Clerical							
g.___Technical, Shop and Other							
Total Salaries and Wages..... ϒ				10,934			
B. Fringe Benefits (If charged as Direct Costs)				66			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)..... ϒ				11,000			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				4,000			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)..... ϒ				15,000			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) ..... ϒ				15,000			
N. Other ..... ϒ							
O. Total Amount of This Request..... ϒ				15,000			
P. Carryover -- (If Applicable).....Federal Funds: $Non-Federal funds: $Total $							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party)..... ϒ Non-Cash Contributions (both Applicant and Third Party)..... ϒ						Leave Blank	
NAME AND TITLE (Type or print)			**SIGNATURE** (required for revised budget only)				**DATE**
Project Director							
Authorized Organizational Representative							
Signature (for optional use)							
ORGANIZATION AND ADDRESS				**USDA AWARD NO.** Year: 2 Objective: 2,4			

Iowa State University 3327 Elings Hall Ames, IA 50011 PROJECT DIRECTOR(S) Dr. Kurt Rosentrater				Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a.___(Co)-PD(s)							
b.___Senior Associates							
2 . No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . . b. ___ Other Professionals							
c.___Paraprofessionals							
d.___Graduate Students.....							
e.___Prebaccalaureate Students.....				3,976			
f.___Secretarial-Clerical							
g.___Technical, Shop and Other							
Total Salaries and Wages..... ϒ				3,976			
B. Fringe Benefits (If charged as Direct Costs)				24			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)..... ϒ				4,000			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				2,500			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)..... ϒ				6,500			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) ..... ϒ				6,500			
N. Other ..... ϒ							
O. Total Amount of This Request ..... ϒ				6,500			
P. Carryover -- (If Applicable)..... Federal Funds: $ Non-Federal funds: $ Total $							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) ..... ϒ Non-Cash Contributions (both Applicant and Third Party) ..... ϒ						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Budget Explanation – Iowa State University
(Rosentrater)

Objective 2&4

Years 1 & 2:

C. Salary, Wages and Fringe Benefits ($15,000): A budget of $11,000 (Year-1) and $4,000 (Year-2) is requested to cover labor needed for feed processing, testing physical quality of pellets, and data collection for report.

D. Nonexpendable Equipment ($1,000): buckets, Rubbermaid containers, scoops, misc. supplies for grinding, mixing, extruding, **and drying of extruded feed products.**

E. Materials and Supplies ($ 6,500): A budget of $4,000 in Year 1 is needed to cover equipment use, feed processing tools, feed ingredients, Rubbermaid containers, scoops, misc. supplies for grinding, mixing, extruding, and drying of extruded feed products and $2,500 in Year 2 is required for ingredient, packing, lab materials for feed physical parameter analysis.

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Department of Agricultural and
Biosystems Engineering
3327 Elings Hall
Ames, Iowa 50011-
515-294-4019

28 March 2019

Dr. Joseph E. Morris, Director
North Central Regional Aquaculture Center
Iowa State University
339 Science II
Ames, Iowa 50011-3221

SUBJECT: Project entitled "Optimization of Dietary Carbohydrate to Improve Fish Health and Production Performance of Yellow Perch (*Perca flavescens*)"

Dear Dr. Morris:

As the Authorized Organizational Representative (AOR) I would like to inform you [Iowa State University's Department of Agricultural and Biosystems Engineering] (ABE) wishes to participate in the above referenced project as a subcontractor to Iowa State University.

I will serve as the Principal Investigator of the subcontract and I have access to all of the necessary equipment, laboratory, and office space to successfully undertake this project.

Upon issuance of approval to the North Central Regional Aquaculture Center for this project, Iowa State University and ISU ABE will enter into a formal agreement.

Sincerely,

Kurt A. Rosentrater
Department of Agricultural and Biosystems Engineering
Iowa State University

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ORGANIZATION AND ADDRESS University of Nebraska-Lincoln 412 Hardin Hall 3310 Holdrege Street, Lincoln, NE 68583 PROJECT DIRECTOR(S) Dr. Jamilynn Poletto				**USDA AWARD NO.** Year: 1 Objective: 3,4 Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a.___(Co)-PD(s)							
b.___Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c.___Paraprofessionals							
d. 2 Graduate Students.....				8,000			
e.___Prebaccalaureate Students.....							
f.___Secretarial-Clerical							
g.___Technical, Shop and Other							
Total Salaries and Wages.....ϒ				8,000			
B. Fringe Benefits (If charged as Direct Costs)				648			
C. Total Salaries, Wages, and Fringe Benefits (A plus B).....ϒ				8,648			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				300			
F. Travel				1,305			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I).....ϒ				10,253			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) .....ϒ				10,253			
N. Other .....ϒ							
O. Total Amount of This Request.....ϒ				10,253			
P. Carryover -- (If Applicable).....Federal Funds: $Non-Federal funds: $Total $							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) .....ϒ Non-Cash Contributions (both Applicant and Third Party) .....ϒ						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

ORGANIZATION AND ADDRESS University of Nebraska-Lincoln 412 Hardin Hall 3310 Holdrege Street, Lincoln, NE 68583 PROJECT DIRECTOR(S) Dr. Jamilynn Poletto				**USDA AWARD NO.** Year: 2 Objective: 3,4 Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS**						
	Calendar	Academic	Summer				
a.___(Co)-PD(s)							
b.___Senior Associates							
2 . No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . . b. ___ Other Professionals							
c.___Paraprofessionals							
d.___Graduate Students.....							
e.___Prebaccalaureate Students.....				8,240			
f.___Secretarial-Clerical							
g.___Technical, Shop and Other							
Total Salaries and Wages..... ϒ				8,240			
B. Fringe Benefits (If charged as Direct Costs)				667			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)..... ϒ				8,907			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				300			
F. Travel				1,305			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)..... ϒ				10,512			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) ..... ϒ				10,512			
N. Other ..... ϒ							
O. Total Amount of This Request ..... ϒ				10,512			
P. Carryover -- (If Applicable)..... Federal Funds: $ Non-Federal funds: $ Total $							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) ..... ϒ Non-Cash Contributions (both Applicant and Third Party) ..... ϒ						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Budget Explanation – Univ. of Nebraska - Lincoln
(Poletto)

Objectives 3&4

Years 1 & 2:

C. Salary, Wages and Fringe Benefits = $17,555 ($8,648 for year 1 and $8,907 for year 2): Two undergraduate student workers, will be responsible for conducting the experiments and assisting in analyzing data. They will be trained the technology developed by this project. Each student will be paid with $10/ for 400 hours. A 3% cost of living increase has been applied to all salaries in year. Personnel benefits are estimated at 8.1% of salary. The actual cost of benefits for each person will be charged to the project.

E. Materials and Supplies ($ 600 for two years): A budget of $300/yearl is needed to cover materials for training.

F. Domestic Travel = $2,610: $1,305 is requested per year of the project to cover the cost of a 3-month rental truck from UNL Transportation Services @ $435/month

April 03, 2019

Dr. Joseph E. Morris, Director
North Central Regional Aquaculture Center
Iowa State University
339 Science II
Ames, Iowa 50011-3221

SUBJECT: Project entitled "Optimization of Dietary Carbohydrate to Improve Fish Health and Production Performance of Yellow Perch (*Perca flavescens*)"

Dear Dr. Morris:

As the Authorized Organizational Representative (AOR) I would like to inform you the Board of Regents, University of Nebraska, University of Nebraska-Lincoln (UNL) wishes to participate in the above referenced project as a subcontractor to Iowa State University.

Dr. Jamilynn B. Poletto will serve as the Principal Investigator of the subcontract and she has access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Dr. Poletto's involvement in this project.

Upon issuance of approval to the North Central Regional Aquaculture Center for this project, Iowa State University and UNL will enter into a formal agreement.

Sincerely,

Archie Clutter, Ph.D
Institute of Agriculture and Natural Resources
Agricultural Research Division
Dean and Director

University of Wisconsin 600 E. Greenfield Ave. Milwaukee, Wisconsin, 53204				**USDA AWARD NO.** Year: 1 Objective: 1,4			
PROJECT DIRECTOR(S) PI Name Dr. Ryan Newton				Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer	833			
a. 1 (Co)-PD(s)			0.1				
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. 1 Prebaccalaureate Students				3,900			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages				4,733			
B. Fringe Benefits (If charged as Direct Costs)				420			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				5,153			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				10,000			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)				15,153			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K)				15,153			
N. Other							
O. Total Amount of This Request				15,153			
P. Carryover -- (If Applicable)	**Federal Funds: $**	**Non-Federal funds: $**	**Total $**				
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)					Leave Blank		
Cash (both Applicant and Third Party)							
Non-Cash Contributions (both Applicant and Third Party)							

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

University of Wisconsin 600 E. Greenfield Ave. Milwaukee, Wisconsin, 53204				**USDA AWARD NO.** Year: 2 Objective: ,1,3,4			
PROJECT DIRECTOR(S) PI Name Dr. Ryan Newton				Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**						
1. No. of Senior Personnel	Calendar	Academic	Summer	1,717			
a. _1 (Co)-PD(s)			0.2				
b.___Senior Associates							
2. No. of Other Personnel (Non-Faculty)							
a. _1 Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c.___Paraprofessionals							
d. _1 Graduate Students.....							
e._1_ Prebaccalaureate Students.....				3900			
f.___Secretarial-Clerical							
g.___Technical, Shop and Other							
Total Salaries and Wages..... ϒ				5,617			
B. Fringe Benefits (If charged as Direct Costs)				740			
C. Total Salaries, Wages, and Fringe Benefits (A plus B)..... ϒ				6,357			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				12,000			
F. Travel							
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I)..... ϒ				18,357			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K)..... ϒ				18,357			
N. Other ..... ϒ							
O. Total Amount of This Request..... ϒ				18,357			
P. Carryover -- (If Applicable)..... **Federal Funds: $ Non-Federal funds: $ Total $**							
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)						Leave Blank	
Cash (both Applicant and Third Party) ..... ϒ							
Non-Cash Contributions (both Applicant and Third Party) ..... ϒ							

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Budget Explanation – Univ. of Wisconsin - Milwaukee
(Newton)

Objective #1, 3 &4

Years 1 & 2:

C. Salary, Wages and Fringe Benefits ($11,778): Total amount is $5,153 and $6,357 required for Year 1 and Year 2, respectively. The budget for Year-1 is needed to cover salary ($4,0253) for an hourly student be trained and working 320 hours annually at a pay rate of $13/ hour (Fringe rate is 3.2%); and 0.1 month salary of the PI ($1,128=$833, salary+$374 fringe); The budget for Year-2 is required to cover an hourly student salary and fringe for 320 hours ($4,025=$3,900 +125, fringe), and 0.2 month salary of the PI ($2,332= $1,717, salary+$615, fringe). The Co-PI will supervise the hourly student and a graduate student to perform sample preparation, DNA extractions, and preparation for microbial community sequencing; and prepare papers for publication.

E. Materials and Supplies ($22,000): A budget of $10,000 for Years 1 and $12,000 for Year 2 is requested to cover the cost of sample preparation, DNA extraction, Polymerase Chain Reaction, illumina sequencing library preparation, and illumina MiSeq sequencing. Sequencing will be conducted at the Great Lakes Genomics Center. This budget provides microbial community analysis for up to 150 samples per year.

Budget Summary

YEAR 1

Institution Name	**University of Wisconsin-Milwaukee**	**Iowa State University**	**University of Nebraska-Lincoln**
Salaries & Wages	31,997	10,934	8,000
Fringe Benefits	2,731	66	648
Total Salaries, Wages, and Fringe Benefits	34,728	11,000	8,648
Nonexpendable Equipment			
Materials and Supplies	28,500	4,000	300
Travel	1,000		1,305
All Other Direct Cost			
Totals	$64,228	$15,000	$10,253

YEAR 2

Institution Name	**University of Wisconsin-Milwaukee**	**Iowa State University**	**University of Nebraska-Lincoln**
Salaries, Wages, and Fringe Benefits	33,999	3,976	8,240
Fringe Benefits	3,475	24	667
Total Salaries, Wages, and Fringe Benefits	37,474	4,000	8,907
Nonexpendable Equipment			
Materials and Supplies	22,500	2,500	300
Travel	3,000		1,305
All Other Direct Cost			
Totals	$62,974	$6,500	$10,512

Schedule for Completion of Objectives

Start date: June 2019

Completion date: May 2011

Objectives and Tasks	Year 1						Year 2					
	J J	A S	O N	D J	F M	A M	J J	A S	O N	D J	F M	A M
Objective 1. Lab testing to determine feed formulation												
System set up, produce fingerlings and test diets for the feeding trial												
Sample analysis (nutrition and microbiology)												
Data analysis & formulation determination												
Objective 2, Practical feed processing and quality checking												
Ingredients analysis and sourcing												
Feed processing, chemical and physical quality checking												
Objective 3 Farm testing of selected feed												
Set up farm testing protocols and train farmer and students on the protocol												
Produce fingerlings for the tests												
Run feeding trials at farm conditions												
Sample analysis and data processing												
Objective 4												
Extension and trainings (students and farmers)												
Economic analysis of feed production												
Economics analysis of fish production												
Data dissemination and presentation												
Delivery												
Annual report												
Article presentations and paper publications												
Final report												

Participating Institutions And Co-Principal Investigators

University of Wisconsin-Milwaukee
Dong-Fang Deng & Ryan Newton

Iowa State University
Kurt A. Rosentrater

University of Nebraska-Lincoln
Jamilynn B. Poletto

VITA

Dong-Fang Deng
University of Wisconsin
600 E Greenfield Ave
Milwaukee, WI 53204

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EDUCATION

Ph.D. University of California, Davis, USA, 1999, Animal Science/ Nutrition& Physiology
M.S. Zhongshan (Sun Yet-Sen) University, P.R.China, 1990, Biology/Aquaculture Nutrition
M.S. University of California, Davis, USA, 1996, Animal Science/Fish Nutrition
B.S. Zhongshan (Sun Yet-Sen) University, P.R.China, 1987, Biology/Zoology

POSITION

2014- Present Senior Research Scientist, University of Wisconsin, Milwaukee.
2013-2014 Interim Director, Oceanic Institute, Waimanalo, Hawaii.
2009-2013 Research Scientist, Oceanic Institute, Waimanalo, Hawaii.
2005-2009 Project Scientist, University of California, Davis.
2003-2005 Postdoctoral Researcher, University of California, Davis.
2000-2002 Postdoctoral Assistant, Mississippi State University
1993-1994 Visiting Scientist, Deakin University, Australia.
1992-1994 Lecturer, Dept. of Biology, Zhongshan University, Guangzhou, P.R. China,
1990-1992 Teaching Assistant, Zhongshan University, Guangzhou, P.R. China,

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

World Aquaculture Society
Asian Fisheries Society
Editor, Aquaculture Nutrition
Associate Editor, Animal Nutrition
Adjunct Professor, Qingdao Agriculture University, Qingdao, P.R.China
Adjunct Professor, Fisheries College of Jimei University, Xiamen, P.R. China
Technical Committee/Research Subcommittee, NCRAC, NIFA-United State Department of Agriculture

SELECTED PUBLICATIONS (PAST 5 YEARS)

Jiang, M., H.H. Zhao, S.W. Zai, B. Shepherd, H. Wen, and D.F. Deng 2018. A defatted microalgae meal *Haematococcus pluvialis* as a partial protein source to replace fishmeal for feeding juvenile yellow perch *Perca flavescens*. Journal of Applied Phyology https://doi.org/10.1007/s10811-018-1610-3

Jiang, M., H. Wen, G.W. Gou, T.L. Liu, X. Lu, and D.F. Deng. 2018. Preliminary study to evaluate the effects of dietary bile acids on growth performance and lipid metabolism of juvenile genetically improved farmed tilapia *Oreochromis niloticus* fed plant ingredient-based diets. Aquaculture Nutrition https://onlinelibrary.wiley.com/doi/full/10.1111/anu.12656.

Ju, Z.Y., D.F. Deng, C.Viljoen, and I. Forster. 2017. Effects of algae-supplemented diets on shell pigmentation, growth performance, and meat composition of Pacific abalone *Haliotis discus hannai*. Journal of the World Aquaculture Society 48: 93-102.

Zhou, P.P., M.Q. Wang, F.J. Xie, D.F Deng, and Q.C. Zhou. 2016. Effects of dietary carbohydrate to lipid ratios on growth performance, digestive enzyme and hepatic carbohydrate metabolic enzyme activities of large yellow croaker *Larmichthys crocea*. Aquaculture 452: 45-5.

K.K. Zheng D.F. Deng, N. De Riu, G. Moniello, & S.S.O. Hung. 2015. The effect of feeding rate on the growth performance of green sturgeon *Acipenser medirostris* fry, Aquaculture Nutrition 21:489-495.

Hemre Gro-Ingunn and Deng. D.F. 2015. Carbohydrate. In: Dietary Nutrients, Additives, and Fish Health, Lee, C-S., Lim, C., Weber, C. and Gatlin, D. (eds.), pp 95-110. Wiley-Blackwell.

Deng, D.F., Z.Y.Ju, W.G. Dominy, L.Conquest, P. J. Bechtel, and S. Smiley. 2014. Effect of replacing dietary menhaden oil with pollock or soybean oil on muscle fatty acid composition and growth performance of juvenile Pacific threadfin *Polydactylus sexfilis*. Aquaculture 422-423:91-97.

VITA

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Assistant Professor
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Email: newtonr@uwm.edu

EDUCATION

Ph.D. University of Wisconsin-Madison, 2008, Microbiology Doctoral Training Program
B.S. University of Nebraska-Lincoln, 2002, Biological Sciences, Minors – Business Administration & Math

POSITIONS

2015-current	Assistant Professor, University of Wisconsin-Milwaukee School of Freshwater Sciences
2012-2015	Visiting Professor, University of Wisconsin-Milwaukee School of Freshwater Sciences
2010-2011	Postdoctoral Research Associate, University of Wisconsin-Milwaukee, Great Lakes WATER Institute
2008-2010	Postdoctoral Research Associate, University of Georgia, Department of Marine Sciences

SCIENTIFC & PROFESSIONAL ORGANIZATIONS

American Society of Microbiology
Association for the Sciences of Limnology and Oceanography
Ecological Society of America
International Association of Great Lakes Research
International Society of Microbial Ecology

SELECTED PUBLICATIONS (33 total)

Bartelme, R.P., P. Barbier, R.S. Lipscomb, S.E. LaPatra, R.J. Newton, J.P. Evenhuis, and M.J. McBride. 2018. Draft genome sequence of the fish pathogen *Flavobacterium columnare* strain MS-FC-4. *Genome Announcements* 6:e00429-18. doi: 10.1128/genomeA.00429-18.

Bartelme, R.P. †, B.O. Oyserman, J.E. Blom, O.J. Sepulveda-Villet, and R.J. Newton. 2018. Stripping away the soil: Plant growth promoting microbiology opportunities in aquaponics. Frontiers in Microbiology 9:8.

Bartelme, R.P., S.L. McLellan, and R.J. Newton. 2017. Freshwater recirculating aquaculture system operations drive biofilter bacterial community shifts around a stable nitrifying consortium of ammonia-oxidizing *Archaea* and comammox *Nitrospria*. Frontiers in Microbiology 8:101.

Bartelme, R.P.†, R.J. Newton, Y. Zhu, N. Li, B.R. LaFrentz, and M.J. McBride. 2016. Complete genome sequence of the fish pathogen *Flavobacterium columnare* Strain C#2. Genome Announcements 4(3):e00624-16. doi:10.1128/genomeA.00624-16.

Bendall, M.L., S.L.R. Stevens, L.-K. Chan, S. Malfatti, P. Schwientek, J. Tremblay, W. Schackwitz, J. Martin, A. Pati, B. Bushnell, J. Froula, D. Kang, S.G. Tringe, S. Bertilsson, M.A. Moran, A. Shade, R.J. Newton, K.D. McMahon, and R.R. Malmstrom. 2016. Genome-wide selective sweeps and gene-specific sweeps in natural bacterial populations. ISME Journal 10:1589-1601.

Newton, R.J., S.L. McLellan, D.K. Dila, J.H. Vineis, H.G. Morrison, A.M. Eren, and M.L. Sogin. 2015. Sewage reflects the microbiomes of human populations. mBio 6(2): e02574-14. doi: 10.1128/mBio.02574-14

Eren, A.M., M.L. Sogin, H.G. Morrison, J.H. Vineis, J.C. Fisher, R.J. Newton, and S.L. McLellan. 2015. A single genus in the gut microbiome reflects host preference and specificity. ISME Journal 9:90-100.

VITA

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Ames, IA 50011

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EDUCATION

Ph.D. Iowa State University, 2001, Agricultural Engineering
M.S. Iowa State University, 1996, Agricultural Engineering
B.S. Iowa State University, 1994, Agricultural Engineering

POSITIONS

2014 – Present	Associate Professor, Iowa State University
2011 – 2014	Assistant Professor, Iowa State University
2004 – 2011	Lead Scientist, USDA, Agricultural Research Service
2002 – 2004	Assistant Professor, Northern Illinois University
1997 – 2002	Process Development Engineer, Todd & Sargent, Inc.

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

ASAE Biological Engineering (BE) 22 Committee (Ecological Engineering). 2004-Present.
ASAE Biological Engineering (BE) 26 Committee (Modeling Biological Processes). 2004-Present.
ASAE Biological Engineering (BE) 28 Committee (Bioconversion and Bioprocesses). 2003-Present.
ASAE Educational Division (ED) 203 Committee (Undergraduate and Graduate Instruction). 2008-Present. (Secretary, 2008-2010).
ASAE Educational Division (ED) 412 Committee (Professional Ethics). 2006-Present. (Secretary, 2007; Vice Chair, 2008; Chair, 2009).
ASAE Food Process Engineering (FPE) 01/02 Committee (Executive and Steering). 2003-Present.
ASAE Food Process Engineering (FPE) 04/041 Committee (Refereed Publications). 2004-Present.

SELECTED PUBLICATIONS

Ayadi, F., K. A. Rosentrater, and K. Muthukumarappan. 2012. A review of alternative protein sources in aquaculture feeds. Journal of Aquaculture Feed Science and Nutrition 4(1):1-26.

Barnes, M. E., M. L. Brown, and K. A. Rosentrater. 2012. Initial observations on the inclusion of high protein distillers dried grain into rainbow trout diets. Open Fish Science Journal 5:21-29.

Barnes, M. E., M. L. Brown, and K. A. Rosentrater. 2012. Juvenile rainbow trout responses to diets containing distillers dried grain with solubles, phytase, and amino acid supplements. Open Journal of Animal Science 2(2):69-77.

Fallahi, P., K. Muthukumarappan, K. A. Rosentrater, and M. L. Brown. 2012. Twin-screw extrusion processing of vegetable-based protein feeds for yellow perch *Perca flavenscens* containing distillers dried grains, soy protein concentrate, and fermented high protein soybean meal. Journal of Food Research 1(3):230-246.

Mjoun, K.,K. A. Rosentrater, and M. L. Brown. 2012. Culture performance and tissue fatty acid compositions of yellow perch (Perca flavenscens) fed different dietary lipids. Aquaculture 360-361:17-24.

Rosentrater, K. A. and E. Kongar. 2012. Biofuel coproduct densification: techno-economic assessment. Encyclopedia of Energy Engineering and Technology DOI:10.1081/E-EEE-120047375.

Schaeffer, T. W., M. J. Hennen, M. L. Brown, and K. A. Rosentrater. 2012. Nutritional composition and use of common carp muscle in yellow perch diets. North American Journal of Aquaculture 74:297-305.

Ayadi, F., K. A. Rosentrater, K. Muthukumarappan, and M. L. Brown. 2012. Twin screw extrusion processing of distillers dried grains with solubles (DDGS)-based yellow perch *Perca flavenscens* feeds. Food and Bioprocess Technology 5(5):1963-1978.

Ayadi, F., K. Muthukumarappan, K. A. Rosentrater, and M. L. Brown. 2011. Single screw extrusion processing of distillers dried grains with solubles (DDGS)-based yellow perch *Perca flavenscens* feeds. Cereal Chemistry 88(2):179-188.

VITA

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EDUCATION

Ph.D University of California Davis, 2014, Animal Behavior
B.S. University of Rochester, 2009, Neuroscience

POSITIONS

2016-Present Assistant Professor, University of Nebraska-Lincoln
2015-2016 Postdoctoral Researcher, University of California Davis (UCD)
2014-2015 Associate Instructor of Record, University of California Davis
2010-2014 National Science Foundation Graduate Research Fellow, UCD

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
British Ecological Society
Society for Experimental Biology
World Sturgeon Conservation Society

SELECTED PUBLICATIONS

Hamda, N., B. Martin, J. B. Poletto, D. E. Cocherell, N. A. Fangue, J. Van Eenennaam, E. A. Mora, and E. Danner. 2019. Applying a simplified energy-budget model to explore the effects of temperature and food availability on the life history of green sturgeon *Acipenser medirostris*. Ecological 395:1-10.

Poletto, J. B., B. Martin, E. Danner, S. E. Baird, D. E. Cocherell, N. Hamda, J. J. Cech Jr., and N. A. Fangue. 2019. Assessment of multiple stressors on the growth of larval green sturgeon *Acipenser medirostris*: Implications for recruitment and management of early life history stages. Journal of Fish Biology 93-5: 952-960.

Poletto, J. B., D. E. Cocherell, N. Ho, J. J. Cech, A. P. Klimley, and N. A. Fangue. 2018. The effect of size on juvenile green sturgeon *Acipenser medirostris* behavior near water-diversion fish screens. Environmental Biology of Fishes 101-1:66-77.

Bjelde, B., L. Komoroske, M. Hansen, J. B. Poletto, E. Perry, N. Miller, S. Ehlman, S. Wheeler, A. Sih, A. Todgham, and N. A. Fangue. 2018. Juvenile rockfish show resilience to CO 2 -acidification and hypoxia across multiple biological scales. Conservation Physiology 6-1:coy038.

Poletto, J.B., D. E. Cocherell, S. E. Baird, T. X. Nguyen, V., Cabrera-Stagno, A. P. Farrell, and N. A. Fangue. 2017. Unusual aerobic performance at high temperatures in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Conservation Physiology 5-1:cow067.

Poletto, J.B., D.E. Cocherell, T. D. Mussen, A. Ercan, H. Bandeh, M. L. Kavvas, J. J. Cech Jr., and N. A. Fangue. 2015. Fish protection devices at unscreened water diversions can reduce entrainment: Evidence from behavioral laboratory investigations. Conservation Physiology 3-1:cov40.