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An Evaluation of Nitrogen Supplementation and Processed Soy Fractions on the Performance of Cultured Fishes

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AN EVALUATION OF NITROGEN SUPPLEMENTATION AND PROCESSED SOY FRACTIONS ON THE PERFORMANCE OF CULTURED FISHES

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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LIST OF ABBREVIATIONS

ADC-P……..Apparent digestion coefficient of protein

ANF…....……Antinutritional factor

ANOVA……Analysis of variance

BP-SBM……....……Bioprocessed soybean meal

°C….....Degree Celsius

DHA…….……..Docosahexaenoic acid

dm…….Dry matter

EAA……..Essential amino acid

EFA…….Essential fatty acid

EPA……Eicosapentaenoic acid

ER…….Expansion ratio

FCR…….Feed conversion ratio

FM…….....Fish meal

g……Gram

hr……Hour

HSI…….....Hepatosomatic index

K…….....Fulton's condition factor

kg…….....…..Kilogram

L…….....……Liter

lb…….....……Pound

mg…….....…….Milligram

min…….....Minute

mm……Millimeter

mmt ……...…….Million metric tons

n…….....Sample size

NFE……..Nitrogen free extract

NSP…….Non-starch polysaccharide

ppm……..…...Parts per million

RAS……..Recirculating aquaculture system

RG……...….Relative growth

SBM……...…….Soybean meal

SCP……...Single cell protein

SE……Standard error

SGR……...……Specific growth rate

SSI……Splenosomatic index

VFI……Visceral fat index

VSI……..Viscerosomatic index

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ABSTRACT

AN EVALUATION OF NITROGEN SUPPLEMENTATION AND PROCESSED SOY FRACTIONS ON THE PERFORMANCE OF CULTURED FISHES

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World population growth has resulted in an increased demand for a sustainable food supply. This rapid growth coupled with an increased per capita consumption of seafood, has resulted in many of the world's marine fisheries being over-exploited in an effort to meet the global demand for seafood. Aquaculture has attempted to fill the supply and demand gap created by the dwindling supply of fish in the world's oceans. Fishmeal (FM) has traditionally been the primary protein ingredient in aquafeeds fed to farm-raised fish, however its unstable supply and increasing price have driven researchers to identify alternative protein sources. Soybean meal offers a viable alternative with a stable supply at a low price. This research focused on how nitrogen inclusions and varying processing treatments may improve nutritional characteristics of soybean meal for use in aquafeeds. Bioprocessing combined with nitrogen supplementation has the potential to reduce antinutritional factors (ANFs) such as trypsin inhibitors, antigens, saponins, lectins, oligosaccharides, and phytate while simultaneously increasing protein levels.

The first two feeding trials were completed to determine a palatable source and concentration of nitrogen for potential inclusion during bioconversion to limit the risk of nutrient limitation of *Aureobasidium pullulans*. In the first palatability study, three

nitrogen sources, ammonium chloride, diammonium phosphate, and urea, were included (1,250 ppm) into diets of rainbow trout (*Oncorhynchus mykiss*) containing a bioprocessed soybean meal (BP-SBM) in place of fish meal (FM) and feed consumption was monitored. No significant differences in consumption occurred, however it was noted that the diet containing urea was consumed slightly more than the other two nitrogen supplemented diets. Therefore, in a follow-up palatability study urea was supplemented at varying inclusion levels (0, 500, 1,000, 1,500, and 2,000 ppm) to rainbow trout diets containing BP-SBM in place of FM. The study revealed nonsignificant palatability responses when consumption and growth parameters were analyzed among treatments. The results indicate that up to 2,000 ppm urea can be potentially supplemented to *Aureobasidium pullulans* during bioprocessing without any adverse effect on palatability of rainbow trout feeds.

In further examining bioprocessing as a feasible approach to enhancing soy, six experimental soy ingredients were processed and formulated into aquafeeds and fed to rainbow trout and hybrid striped bass (*Morone saxatilis x M. chrysops*) in separate feeding trials. The performance of these experimental ingredients was tested against a FM control through digestibility and growth trials (105-day). The optimal experimental soy ingredient in each trial was determined based on consumption, growth, feed efficiency, apparent digestibility, and health indices. The experimental ingredient with the highest apparent digestion coefficient of protein (ADC-P) when fed to hybrid striped bass was the BP-SBM ingredient with an enzyme inclusion (Diet 4). This ingredient was found to have a significantly higher ADC-P than the soybean meal

negative control ingredient (SBM). Growth trial experiments revealed that Diets 1 and 2 (BP-SBM fraction #1 and BP-SBM fraction #2) were the top performing diets fed to rainbow trout when comparing growth parameters and health indices. Diets 1 and 5 (BP-SBM fraction #1 and the washed base BP-SBM ingredient) were the top performing HSB diets when comparing growth parameters and health indices.

CHAPTER 1. SOY AS AN ALTERNATIVE PROTEIN SOURCE IN AQUAFEEDS

Introduction

World population growth has resulted in an increased demand for a sustainable food supply. The global population continues to grow at a rate of 1.6% annually (FAO 2014), resulting in a continually increasing number of people who regularly consume seafood. Per capita consumption of seafood also continues to increase from 9.9 kg in the 1960's to 19.7 kg in 2013 and preliminary estimates of more than 20 kg today (FAO 2016). As a result of the increased population growth and seafood consumption, many of the world's fisheries have been exploited in an attempt to meet the overwhelming demand for seafood. Currently 89.5% of marine fish stocks are fully fished or overfished at biologically unsustainable levels (FAO 2016). The exploitation of the world's marine fisheries has had detrimental effects on the fish populations inhabiting them. Marine fishery production peaked in 1996 at 86.4 million metric tons (mmt) and has since declined to 80.9 mmt in 2013 (FAO 2016).

Aquaculture attempts to fill the supply and demand gap that is created by the marine fisheries' dwindling production. The aquaculture industry's fish production expanded annually at a rate of 6.2% from 2000 to 2012 and now accounts for 44.1 percent of total fish production, up from 31.1% in 2004 (FAO 2016). Total production of aquatic animals raised in aquaculture in 2014 amounted to 73.8 mmt of which 49.8 mmt was from finfish, a $99.2 billion industry (FAO 2016). Aquaculture production of fish for human consumption surpassed the production of wild-caught fish for human consumption for the first time in 2014.

Fishmeal (FM) is the primary protein ingredient in traditional aquafeeds. It can be made from any type of seafood but is generally manufactured from small marine fish that are not suitable for human consumption because of their high oil content and bony nature (Miles and Chapman 2006). Common species of fish that are usually manufactured into FM are often termed "industrial fish" and include those in the groups of anchovies, herrings, menhaden, sardines, shads, and smelt (Miles and Chapman 2006). The majority of commercial FM is produced using a mixture of these species, however if only one species of industrial fish comprises more than 50% of the raw material then the FM can be classified as pure (Hertrampf and Piedad-Pascual 2000).

Due to a diminishing global supply and recent increases in the market price, it is believed that future use of FM in compound aquafeeds will continue to decrease (Tacon and Metian 2008). The diminishing global supply of FM is primarily due to high demand and the great amount of fish it takes to manufacture FM. Approximately 4 to 5 tons of whole fish are required to produce 1 ton of dry FM (Miles and Chapman 2006). The elevated ratio of whole fish input to FM output is primarily due to the high water content of industrial fishes. Industrial fishes processed into FM typically are comprised of 22% solids, 6% oil, and 72% water (Hertrampf and Piedad-Pascual 2000).

The FM market is highly variable due to supply, demand, source, and quality of the FM. FM is generally considered high quality if it contains between 60% and 72% crude protein (Miles and Chapman 2006). The price of FM sourced from Peru, a

product containing 65% crude protein, peaked in December of 2014 at $2,390 per metric ton and has since declined to $1,070 per metric ton in March of 2017 (World Bank 2017).

Due to the fluctuating FM market, researchers have been driven to identify plant-based alternatives as viable protein sources in aquafeeds. One possible plant-based alternative is soybean meal (SBM). The SBM supply is more stable resulting in a lower price and a more stable market than FM. In March of 2017, a metric ton of SBM was approximately $356.89, only 33.4% of the value of a metric ton of FM (World Bank 2017). Unlike FM, SBM is produced in the Midwest making it more readily available and cheaper for fish producers in central North America because the soybeans are grown locally and transportation costs are lower. The soybean industry was a $38.7 billion industry in the United States in 2012 and 77% of sales were from the top 10 soybean producing states including Iowa, Minnesota, Nebraska, North Dakota, and South Dakota, which were all among the top 10 in 2012 (USDA 2012).

Soy products have shown to be useful as protein ingredients in animal feeds and they are routinely manufactured into commercial diets for various species of fish (Forster 2002). Soybeans, however, cannot be included into aquafeed formulations without first being processed into a more concentrated product. An assortment of soy protein products can be produced from processing soybeans such as full-fat soybean meal, soy flour, soybean meal, soy protein concentrate, and soy protein isolate, but soybean meal is the primary form that is used in animal feeds (Gatlin et al. 2007). Soy products have been the preferred plant-based alternative because they contain high

levels of crude protein ranging between 38 and 49% (Gatlin 2002) and have a well-balanced amino acid profile relative to other plant-based sources (Dersjant-Li 2002).

Despite the positive attributes of soy products as FM replacers, there are restrictions in their use. The constraints are especially prevalent when including SBM in diets of carnivorous fish species which are more sensitive than omnivorous or herbivorous species to the antinutritional factors (ANFs) present in SBM (Dersjant-Li 2002). ANFs present in SBM that have negative effects on various fish species include protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, and allergens (NRC 2011). Other nutritional limitations may include non-starch polysaccharides, oligosaccharides, fibers, and deficiencies in amino acids, namely methionine and lysine (Gatlin et al. 2007).

Nutrient limitations and ANFs can be successfully reduced and even eliminated through regular processing methods resulting in a highly digestible product with a high protein content and low antinutritional components (Dersjant-Li 2002). ANFs can be split into two primary categories, heat labile or heat stable compounds, based on their ability to withstand thermal processing (Francis et al. 2001). Heat-labile compounds present in soybean meal such as protease inhibitors, phytates, lectins, and antivitamins are easily destroyed by heat treatments like extrusion (Drew et al. 2007; Francis et al. 2001). Oligosaccharides are also affected by thermal treatments and may become more digestible after processing. However, other ANFs that are heat stable are not affected by extrusion or other heat treatments and require further processing for removal. Heat stable ANFs present in soybean meal include saponins, non-starch polysaccharides,

antigenic proteins, estrogens, and phenolic compounds (Francis et al. 2001). Caution needs to be used when ridding plant-based ingredients of ANFs though, because thermal treatments and extrusion may have unintended adverse effects like decreasing the nutritional quality of proteins and carbohydrates (Francis et al. 2001).

One method of further processing to increase the value and utilization of a soy-based protein product for fish feeds is fermentation or bioprocessing. The process of fermentation and use of microorganisms produces single cell protein (SCP). The cultivation of SCP is done by multiplying selected strains of microorganisms on raw materials while directing growth of culture and cell mass followed by a separation process to harvest the cells (Suman et al. 2015). Biological enhancement of SBM shows great potential for reducing ANFs, such as oligosaccharides, and adding essential nutrients by involving the use of microorganisms such as yeast, bacteria, or fungi to change the chemical composition of the ingredient (Gatlin et al. 2007). SCP's have been successfully added to aquafeed diets as partial replacements to FM due to their good source of protein, fats, carbohydrates, ash ingredients, water, phosphorus, and potassium (Suman et al. 2015).

Fermentation has been shown to reduce phytic acid concentrations in SBM due to the phytases that are produced by yeast or lactic acid bacteria during the conversion process (Francis et al. 2001). Indigestible oligosaccharides and trypsin inhibitors can be eliminated from SBM during the fermentation process as well (Barnes et al. 2012). However, there may be some drawbacks to fermentation and the use of SCP. Some limitations of SCP include cell walls in algal species, mycotoxins in fungal species,

and the high cost of bacterial species (Ravindra 2000). High nucleic acid content and unacceptable color and flavors have also been noted as disadvantages to the use of SCP as a protein supplement (Suman et al. 2015).

Fermented soy products that have been included into diets as the primary protein source have exhibited significant improvements over diets containing SBM as the primary protein source. Rainbow trout *Oncorhynchus mykiss* that were fed a diet with up to 62% of the FM replaced with fermented SBM exhibited similar growth performance metrics to the FM control diet (Barnes et al. 2014). Yamamoto et al. (2010) fed rainbow trout a diet containing only fermented SBM (100% replacement of FM) as the primary protein source and found that the fish exhibited significantly greater body weight, weight gain, and specific growth rate (SGR) as compared to diets containing an unfermented product at the same inclusion level.

In a similar study, diets were made with incremental inclusions of fermented SBM and fed to a warm water species, hybrid striped bass *Morone chrysops x M. saxatilis.* Growth performance results indicated that fish fed the fermented soy diets outperformed fish fed the SBM diets at every corresponding inclusion level (Rombenso et al. 2013). Also, comparable growth was maintained in fish fed the fermented soy diets as compared to the fish fed the FM control when the diet constituted up to 56% of the fermented soy protein source in the experimental diets (Rombenso et al. 2013). However, the paradigm indicates that it is difficult to completely replace FM in diets while maintaining long-term production performance.

During fermentation, the selected microorganism being used may become nutrient limited. Nutrient supplementation may be required depending on the substrate and type of microorganism. Some substrates may be a suitable carbon feedstock but will require ammonia salts and phosphorus salts in order to fulfill the microbe's nutritional demands (Suman et al. 2015). Additionally, the type of microorganism may vary in its nutritional needs. Nitrogen sources such as ammonia, ammonia salts, urea, nitrates, and organic nitrogen wastes can be useful for bacterial growth during SCP production (Suman et al. 2015).

Enzyme saccharification is another means of further processing SBM to produce a more palatable and digestible feed ingredient with few ANFs. The primary focus of previous enzyme saccharification research has been the use of phytase to hydrolyze phytate. SBM and other plant-based protein sources contain phytate or phytic acid, a form of phosphorus that is unavailable to fish. Phytate reduces the hydrolysis of protein and absorption of minerals and is a pollution problem in fresh water (Storebakken et al. 2000). Rainbow trout that were fed soy-based diets with no phytase pretreatments and low phosphorus supplementation exhibited signs of phosphorus deficiencies (Cain and Garling 1995). Phytase treatments to SBM have reduced the concentration of phytic acid from 8g kg^{-1} to less than 0.5g kg^{-1}, resulting in increased availability of phosphorus to the fish (Storebakken et al. 1998). As a result of the improved bioavailability of phosphorus in the phytase treated diets, fish growth performance has also improved. Rainbow trout that were fed a phytase treated diet without any phosphorus supplementation had excellent weight gain and feed

conversion and exhibited an 88% reduction in phosphorus discharge as compared to fish fed a commercial FM based diet (Cain and Garling 1995). Similar results were also observed in Atlantic salmon *Salmo salar* with a good feed conversion ratio (FCR), 100% survival, and rapid growth (Storebakken et al. 1998). Cao et al. (2008) found that a phytase treatment of 1000 U kg^{-1} was able to replace inorganic phosphorus supplementation of 12.5 g kg^{-1} and was the most cost effective and efficient dosage level in converting phytic acid to available phosphorus in Nile tilapia *Oreochromis niloticus* diets.

Rainbow Trout Culture

Rainbow trout are a freshwater species native to the Pacific Northwest and along the west coast of North America up to Alaska and the Aleutian Islands where they were traditionally sought as a food fish to meet regional market demands. However, due to the increased demand for seafood and the declining production from marine fisheries, researchers have turned to aquaculture and rainbow trout have been globally recognized as an optimal culture species. Rainbow trout, the most widely produced species of trout, are a hearty species that grow rapidly and thrive in well oxygenated water that is in the range of 13 to 18°C (Hardy 2002). They are easy to spawn and produce fry that are large at first feeding. Another advantage of rainbow trout culture is that hatcheries have the opportunity to provide eggs at all times of the year by adjusting the photoperiod of the broodstock to change their spawning time.

Farm production of rainbow trout is not a new idea, but it is a practice that has grown rapidly since the 1950's. Global annual production of rainbow trout was less

than 20,000 metric tons in the 1950's and has since soared to over 800,000 metric tons in 2014 (FAO 2014). France, Chile, Denmark, and Italy are the major rainbow trout producers, producing nearly half of the total global production while the United States contributes only 7% (Hardy 2002). The top producing state in the United States, Idaho, produces 67% of the nation's total production (Hardy 2002). This cost-efficient production is due to the many natural springs in the Snake River Valley that provide a suitable supply of water that meets the water quality requirements of rainbow trout and the flow requirements to stock high densities of trout in concrete or earthen raceways without the use of pumps.

The production cycle begins with the hatching of eggs that have been artificially spawned from broodstock. Hatching time varies based on water temperature and can occur in as few as 21 days at 14.4°C or as long as 100 days at colder temperatures around 3.9°C (FAO 2005). One obstacle that researchers have overcome is the production of same-sex or sterile offspring. If male and female rainbow trout are raised together the males will be susceptible to early maturation and in turn begin to utilize nutritional resources for gonadal growth instead of somatic growth. As a result, producers prefer stocks of rainbow trout that are all-female or triploid stocks that are sterile. Triploid stocks are produced by exposing the eggs to heat or pressure shock treatments (FAO 2005). All-female triploid stocks of rainbow trout can be produced by fertilizing normal female eggs with the milt from masculinized females in combination with a heat shock treatment (Lincoln and Scott 1983). However, all-female stocks are

usually preferred over triploid stocks because the advantageous effects of the triploid stocks are not realized until after the rainbow trout is 500 g or larger (Hardy 2002).

Once hatching is complete and the yolk sacs are completely absorbed, fry are moved into rearing tanks. Fry should be fed continuously with a mechanical belt feeder at a rate of 10% of the fish weight daily for 2 to 3 weeks (FAO 2005). During these initial weeks of first feeding fish are typically fed small feed particles that are produced by crushing feed pellets and sifting the crushed product to obtain a desirable particle size. Once fish become larger they can then be moved to grow out tanks and be fed a larger pellet. Fingerlings are fed mechanically, by hand, or with demand feeders frequently throughout the day up to 4 times per hour (Hardy 2002). During this phase fish should be gradually switched from the crushed diet to an extruded pellet while mixing pellets and the crushed diet to ensure that the smallest fish still have the opportunity to eat. The pellet size that is offered should continue to increase in this manner until the fish obtain a harvestable size. Feeding rate and frequency should be adjusted as necessary as they will decrease with increased fish weight and decreased temperature (Hardy 2002).

Cannibalism can become a major problem if fish size variation becomes too large. Cannibalism can be minimized by offering feed sizes that can be readily accepted by all of the fish as previously described, and by regularly grading of the stock. Rainbow trout should be graded at least 4 times as they are being raised to market size. Recommended sizes intervals for when grading should occur include 2 to 5 g, 10 to 20 g, 50 to 60 g, and at 100 g or greater (FAO 2005). Grading as well as

regular fish quantity and biomass sampling should continue until fish reach a marketable size. Marketable size in the United States is 500 to 600 g which is usually obtained within 6 to 9 months (FAO 2005; Hardy 2002).

Hybrid Striped Bass Culture

Hybrid striped bass are crosses between white bass *Morone chrysops* and striped bass *Morone saxatilis*. When the female in the cross (reciprocal) is a white bass the hybrid is known as a sunshine bass. Conversely, when the female in the cross (original) is a striped bass the hybrid is known as a palmetto bass. Hybrid striped bass, unlike many other hybrid species, are fertile.

The sunshine hybrid is the more commonly produced cross today because the female white bass are smaller making them easier to handle, produce viable eggs in captivity, and mature nearly a year earlier than the female striped bass allowing them to spawn with male striped bass that mature at the same age (Ludwig 2004). Also, it has been documented that the sunshine hybrid grows faster in closed-circuit systems and is more tolerant to salinity changes than the original hybrid, making them the more favorable cross (Guner et al. 2016). McEntire et al. (2015) also found FCR to be improved in sunshine bass, yet no differences in production were found when sunshine and palmetto bass were reared in earthen ponds.

Similar to many other cultured species, hybrid striped bass came to be an important species in aquaculture when natural fisheries production began to decline. The culture of this species became prominent when commercial harvest of one of its parent species, the striped bass, declined drastically and then later was strictly

regulated and finally outlawed (Hodson 1989). Consequently, global annual aquaculture production has expanded in order to supply hybrid striped bass in replacement of the traditional wild harvest of striped bass. Annual aquaculture production of hybrid striped bass in the United States increased from 181,500 kg in 1987 to 4.81 million kg in 2001 (Ludwig 2004). Other major global producers include Taiwan, Israel, and Italy.

Hybrid striped bass can tolerate wide ranges of various water quality parameters making them an ideal culture species. Their thermal tolerance ranges from 4 to 33°C but optimum growth occurs between 25 to 27°C (Hodson 1989). Similarly, they can tolerate very low dissolved oxygen levels, as low as 1 mg/L, but preferred levels are 6 mg/L or greater at optimal temperatures (Hodson 1989). During pond production of hybrid striped bass it is important to closely monitor water quality, especially during the fry and fingerling stages when they require zooplankton as their primary food source. In order to have a healthy and plentiful zooplankton stock, ponds are typically fertilized with organic and inorganic fertilizers to produce phytoplankton. Also, alkalinity should be maintained above 50 mg/L to stabilize pH and ensure adequate nutrient uptake of phytoplankton (Ludwig 2004). Lime may be added to the pond if alkalinity becomes too low. Hybrid striped bass can survive short term exposures to pH levels as low as 2.5 but optimal growth occurs between the range of 7.0 and 8.5 (Hodson 1989).

Hybrid striped bass can be grown in intensive systems (raceways, cages, indoor recirculating systems) but most fingerlings raised for grow-out are grown in ponds

while fish that are cultured for food-fish are commonly produced in tanks (Webster et al. 2002). Good fingerling production is possible in ponds but water quality parameters are highly variable making it difficult to yield consistent crops. Temperature swings, high pH and unionized ammonia levels that accompany phytoplankton blooms, low dissolved oxygen levels, and insect predation all inhibit fingerling production (Ludwig 2003). Also, production is limited in the winter months of temperate climates when temperatures become too low to allow for growth. Therefore, it may be more practical to culture larvae indoors in tanks where conditions are less variable and easily controlled (Ludwig 2003). However, fingerling pond production may be less risky at southern latitudes than tank production due to improved survival rates in pond production (Engle and Sapkota 2012).

The production cycle of hybrid striped bass begins with hatching. Broodstock produce eggs and sperm in the spring when water temperature is between 18 and 20°C (Hodson 1989). Eggs hatch in approximately 2 days when the water temperature is in the range of 18 to 20°C but may take longer than 3 days if water temperatures are colder (Hodson 1989). First feeding does not occur until mouth parts are fully developed, about 5 days after hatching when fry of the sunshine hybrid are 3 to 5 mm and fry of the palmetto hybrid are 6 to 9 mm in length (Ludwig 2004). The fry are then fed a live zooplankton diet until they are grown to fingerling size (15 to 20 mm) and then they are stocked in fingerling rearing ponds where they are reared for 5 to 9 months when they reach 100 to 140 mm in length. After 30 to 45 days the fingerlings should be graded to prevent cannibalism (Ludwig 2004).

The final stage in production occurs when the fingerlings are grown to a market size of 700 to 1,200 g (Webster et al. 2002). Growth to market size usually takes 18 to 24 months (Hodson 1989). Growth time varies, however, due to water quality parameters (primarily temperature) and sex of the fish. Male and female hybrid striped bass mature at different times so it may be advantages to produce all-female populations that are characteristic of late maturation (Guner et al. 2016).

Nutritional Requirements of Cultured Fishes

Fish species vary greatly in their morphology and physiology. They are poikilothermic so they can either be adapted for warm water or cold water environments as well as fresh or salt water environments, within species tolerances. They also vary by their trophic status which may be carnivorous, omnivorous, or herbivorous in nature, therefore it is not appropriate to define the nutritional requirements of fish in general but rather by species (Storebakken et al. 2000).

The manufactured diets that are fed to cultured fishes are composed of multiple ingredients that all play a role in meeting the protein and amino acid, lipid, carbohydrate, mineral, and vitamin requirements. These ingredients may also serve other functions such as feed functionality or attractants for fish. Complete diets should be palatable, digestible, and fish should be able to readily utilize the nutrients present in the ingredients (Glencross et al. 2007).

There has been a recent interest to partially or completely replace FM with plant feedstuffs in aquafeeds due to the inflated cost and wavering availability of FM. Soy has been identified as an ideal candidate to be included into aquafeeds. However,

the inclusion of soy further complicates feed formulation to meet the nutritional demands of some fish, especially carnivorous species. An ideal ingredient should have low levels of fiber, starch, non-soluble carbohydrates, and antinutrients, plus have a high protein content, good amino acid profile, high nutrient digestibility, and reasonable palatability (Gatlin et al. 2007).

Proteins and Amino Acids

Protein is arguably the most important constituent in the diets of cultured fishes. Proteins are essential for every type of cell in the body including muscles, bones, organs, tendons, and ligaments (NRC 2011). Amino acids, the building blocks of proteins, are needed in the correct quantities in the diet relative to the amino acid requirements of the fish. Fish require the same 10 essential amino acids (EAA) as all other vertebrates including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (NRC 2011). Lysine and methionine are usually limiting in nearly all practical feed ingredients that are commonly used when formulating the diets of fish, including rainbow trout and hybrid striped bass (Hardy 2002; Webster et al. 2002).

The exact dietary protein requirement of fishes is variable due to several factors. The protein requirements of fish change with fish size, age, reproductive status, and dietary energy level (Hardy 2002). Despite the ever changing dietary protein requirement of fish, NRC reports that the digestible protein requirement of hybrid striped bass and rainbow trout is 36% and 38%, respectively (2011). Also, the requirements of lysine, one of the first two limiting amino acids, are 1.6% and 2.4%

for hybrid striped bass and rainbow trout, respectively, and the other limiting amino acid, methionine, is required at 0.7% of the diet for both species (NRC 2011).

FM, the traditional primary protein ingredient in aquafeeds, is reported to have between 59% and 72% crude protein, 4.22% to 7.38% lysine, and 1.47% to 3.04% methionine depending on the source (NRC 2011). The crude protein content of solvent-extracted soybean meal (SBM), on the other hand, is usually in the range of 45% to 50% (Storebakken et al. 2000) and the lysine and methionine levels of SBM is 2.83 and 0.61, respectively (NRC 2011).

Soybeans are subjected to various heat treatments and mechanical processing during the production of SBM that limits the availability of individual amino acids. Temperature, moisture, and duration of the heat treatment are the most important factors in the degree of the effect that heat treatments have on amino acid availability. A mild heat treatment of up to 90°C has the potential to benefit ingredient digestibility and palatability by reducing ANFs and inactivating heat labile compounds but higher temperatures may negatively affect amino acids such as lysine and serine (Storebakken et al. 2000).

Lipids

Protein is the most expensive component of the diet so it should not be over formulated in excess of requirements because fish may use the extra protein as energy, instead lipids should be included into the diet (Webster et al. 2002). Essential fatty acids (EFA) of the omega-3 group (n-3) and omega-6 group (n-6) are required in the diets of rainbow trout and hybrid striped bass. Fish use EFA such as eicosapentaenoic

acid (EPA), 20:5n-3, and docosahexaenoic acid (DHA), 22:6n-3, to metabolize into phospholipids (NRC 2011).

Similar to protein requirements, EFA requirements vary with reproductive and physiological stage. The n-3 requirements of juvenile rainbow trout have been reported to be approximately 1% which can be achieved by adding marine fish oil to the diet at an inclusion rate of 4 to 5% of the diet (Hardy 2002). Hybrid striped bass have similar n-3 requirements of around 1% but this requirement may be achieved in their diet without the inclusion of marine fish oils if at least 30% of the diet is composed of menhaden FM (Webster et al. 2002). However, if the primary protein source is plant derived or if the menhaden FM inclusion is low, fish oils will need to be included in the diet to meet the n-3 EFA requirement.

Caution should be used when including soy oil as a lipid source in the diets of fish, especially cold-water carnivorous species that have a high requirement for n-3 fatty acids (Storebakken et al. 2000). Soy oil does contain α-linolenic fatty acid, 18:3n-3, at a concentration of 6.8% of its total fatty acids, but it lacks the longer chain n-3 fatty acids, EPA and DHA (NRC 2011). Conversely, fish oil contains a higher content of n-3 fatty acids as compared to soy oil. Menhaden fish oil contains EPA and DHA at 11.0% and 9.1%, respectively, of the total fatty acid content (NRC 2011). The major fatty acid that is present in soy oil is linoleic acid, 18:2n-6, that comprises over half of the total fatty acid content (Storebakken et al. 2000). Disease resistance and physiological functionality may be compromised in fish with high n-3 requirements if

soy oil is the main lipid source resulting in fatty acid deficiencies (Storebakken et al. 2000).

Carbohydrates

Carbohydrates are the cheapest form of energy in aquaculture diets. However, unlike protein and lipid sources that are formulated to meet minimum nutritional requirements, carbohydrates are included into diets at maximum tolerance levels for each specific fish species, especially carnivorous fishes. This is mainly due to the fact that carnivorous fishes have low glucose uptake rates and slow blood glucose clearance (Kamalam et al. 2017).

Carbohydrates are classified based on their degree of polymerization and can therefore be identified as monosaccharides, disaccharides, oligosaccharides, or polysaccharides (NRC 2011). Among all of the forms of carbohydrates that are present in plant-based ingredients, however, only sugars and starches have a nutritional value to fish (Kamalam et al. 2017). Hybrid striped bass can effectively utilize both lipid and carbohydrates as energy sources and the type of carbohydrate did not have an effect when glucose, maltrose, and dextrin were compared in diets with a 25% dietary carbohydrate inclusion (Webster et al. 2002). Rainbow trout are generally intolerant to dietary carbohydrates, however it has been found that they can adapt to dietary carbohydrate inclusions by increasing endogenous enzyme levels (Krogdahl et al. 2005).

Carbohydrates are included as an energy source to spare more expensive protein and lipid sources, yet at low enough inclusion levels as to not have a negative

effect on digestion or physiological functionality. However, more carbohydrates will inevitably be added to the diets of cultured fishes if the common trend of replacing FM with plant derived protein sources continues in aquaculture. Soybeans contain approximately 30% carbohydrate of which 10% are oligosaccharides such as sucrose, raffinose, and stachyose but sucrose is the only form that is nutritionally available to fish (Gatlin et al. 2007; Storebakken et al. 2000). Raffinose and stachyose are not able to be utilized by fishes or other monogastric animals because they lack α-galactosidases and therefore are not able to hydrolyze these oligosaccharides (Storebakken et al. 2000).

Minerals

Many fishes require the same minerals as terrestrial animals. However, fish differ from terrestrial animals in their dietary mineral requirements because they have the ability to obtain essential minerals through the water in which they are reared, with the exception of phosphorus and iodine (Hardy 2002). Therefore, it can be difficult to quantify the mineral requirements of fishes such as rainbow trout and hybrid striped bass.

Minerals can be grouped into two main categories, macrominerals and microminerals. The main macrominerals that are required by cultured fishes include calcium, chlorine, magnesium, phosphorus, potassium, and sodium. These macrominerals are essential for osmoregulation as well as forming bones, scales, fins, and other hard tissues (NRC 2011). The other mineral category, microminerals, includes minerals that are commonly recognized to be essential in lower

concentrations such as chromium, copper, iodine, iron, manganese, selenium, and zinc. Microminerals are necessary for a variety of biochemical processes and are important components of hormones and enzymes (NRC 2011).

Only five microminerals (copper, iodine, iron, manganese, and zinc) need to be supplemented to the diet when rainbow trout are fed a FM based diet because the FM provides the rest of the minerals in excess (Hardy 2002). However, when FM is increasingly replaced with plant-based ingredients such as SBM, both macrominerals and microminerals will need to be supplemented in higher quantities. Macrominerals including calcium, phosphorus, sodium, and chlorine, and microminerals including iron, selenium, and zinc are all present in much lower quantities on an as-fed basis in SBM as compared to FM (NRC 2011). Thus, caution should be used and mineral supplementation may be necessary when formulating diets with increased amounts of SBM to avoid mineral deficiencies.

Vitamins

There are two classifications of vitamins, water-soluble and fat-soluble. Water-soluble vitamins are easily absorbed and any that are consumed in excess of nutritional requirements are excreted. Choline, inositol, and vitamin C (ascorbic acid) are all water-soluble vitamins that are required in large quantities in fish diets (NRC 2011). Fat-soluble vitamins are stored in the body once they are absorbed through the intestine and they do not need to be replenished nearly as often as water-soluble vitamins. Fat-soluble vitamins include vitamins A, D, E, and K. It is important to not

supplement fat-soluble vitamins in excess as to avoid vitamin toxicity, termed hypervitaminosis (NRC 2011).

Rainbow trout require ascorbic acid (50 mg kg^{-1}), myo-inositol (300 mg kg^{-1}), and choline (1000 mg kg^{-1}) in much larger quantities as compared to the other water-soluble vitamins that are required in the diet (Hardy 2002). Hybrid striped bass have similar water-soluble vitamin requirements with high requirements for ascorbic acid and choline (22 mg kg^{-1}, 500 mg kg^{-1}) as well as a niacin requirement of 200 mg kg^{-1} that is relatively high (Webster et al. 2002). Vitamin E is the primary fat-soluble vitamin that rainbow trout and hybrid striped bass require. Rainbow trout require 50 mg kg^{-1} and hybrid striped bass require 28 mg kg^{-1} of vitamin E in their diet (NRC 2011).

Similar to many other nutrients in aquafeeds, the vitamin aggregate declines in aquafeeds when FM is replaced with plant derived products such as SBM. Also, vitamin quality and quantity from ingredient sources could be negatively affected if feeds are stored too long, under poor conditions, or if the ingredient is cooked or extruded (Barrows et al. 2008). Hence, diets should be supplemented with a heat-stable vitamin premix as well as other choline sources.

Research Objectives

The research presented in this thesis was obtained by examining a cold-water species, rainbow trout, and a warm-water species, hybrid striped bass, fed various experimental diets through three main trial designs. The trial designs included palatability, digestibility, and growth trials. The species and trials were used to assess

diets where FM was replaced with bioprocessed soybean meal (BP-SBM). The diets with BP-SBM inclusions varied with nutrient supplementation and fractions of the BP-SBM to determine growth performance in subsequent trials. The experimental design of each trial included at least one control diet as a means for comparing the BP-SBM diets to a base-line ingredient such as FM or SBM. The three main research objectives were as follows:

1.) Determine a palatable source of nitrogen for potential inclusion during bioconversion to limit the risk of nutrient limitation of a microbe, *Aureobasidium pullulans*. Three nitrogen sources, ammonium chloride, diammonium phosphate, and urea, were included (post-conversion) into diets of rainbow trout containing BP-SBM in place of FM and feed consumption was monitored. In a following trial, urea was further investigated to determine the maximum concentration that could be included into the diets of rainbow trout before affecting palatability.
2.) Determine apparent digestion coefficient of protein (ADC-P) in BP-SBM fractions. Experimental diets were formulated with BP-SBM fractions to meet the nutritional requirements of hybrid striped bass and an apparent digestibility trial was completed to determine the BP-SBM fraction with the highest protein digestibility.
3.) Evaluate growth performance of rainbow trout and hybrid striped bass fed experimental diets with BP-SBM fractions included into the diet. Two trials, one trial for each species, were completed to evaluate and compare

growth performance and health indices of the two species when fed experimental diets.

CHAPTER 2. EFFECTS OF INORGANIC NITROGEN SOURCES AND CONCENTRATION ON FEED PALATABILITY

Introduction

Traditionally fishmeal (FM) has been the primary protein source in aquafeeds. However, the future use of FM is uncertain and may decrease due to a fluctuating market price and wavering supply (Tacon and Metian 2008). This is currently a topic of great concern in aquaculture as the global population and per capita consumption of seafood continue to increase while wild marine fishery production has plateaued and begun to show a downward trend due to overexploitation of the world's fisheries (FAO 2016). The combinations of these factors has provoked researchers to identify alternative, sustainable protein sources for use in aquafeeds.

An abundance of research has focused on replacing FM in aquafeeds with plant-based protein sources, primarily soy-derived protein. Soybean meal (SBM) has a well-balanced amino acid profile, favorable crude protein level, and few antinutritional factors (ANF) relative to other plant-based protein sources (Dersjant-Li 2002; Gatlin et al. 2007). However, SBM does have limitations on inclusion levels especially in the diets of carnivorous fishes. Protease inhibitors, non-starch polysaccharides (NSP), lectins, saponins, phytic acid, phytoestrogens, and allergens are all ANFs that are present in SBM that limit growth and nutrient uptake in carnivorous fishes (Francis et al. 2001; NRC 2011). Amino acids including methionine and lysine may also be limiting in the diets of carnivorous fishes if too much FM is replaced with SBM

(Gatlin et al. 2007). Therefore, further processing may be required if soy ingredients are to be included into aquafeeds at high inclusion levels.

Bioprocessing or fermentation reduces ANFs and frees up nutrients to produce a more digestible and palatable product by using microorganisms to produce single cell protein (SCP). SCP is dried cells of microorganism which can be used as a protein source in animal feeds. SCP has a high protein content and contains fats, carbohydrates, nucleic acids, vitamins, minerals, and is rich in lysine and methionine which are often limiting amino acids in animal diets (Suman et al. 2015).

A variety of microorganism and substrate combinations can be used during bioprocessing. Algae, fungi, and bacteria are the principal sources of microbial protein that can be used in order to make SCP during bioprocessing (Ravindra 2000). SCP can be produced with any of these aforementioned microbes on a variety of substrates. Common substrates that are used are generally inexpensive, low-grade materials that supply adequate carbon and nitrogen for microbe growth (Ravindra 2000).

Depending on the type of microbe, growth rate, incubation duration, and substrate source, the substrate may become nutrient limiting during fermentation. Some substrates may be a suitable carbon feedstock but microbes may require supplementation of phosphorus salts and further require bioavailable nitrogen sources such as ammonia, ammonium salts, urea, nitrates, and organic nitrogen wastes (Suman et al. 2015). Bioprocessing can be a useful technique to enhance the nutritional profile of SBM while lowering ANFs to successfully replace more FM in the diets of rainbow

trout *Oncorhynchus mykiss* and hybrid striped bass *Morone chrysops x M. saxatilis* (Gatlin et al. 2007; Rombenso et al. 2013; Yamamoto et al. 2010).

The primary objective of this study was to identify a palatable source of nitrogen in rainbow trout diets for potential inclusion during bioprocessing to reduce the risk of nutrient limitation for the microbe, *Aureobasidium pullulans*. We monitored feed consumption to determine the most palatable nitrogen source of three sources, ammonium chloride, diammonium phosphate, and urea. Based on those results, we down selected to urea and further investigated the compound by varying inclusion levels in experimental diets to determine maximum concentration.

Methods and Materials

Experimental Design

Two palatability trials were conducted to identify an appropriate nitrogen source and maximum nitrogen concentration in rainbow trout diets. In Experiment A, a total of four diets, three experimental and one control diet were formulated (Table 2-1). Three nitrogen sources, ammonium chloride, diammonium phosphate, and urea, were included (pre-extrusion) into the three experimental bioprocessed soybean meal (BP-SBM) diets at a set inclusion level of 1250 ppm based on the nitrogen aggregate of each compound. The nitrogen portion of ammonium chloride, diammonium phosphate and urea were 26.19%, 21.27%, and 46.64%, respectively. The remaining control diet was a BP-SBM control diet with no nitrogen supplementation (0 ppm). The three experimental diets and the BP-SBM control diet were all manufactured with a 40% inclusion of BP-SBM (100% replacement of FM).

In Experiment B, a total of five diets were formulated, including four experimental diets and one control diet (Table 2-2). The four experimental diets and the control diet were all manufactured with a 40% inclusion of a BP-SBM ingredient (100% FM replacement). Urea was then added to the experimental diets post-bioprocessing and blended into the diets at increasing inclusion levels (500, 1,000, 1,500, and 2,000 ppm) based on the nitrogenous portion of the compound (46.64%). The urea inclusion levels were determined based on palatability responses from the previous study, Experiment A, and levels that would be in excess of the potential nitrogen requirements of the microbe, *Aureobasidium pullulans*, during bioprocessing. The remaining diet was a BP-SBM control diet with no nitrogen supplementation (0 ppm).

All diets were manufactured using identical methods for both experiments. Dry ingredients were ground using a Fitzpatrick Comminutator (Elmhurst, IL) with a 1.27 mm screen prior to blending. Complete ingredient mixes were then transferred to a ribbon mixer (Patterson Equipment, Toronto, ON) and blended for five minutes. The homogenous feedstuff was then extruded using an Extru-Tech E325 single-screw extruder (Sabetha, KS) equipped with 2.5-mm die inserts that produced 3.2-mm diameter floating pellets. Extruded pellets were dried with a conveyor oven drier (Colorado Mill Equipment, Canon City, CO), screen sifted with a Rotex screener (Rotex Inc., Cincinnati, OH) and then lipid coated using a Phlauer vacuum coater (A&J Mixing, Oakville, Ontario). The diets were then bagged and stored at room temperature until use.

Feeding Trial

In Experiment A, naive (FM feed trained) juvenile rainbow trout (mean ± SE, 36.01 ± 0.35 g) were randomly stocked (n=240) at a density of 15 fish per tank. Each dietary treatment was randomly assigned to 4 replicate tanks using a random number generator in Microsoft Excel. The diets were offered 3 times per day (0730, 1130, and 1530 hr) for 21 days. In Experiment B, juvenile rainbow trout (56.74 ± 0.34 g) were randomly stocked into 25 tanks (n=250) at a density of 10 fish per tank. Each treatment was randomly assigned 5 replicate tanks and the diets were offered 3 times per day (0800, 1200, and 1600 hr) for 10 days.

In both experiments all fish were fed a FM-based holding feed for a 5-day pretrial period before being fed their respective treatment diets. The same ration of the holding feed was fed to each tank during the pretrial period. On the first day of the trial, fish were switched from the holding feed to their respective experimental treatments and a known amount of feed was given to the replicate tanks in excess of satiation. The remaining uneaten pellets were gathered and counted 20 minutes after feeding to monitor consumption after each feeding event for the duration of the trial. Feeding rates were adjusted each day based on the previous days feeding activity to ensure fish were continually fed in slight excess of satiation. Counted pellets were used to monitor daily consumption as an indirect inference of palatability. Consumption data were then used to calculate feed conversion ratio as follows:

Feed conversion ratio (FCR) was calculated as:

$$\text{FCR} = \frac{\text{mass of feed consumed (dry, g)}}{\text{fish biomass gain (wet, g)}}$$

Mass measurements were sampled when stocking occurred (Day 0) and again upon completion of each trial. Mass measurements included total tank biomass of all tanks in the system and individual weights and lengths of all fish in one tank per treatment on Day 0. Mortalities were accounted for by recording length and weight of the fish as well as date of death so that feeding rates, consumption as a percentage of bodyweight, and FCR could be adjusted on an average weight per fish basis. Mass measurements were obtained to track growth and condition parameters at the end of each study.

Relative growth (RG) was calculated by tank as:

$$\text{RG} = \frac{\Delta \text{ mass (wet,g)}}{\text{initial mass (wet,g)}} \times 100\%.$$

Specific growth rate (SGR) was calculated by tank as:

$$\text{SGR} = \frac{[\ln(\text{final wt(g)}) - \ln(\text{initial wt (g)})] \times 100}{\text{n (days)}}.$$

Fulton's condition factor (K) was calculated for individuals as:

$$\text{K} = \frac{\text{weight (g)}}{\text{length (mm)}^3} \times 100{,}000.$$

Culture System

The experiments were conducted in identically designed recirculating aquaculture systems (RAS) with the exception of tank quantity and total system volume. The system in Experiment A was a 16-tank 2,660 L system and the system in

Experiment B was a 25-tank 3,686 L system, each equipped with 110-L circular tanks. The tanks in both systems were each equipped with a "recirculating" drain which withdrew water from the subsurface and a "sludge" drain which was affixed to the lowest point in the bottom at the center of the tank. Each tank also contained forced air diffusers fed by a blower and half covers to minimize disturbance. Each RAS was also equipped with a pump, bead filter, UV filter, biofilter, solids settling sump, clarifying sump, water inlet float valve, and heater/chiller unit.

The RAS used in Experiment A was supplied with water sourced from a shallow well while the RAS used in Experiment B was supplied with a municipal water source that was dechlorinated with sodium thiosulfate and stored in head tanks. Temperature was maintained between 14 and 15°C, dissolved oxygen at 7 mg L^{-1}, and pH at 7.5 to 8 throughout both experiments. Temperature, dissolved oxygen, and pH were monitored daily while ammonia (NH_3) and nitrite (NO_2) were monitored less frequently, on average three times per week.

Statistical Analysis

Statistical analyses were conducted with JMP, Version 12 (SAS Institute Inc., Cary, North Carolina). All data were first checked for normality with a Shapiro-Wilk test and then for equal variances with a Levene's test. Analysis of Variance (ANOVA) was used for normally distributed data with equal variances and a Kruskal-Wallis test was used for non-normal data. Any significant outcomes were further investigated with a Tukey's HSD post hoc test for multiple comparisons on normal data or a Steel-

Dwass post hoc test for multiple comparisons on non-normal data. An *a priori* alpha value of $\alpha=0.05$ was used for all statistical analyses.

Results

Experiment A

There were no significant differences among treatments when feed consumption was analyzed in Experiment A (P=0.38) (Table 2-3). All diets were readily accepted by the fish indicating no problems in palatability. The highest consumption, based on average consumption per tank over the duration of the trial, was for the control diet with no nitrogen supplementation (584.7 g). The experimental diet containing urea was the highest consumed diet (580.7 g) of the nitrogen supplemented diets. This was followed by the diet supplemented with diammonium phosphate (559.5 g) and the diet supplemented with ammonium chloride (531.8 g), the least consumed diet (Table 2-3).

Similar results were found when consumption was corrected for tank biomass (P=0.383). The highest average consumption occurred in the control treatment, 2.94% of fish biomass per day. The urea treatment was again the highest consumed diet with nitrogen supplementation (2.88% day^{-1}) when consumption was corrected by tank biomass. The diammonium phosphate and ammonium chloride treatments were the lowest consumed diets, an average daily consumption of only 2.84% and 2.79% of tank biomass, respectively.

Survival was high for all fish among the treatments in this study. A total of 5 mortalities were recorded, 3 of which were from fish that were fed the diet containing

ammonium chloride (95% survival). The remaining 2 mortalities were fed the control diet without nitrogen supplementation (98.3% survival) and the diet that was supplemented with diammonium phosphate (98.3% survival). No mortalities occurred in the fish that were fed the urea supplemented diet (100% survival).

Average biomass gained per tank revealed no significant differences among treatments (P=0.35). The greatest biomass gain was observed in the fish that were fed the control diet (656.3 g). The fish fed the urea supplemented diet grew an average of 652.7 g per tank, the second highest biomass gain in the study. Fish exhibiting the lowest gain were fed diets supplemented with diammonium phosphate and ammonium chloride, the latter showing the lowest growth among all treatments (601.9 g).

Relative growth (RG) and specific growth rate (SGR) showed similar results in fish performance among treatments (Table 2-3). No significant differences were noted among treatments for RG (P=0.07) or SGR (P=0.07), however it should be noted that the fish fed the urea supplemented diet exhibited the best growth (RG=125.9 %) (SGR=3.13 g day^{-1}). The fish fed the control diet were the next highest performers in both categories, followed by the diammonium phosphate and ammonium chloride treatments, respectively.

Fulton's condition factor (K) revealed significant differences among treatments (P<0.001). The treatment supplemented with diammonium phosphate displayed an average K value of 1.21, significantly lower than all other treatments. The fish with the highest average K were fed the control diet (1.30). Fish fed the control diet were

followed closely in performance by the urea supplemented diet (1.29) and the ammonium chloride supplemented diet (1.27).

Feed conversion ratio (FCR) differences were nonsignificant (P=0.50). However, a similar trend was noted in comparison of K and FCR in that the fish fed the diet supplemented with diammonium phosphate were the poorest performers. The fish fed the diammonium phosphate supplemented diet had an FCR of 1.06. The lowest FCRs were exhibited in the control diet and the urea supplemented diet which both had similar FCRs of 1.03. The FCR of the fish fed the ammonium chloride supplemented diet was 1.04.

Experiment B

There were no significant differences among treatments when feed consumption was analyzed in Experiment B (P=0.32) (Table 2-4). All diets were readily accepted by the fish indicating no problems in palatability. The highest consumed diet based on average consumption per tank over the duration of the trial was the control diet (0 ppm) (452.9 g). The fish that were fed the remaining experimental diets supplemented with urea consumed similar amounts of feed during the 10 days of the trial. Total average consumption ranged from 402.0 to 410.8 g per tank in the urea supplemented diets (500, 1,000, 1,500, and 2,000 ppm) over the duration of the trial (Table 2-4).

Consumption was also corrected for biomass of fish in each tank and analyzed on a percentage of biomass consumed per day basis (P=0.40). This method of consumption analysis produced very similar results as the average total tank

consumption analysis. The fish that were fed the control diet, the highest consumed diet, consumed an average of 3.60% of their biomass each day. The lowest consumed diet contained 1,500 ppm of urea (3.28% day^{-1}) and the remaining experimental treatments averaged between 3.32% and 3.41% of biomass consumption per day.

Survival was high among all treatments during this study. Only 2 mortalities occurred, one in the 500 ppm urea supplemented diet (98% survival) and the other in the 2,000 ppm urea treatment (98% survival). No other mortalities occurred in the control diet or the diets supplemented with 1,000 or 1,500 ppm urea (100% survival).

Average biomass gain per tank did not significantly differ among diets during this study (P=0.34). The fish that gained the most biomass per tank were fed the control diet with no urea supplementation (442.0 g). The remaining urea supplemented diets all performed similarly based on biomass gain ranging from 376.2 to 404.0 g of gain per tank over the duration of the study.

RG (P=0.27) and SGR (P=0.29) displayed similar results to one another when they were analyzed. The fish fed the control diet displayed the highest RG of 77.9 and the highest SGR of 2.21. The 500 ppm diet exhibited the second highest RG and SGR (71.9, 2.08) followed by the remaining experimental diets which all performed similarly.

Condition factor did not significantly differ among treatments (P=0.25). The fish that were fed the 500 ppm diet had the highest K of 1.36 followed by the fish fed

the control diet with a K of 1.35. The fish that were fed the remaining treatment diets had similar K values ranging between 1.30 and 1.33.

FCR was not significantly affected by urea supplementation (P=0.73). However, the highest FCR was found in the fish that were fed the 2,000 ppm urea supplemented diet (1.51). The lowest FCR value was observed in the control diet (1.36). All other treatments had FCR values ranging from 1.37 to 1.43.

Discussion

Palatability assessment of a feed ingredient is very important when examining its potential use in a commercial aquafeed. Digestibility, nutrient availability, and energy content of a feed ingredient are all irrelevant in the assessment of the quality of a feed ingredient if the ingredient inhibits feed intake due to poor palatability (Glencross et al. 2007). A pellet counting method was used in both experiments to estimate consumption by calculating the difference between the amount fed and waste feed collected (Helland et al. 1996). This method was chosen due to the high pellet consistency among treatments and ideal durability of pellets to withstand mechanical breakdown during feeding (Helland et al. 1996). The fish were fed experimental diets in excess of satiation for several days because it is imperative that fish are fed beyond apparent satiety to give them the opportunity to reject feed in order to compare the daily feed intake response against a positive control (Glencross et al. 2007).

These two experiments were completed to identify any negative palatability effect of nitrogen source and concentration. The first study, Experiment A, explored the palatability of three nitrogen sources: ammonium chloride, diammonium

phosphate, and urea against a positive control with no nitrogen supplementation. No significant palatability responses were noted among treatments based on consumption and growth ($P>0.05$) indicating that any of the three sources could be potentially used for nutrient supplementation to *Aureobasidium pullulans* during bioprocessing. However, a significant response was detected in this trial was when K was analyzed. The fish that were fed the diet containing diammonium phosphate had a significantly lower K than all other treatment groups. This indicates that diammonium phosphate may be the nitrogen source with the least potential and should be avoided when selecting a nutrient source based on the condition of rainbow trout.

It was discovered that the fish fed the urea supplemented experimental diet had consistently improved feed consumption, biomass gain, RG, SGR, K, and FCR over the other nitrogen supplemented diets despite the general lack of significant responses. Therefore, urea was determined to be the better nitrogen source and a subsequent study, Experiment B, was designed to further explore the effects of urea on the palatability of rainbow trout feeds.

In Experiment B various concentrations of urea (500, 1,000, 1,500, and 2,000 ppm) were supplemented to experimental diets in order to examine a palatable effect of the nitrogen source against a positive control with no nitrogen supplementation. The study revealed nonsignificant palatability responses when consumption and growth parameters were analyzed among treatments ($P>0.05$). The results indicate that up to 2,000 ppm urea can be potentially supplemented to *Aureobasidium pullulans* during bioprocessing without any adverse effect on palatability of rainbow trout feeds.

Minimal research has been completed in the area of nitrogen source and concentration effects on the palatability of rainbow trout diets. However, other nutrient supplementation studies used similar experimental designs to assess palatability and growth performance of rainbow trout. Specifically, previous research has used similar methods to assess the supplementation of copper and zinc in rainbow trout diets by first identifying an ideal source and then examining increasing concentration levels to identify maximum inclusion (Read et al. 2014; Welker et al. 2016).

This research provides insight to the potential nutrient supplementation for a microbe, *Aureobasidium pullulans,* during bioprocessing to limit the risk of a nutrient deficiency. A potential protein loss may occur if the microbe is not supplemented with a bioavailable nitrogen source during bioprocessing (Suman et al. 2015). Urea supplemented up to 2,000 ppm during bioprocessing does not adversely affect palatability of rainbow trout and therefore may potentially be used during bioprocessing to limit the risk of *Aureobasidium pullulans* becoming nutrient limiting during bioprocessing of SBM. Continued research in the area of nitrogen supplementation is needed in order to more completely understand the effect of nitrogen source and concentration on rainbow trout palatability and its potential use during bioprocessing.

Table 2-1. Experiment A diet formulations including Control Diet (0 ppm), Ammonium Chloride Diet (NH_4Cl, 1,250 ppm), Diammonium Phosphate Diet ($(NH_4)_2HPO_4$, 1,250 ppm), and Urea Diet (CH_4N_2O, 1,250 ppm). All values reported as g 100 g^{-1} dm.

	Diet			
Ingredient	Control	NH_4Cl	$(NH_4)_2HPO_4$	CH_4N_2O
Ammonium Chloride	0.00	1.87	0.00	0.00
Diammonium Phosphate	0.00	0.00	2.30	0.00
Urea	0.00	0.00	0.00	1.06
BP-SBM[a]	40.00	39.25	39.08	39.58
Whole Cleaned Wheat[b]	29.09	28.55	28.42	28.78
Poultry Meal[c]	12.04	11.82	11.77	11.92
Empyreal[d]	5.00	4.91	4.89	4.95
Vitamin Premix[e]	0.91	0.89	0.89	0.90
Lysine[f]	1.36	1.34	1.33	1.35
Methionine[f]	0.23	0.22	0.22	0.22
Choline Chloride[g]	0.68	0.67	0.67	0.67
StayC[h]	0.23	0.22	0.22	0.22
Soy Oil[i]	4.89	4.79	4.77	4.83
Fish Oil[j]	4.89	4.79	4.77	4.83
Taurine[k]	0.45	0.45	0.44	0.45
Threonine[k]	0.23	0.22	0.22	0.22
Totals	100.00	100.00	100.00	100.00

[a]South Dakota State University, Brookings, SD, [b]Ag First Farmer's Cooperative, Brookings, SD, [c]Tyson Foods, Springdale, AR, [d]Cargill Corn Milling, Blair, NE, [e]ARS 702 premix, Nelson and Sons, Murray, UT, [f]Pure Bulk, Roseburg, OR, [g]BalChem Corporation, New Hampton, NY, [h]DSM Nutritional Products, Parsippany, NJ, [i]South Dakota Soybean Processors, Volga, SD, [j]Virginia Prime Gold, Omega Protein, Houston, TX, [k]Nutra blend LLC, Neosho, MO.

Table 2-2. Experiment B diet formulations including Control Diet (0 ppm) and each experimental diet with increasing concentrations of urea (500, 1,000, 1,500, and 2,000 ppm). All values reported as g 100 g^{-1} dm.

	Diet (ppm)				
Ingredient	0	500	1000	1500	2000
Urea	0.00	0.43	0.86	1.30	1.70
BP-SBM	40.00	39.57	39.14	38.70	38.30
Whole Cleaned Wheat	29.09	29.09	29.09	29.09	29.09
Poultry Meal	12.04	12.04	12.04	12.04	12.04
Empyreal	5.00	5.00	5.00	5.00	5.00
Vitamin Premix	0.91	0.91	0.91	0.91	0.91
Lysine	1.36	1.36	1.36	1.36	1.36
Methionine	0.23	0.23	0.23	0.23	0.23
Choline Chloride	0.68	0.68	0.68	0.68	0.68
StayC	0.23	0.23	0.23	0.23	0.23
Soy Oil	4.89	4.89	4.89	4.89	4.89
Fish Oil	4.89	4.89	4.89	4.89	4.89
Taurine	0.45	0.45	0.45	0.45	0.45
Threonine	0.23	0.23	0.23	0.23	0.23
Totals	100.00	100.00	100.00	100.00	100.00

[a]South Dakota State University, Brookings, SD, [b]Ag First Farmer's Cooperative, Brookings, SD, [c]Tyson Foods, Springdale, AR, [d]Cargill Corn Milling, Blair, NE, [e]ARS 702 premix, Nelson and Sons, Murray, UT, [f]Pure Bulk, Roseburg, OR, [g]BalChem Corporation, New Hampton, NY, [h]DSM Nutritional Products, Parsippany, NJ, [i]South Dakota Soybean Processors, Volga, SD, [j]Virginia Prime Gold, Omega Protein, Houston, TX, [k]Nutra blend LLC, Neosho, MO.

Table 2-3. Experiment A performance characteristics. Mean consumption of feed per tank (Consumption, g), mean biomass gain per tank (Wt Gain, g), mean relative growth (RG, %), specific growth rate (SGR), Fulton-type condition factor (K), and feed conversion ratio (FCR) for Rainbow Trout fed experimental diets. Diets include control diet with no nitrogen supplementation (Control, 0 ppm), ammonium chloride (NH_4Cl, 1,250 ppm), diammonium phosphate ($(NH_4)_2HPO_4$, 1,250 ppm), and urea (CH_4N_2O, 1,250 ppm). Values given are treatment means±SE. Significant differences ($P<0.05$) are indicated by different letters within a given column.

	Performance Characteristic					
Diet	Consumption	Wt. Gain	RG	SGR	K	FCR
Control	584.7±34.6	656.3±39.2	124.5±6.3	3.11±0.11	1.30±0.01[a]	1.03±0.02
NH_4Cl	531.8±24.0	601.9±22.2	106.7±5.7	2.79±0.11	1.27±0.01[a]	1.04±0.01
$(NH_4)_2HPO_4$	559.5±22.2	615.1±10.8	114.3±3.5	2.93±0.06	1.21±0.01[b]	1.06±0.01
CH_4N_2O	580.7±29.5	652.7±17.9	125.9±4.4	3.13±0.08	1.29±0.01[a]	1.03±0.01

Table 2-4. Experiment B performance characteristics. Mean consumption of feed per tank (Consumption, g), mean biomass gain per tank (Wt Gain, g), mean relative growth (RG, %), specific growth rate (SGR), Fulton-type condition factor (K), and feed conversion ratio (FCR) for Rainbow Trout fed experimental diets. Diets are listed as the urea inclusion level (ppm). Values given are treatment means±SE. All listed values are statistically insignificant (P>0.05).

	Performance Characteristic					
Diet	Consumption	Wt. Gain	RG	SGR	K	FCR
0	452.9±8.1	442.0±19.9	77.9±2.9	2.21±0.06	1.35±0.02	1.36±0.06
500	405.8±19.4	395.8±23.5	71.9±3.7	2.08±0.08	1.36±0.02	1.40±0.05
1,000	402.0±12.9	378.8±11.4	66.6±2.6	1.96±0.06	1.30±0.01	1.44±0.04
1,500	410.8±19.4	404.0±11.8	69.7±2.9	2.03±0.06	1.32±0.01	1.37±0.03
2,000	410.0±27.0	376.2±41.5	68.2±5.7	1.99±0.13	1.33±0.02	1.51±0.13

CHAPTER 3. PERFORMANCE OF RAINBOW TROUT *Oncorhynchus mykiss* FED ENHANCED SOY PRODUCTS

Introduction

Marine fishery production peaked at 86.4 million metric tons (mmt) of annual production in 1996 and has since plateaued and shown a declining trend (FAO 2016). This is largely due to the overexploitation of the world's marine fisheries as we have attempted to fill the seafood supply and demand gap. The increased demand has been created largely in part by the annual increase of the world's population at a rate of 1.6% accompanied with an increased annual global per capita consumption of seafood which is near 20 kg today (FAO 2016). Consequently, producers have intensified efforts to farm raise fish through the practice of aquaculture. Aquaculture, an industry which only accounted for 31.1% of total fish production in 2004, grew substantially to yield 44.1% of total fish production in 2014 and has now exceeded wild caught fish for production of fish used for human consumption (FAO 2016).

Farm-raised fish are commonly fed a pelleted feed. Traditionally the principal protein source in pelleted feeds was fish meal (FM). FM is a product that is commonly derived from marine fish that have been de-oiled and processed into a fine powder. However, the continued use of FM in pelleted aquafeeds is uncertain as the supply is unstable resulting in a fluctuating market that is not dependable for fish producers. Instead FM may soon only be included in starter or specialty diets for farm-raised fish (Tacon and Metian 2008).

Due to the dynamic FM supply and its unpredictable market, increased efforts have focused on identifying plant-based, alternative protein sources for use in aquafeeds. One such plant-based source that has recognized potential is soybean meal (SBM). Soybeans are a primary crop grown in the Midwest with low transportation costs resulting in an inexpensive commodity, providing a steady supply relative to FM (Asche et al. 2013). SBM also has a well-balanced amino acid profile, favorable protein content, and a relatively low antinutrient constituent as compared to other plant-based ingredients (Dersjant-Li 2002; Gatlin et al. 2007).

Despite the potential benefits that SBM offers for use in aquafeeds, there are limitations to its inclusion, especially in the diets of carnivorous fishes. Antinutritional factors (ANF) that are present in SBM limit growth and nutrient absorption in cultured fishes by negatively affecting the palatability, digestibility, or bioavailability of nutrients in the diet. ANFs contained in SBM include protease inhibitors, lectins, saponins, phytic acid, phytoestrogens, and allergens (Francis et al. 2001; NRC 2011). Also, SBM is limiting in select essential amino acids (EAA), namely lysine and methionine, that need to be supplemented to the diet to ensure proper growth (Gatlin et al. 2007).

Bioprocessing or fermentation can be used as a means of enhancing soy products. ANFs in SBM can be reduced with pretreatment and bioprocessing methods which in turn provides a more palatable and digestible ingredient for use in aquafeeds. Microorganisms such as algae, fungi, or bacteria are used during bioprocessing to produce single cell protein (SCP). SCP has a high protein content and contains fats,

carbohydrates, nucleic acids, vitamins, minerals, and improved levels and lysine and methionine which are often limiting in the diets of carnivorous fishes (Suman et al. 2015). Bioprocessing may be a practical approach to reduce ANFs and enhance the nutritional profile of SBM that allows higher inclusion levels.

The primary objective of this study was to compare various bioprocessed soybean meal (BP-SBM) products with a FM based diet on the growth performance of rainbow trout *Oncorhynchus mykiss*. We monitored growth over the duration of the trial as well as overall health by obtaining hematocrit samples and weights of liver, spleen, fat, viscera, and muscle tissue to compare respective health indices.

Methods and Materials

Experimental Design

This study was completed to assess rainbow trout growth performance when fed various enhanced soy ingredients. Six of the diets contained BP-SBM test ingredients and the remaining diet was a FM control diet. The 6 experimental diets were manufactured with a 25% inclusion of BP-SBM in the diet (46.3% replacement of FM with experimental ingredient) (Table 3-1). The control diet was manufactured with a 15% inclusion of FM in the diet (0% replacement of FM). The dietary treatments included 3 fractions of a base BP-SBM ingredient (Diets 1, 2, and 3), a BP-SBM ingredient with an enzyme inclusion during fermentation (Diet 4), the base BP-SBM diet with an extra wash step (Diet 5), the base BP-SBM diet (Diet 6), and the FM control diet (Diet 7) (Table 3-1). All diets were formulated on an isonitrogenous (44% crude protein) and isocaloric (14% crude fat) basis.

Dry ingredients were ground using a Fitzpatrick Comminutator (Elmhurst, IL) with a 1.27 mm screen prior to blending. Milled ingredients were then transferred to a ribbon mixer (Patterson Equipment, Toronto, ON) and blended for five minutes. The homogenous feedstuff was then extruded using an Extru-Tech E325 single-screw extruder (Sabetha, KS) equipped with 2.5 mm die inserts to produce 3.2 mm diameter floating pellets. Extruded pellets were dried with a conveyor oven drier (Colorado Mill Equipment, Canon City, CO), screen sifted with a Rotex screener (Rotex Inc., Cincinnati, OH) and then lipid coated using a Phlauer vacuum coater (A&J Mixing, Oakville, Ontario). The diets were then bagged and stored at room temperature until use.

Feeding Trial

Juvenile rainbow trout (mean ± SE, 12.25 ± 0.06 g) were randomly stocked (n=1,050) at a density of 30 fish per tank. Each treatment was randomly assigned to 5 replicate tanks using a random number generator in Microsoft Excel. The experimental diets were offered 3 times per day (0800, 1200, and 1600 hr) for 105 days.

All fish were fed the same ration of a FM-based control diet prior to the start of the trial. Upon the start of the trial each tank of fish was switched from the control diet to their respective experimental diets. A known amount of feed was offered to each tank at the beginning of each feeding. After 10 minutes, tanks were checked for remaining floating pellets. If pellets were left floating in the tank, the fish in that tank were assumed to have reached apparent satiation. If no pellets remained in the tank, the fish in that tank were again fed a predetermined ration and allowed 10 minutes to

consume the feed and then checked again. Feeding continued in this manner until all tanks had feed remaining and it was determined that all fish had reached apparent satiation. Care was taken to avoid feeding in extreme excess of satiation to avoid any detrimental effects on water quality or any feed waste.

Upon completion of the final feeding each day, feed containers were gathered and remaining feed was weighed in order to monitor daily consumption over the course of the trial. Total feed consumption was used to estimate feed conversion ratio (FCR) as follows:

$$FCR = \frac{mass\ of\ feed\ consumed\ (dry, g)}{fish\ biomass\ gain\ (wet, g)}$$

Mass measurements were recorded when stocking of fish occurred (Day 0) and again at 3-week intervals until completion of the trial. Mass measurements included total tank biomass of all tanks in the system during each sampling event. Individual weights and lengths were measured for fish in one tank of each treatment on Day 0 and all fish in three replicate tanks from each treatment for all remaining 3-week sampling intervals and upon completion of the trial. Mortalities were accounted for by recording length and weight of the fish, as well as date of death, so that feeding rates and FCR could be adjusted on an average weight per fish basis.

Total tank biomass measurements were used to calculate relative growth (RG), specific growth rate (SGR), and biomass gain over the course of the trial. Weights and lengths of individual fish were used to calculate Fulton's condition factor (K) and to

monitor growth variation among individuals within tanks. RG, SGR, and K were calculated as follows:

$$RG = \frac{\Delta\, mass\ (wet, g)}{initial\ mass\ (wet, g)} \times 100\%$$

$$SGR = \frac{[\ln(final\ wt\ (g)) - \ln(initial\ wt\ (g))]\ \times 100}{n\ (days)}$$

$$K = \frac{weight\ (wet,g)}{length\ (mm)^3} \times 100{,}000$$

Three fish per tank were euthanized at the end of the study using 250 ppm buffered MS-222 (IACUC Approval Number 16-041 A). Samples including blood, liver, spleen, viscera, fat, and muscle tissue were gathered from fish to obtain hematocrit values, hepatosomatic index (HSI), splenosomatic index (SSI), viscerosomatic index (VSI), visceral fat index (VFI), and fillet yield, respectively. Organosomatic indices were calculated as follows:

$$HSI = \frac{Liver\ weight}{Body\ weight} \times 100$$

$$SSI = \frac{Spleen\ weight}{Body\ weight} \times 100$$

$$VSI = \frac{Viscera\ weight}{Body\ weight} \times 100$$

$$VFI = \frac{Visceral\ fat\ weight}{Body\ weight} \times 100$$

$$Fillet\ Yield = \frac{Weight\ of\ 1\ fillet\ (skin\ attached) \times 2}{Body\ weight} \times 100$$

Culture System

The experiment was conducted in an 8,246 L recirculating aquaculture system (RAS). The system was equipped with 35, 190 L semi-square tanks each equipped with a recirculating drain which withdrew water from the subsurface and a sludge drain which was affixed to the lowest point in the bottom at the center of the tank. Each tank also had forced air diffusers fed by a blower, a water inlet flow bar that controlled the direction of current, and covers which provided darkness to half of the tank and netting which allowed light to penetrate the other half of the tank. The RAS was also equipped with a centrifugal water pump, bead filter, UV filter, biofilter, 3 solids settling sumps, clarifying sump, water inlet float valve, and a heater/chiller unit.

The RAS replacement water was sourced with well water. Water flow to each tank was maintained at 6 to 7 L min^{-1} and water temperature was maintained between 14 and 15°C. Dissolved oxygen was maintained above 6 mg L^{-1} and pH was held between 7 and 8 throughout the experiment. Temperature, dissolved oxygen, and pH were monitored daily (0800 hr) while ammonia (NH_3) and nitrite (NO_2) were monitored three times per week.

Statistical Analysis

Statistical analyses were conducted with JMP, Version 12 (SAS Institute Inc., Cary, North Carolina). All data were first checked for normality with a Shapiro-Wilk test and then for equal variances with a Levene's test. Analysis of Variance (ANOVA) was used on all normally distributed data and a non-parametric Kruskal-Wallis test was used on non-normal data. Any significant outcomes were further investigated with

a Tukey's HSD post hoc test for multiple comparisons on normal data or a Steel-Dwass post hoc test for multiple comparisons on non-normal data. An *a priori* alpha value of $\alpha=0.05$ was used for all statistical analyses.

Results

There were evident differences in proximate composition among the experimental ingredients used in this study. The FM ingredient that was used in Diet 7 had the highest concentrations of protein, fat, ash, lysine, and methionine while also having a substantially lower fiber content as compared to all other BP-SBM ingredients (Table 3-2). The BP-SBM ingredients used in the soy fraction diets (Diets 1, 2, and 3) contained the lowest levels of crude protein, on average 8.2% lower than the FM ingredient. The lowest level of fat (0.32%, dm) and the lowest lysine concentration (3.45%, dm) were observed in the experimental ingredient used in Diet 6. The highest fiber was found in the experimental ingredients used in Diets 1 and 3, an average of 10.5% higher than the FM ingredient. The lowest methionine level (0.91%, dm) was noted in the Diet 1 experimental ingredient and the experimental ingredient with the lowest ash content (3.3%, dm) was used in Diet 5.

The diets used in this study were formulated on an isonitrogenous and isocaloric basis. Therefore, the protein, fat, fiber, and ash concentrations were more consistent in the final diets relative to the experimental ingredients (Table 3-1). Crude protein values varied 1.5% among all diets from the highest crude protein found in Diet 7 (47.5%, dm) to the lowest crude protein concentration in Diet 5. Conversely, Diet 5 contained the highest crude fat level (15.5%, dm) which was only 1.0% higher

than Diet 6, the diet with the lowest crude fat concentration. Ash ranged 3.4% and fiber ranged 2.2% among all diets.

At the end of the first growth phase, the only differing performance response was Fulton's condition factor (K) ($P<0.01$). Diet 1 and Diet 4 had average K values of 1.46 and 1.45, respectively, significantly greater than Diet 6 which had an average K of 1.39. No other significant responses were noted among treatments for consumption, biomass gain, relative growth (RG), specific growth rate (SGR), and feed conversion ratio (FCR) ($P>0.05$). Also, no significant differences were found among treatments at the 6-week sampling when all of the aforementioned performance parameters were analyzed ($P>0.05$).

The greatest separation in treatment responses was observed at the 9-week sampling for RG and SGR. Fish fed Diets 1, 2, 3, 4, and 5 all had significantly higher average RG than fish fed Diet 7; fish fed Diets 1 and 2 also had significantly greater RG than fish fed Diet 6 ($P=0.03$). The highest RG was observed in Diet 1 (489.8) and the lowest in Diet 7 (436.7). Similarly, fish fed Diets 1 and 2 had a significantly higher average SGR than fish fed Diets 6 and 7 and fish fed Diets 3, 4, and 5 were also significantly greater than Diet 7 for SGR ($P=0.03$). The highest SGR of 2.86 was noted for the Diet 1 treatment while the lowest SGR of 2.71 was found in the Diet 7 treatment. Consumption, biomass gain, K, and FCR did not significantly differ at the 9-week sampling period ($P>0.05$).

RG was the only performance characteristic to reveal a significant response at the 12-week sampling period ($P=0.046$). Diet 7 had an average RG of 711.0,

significantly lower than Diets 1, 2, 3, 4, and 5 which had RG values that ranged between 776.7 and 799.1. The RG of Diet 6 (740.2) did not significantly differ from any other treatment ($P>0.05$).

At the final sampling event (15-week), FCR provided the only significant result ($P=0.03$). Fish fed Diet 5 had a significantly lower average FCR than fish fed Diets 6 and 7. Fish fed Diets 2, 3, and 4 were also significantly lower than Diet 7 when FCR was analyzed. The lowest FCR was observed for fish fed Diet 5 (1.11) and the highest FCR for Diet 7 (1.27) (Table 3-3).

Biomass gain did not significantly differ among treatments throughout the trial ($P=0.40$). The highest average biomass gain per tank was found in the fish that were fed Diet 1 (4,083.2 g) and the lowest average gain per tank was found in the fish fed Diet 7 (3,778.3 g). Biomass gain was also examined on a weight gain per fish basis as opposed to a gain per tank basis. When weight gain was analyzed on a per fish basis results were still insignificant ($P=0.24$), however it should be noted that the fish fed Diet 2 had the greatest average gain per fish of 143.5 g, followed by Diet 5 (139.2), and then Diet 1 (138.8 g). Diet 7 remained as the treatment with lowest average of 128.2 g of gain per fish over the duration of the study. The changes in performance between the two growth parameters are due to mortalities that occurred among treatments during the trial.

Survival was high for all treatments in this study and was not significantly affected by treatment ($P=0.42$), however it did have an impact on some treatments. A total of 30 mortalities occurred during the trial resulting in 97.1% overall survival.

Five of the mortalities were caused by escapes, 8 from handling stress during sampling, 1 from a physical deformity, and the remaining from an unexplained death. The highest survival was documented in the fish fed Diets 1, 3, and 7 which only had 2 mortalities occur per treatment (98.7% survival). The lowest survival was observed in the fish fed Diet 5 which had a total of 9 mortalities resulting in 94% survival.

Consumption was not significant among treatments (P=0.23). Fish fed Diet 7 consumed the most feed (4,803.4 g). The fish that were fed Diet 5 only consumed 4,320.2 g of feed during the trial, 10.1% less than Diet 7. Consumption was also evaluated as a percentage of fish body weight per day to account for mortalities and size of individuals (P=0.13). Diet 7 produced an average of 1.94% of bodyweight consumed per day. The remaining diets followed closely to Diet 7 ranging from 1.77% to 1.91% of bodyweight consumption per day.

RG and SGR, which previously exhibited significant responses among treatments in previous samplings, showed no significant differences upon completion of the trial (P=0.211, 0.208). The highest RG was observed in fish fed Diet 2 (1,159.5) followed closely by fish fed Diet 1 (1,149.0). The lowest RG, 1,029.0, was from the fish that were fed Diet 7. SGR performance was similar to RG among treatments. The highest SGR was from Diet 2 (2.435) and the lowest from Diet 7 (2.329). Fulton's condition (K) also did not significantly differ among treatments (P=0.96). The highest K was from fish fed Diet 6 (1.513), however K from all fish were very similar with the lowest value found in the fish fed Diet 5 (1.497).

A final necropsy occurred to obtain blood for hematocrit and weights of fat, tissue, liver, spleen, and viscera to calculate visceral fat index (VFI), fillet yield, hepatosomatic index (HSI), splenosomatic index (SSI), and viscerosomatic index (VSI), respectively. HSI was the only index to yield a significant result (P=0.04) (Table 3-4). HSI was found to be significantly higher in the fish fed Diet 7 (1.327) than the fish fed Diet 2 (1.083). No significant results were revealed when SSI was analyzed (P=0.10). Average SSI ranged from 0.078 to 0.107 across all treatments. VSI also was not significantly affected across treatments (P=0.72). The largest VSI of 14.6 was observed in the fish that were fed Diet 5 and the smallest in Diet 2 (13.4).

Hematocrit analysis displayed no significant responses among diets (P=0.08). However, fish fed Diet 1 had a slightly increased average hematocrit of 51.9% relative to the rest of the fish fed the remaining treatment diets. The fish fed Diet 1 also had the highest VFI (4.86%) among all treatments yet not significantly greater than other treatments (P=0.07). Fillet yield varied from 47.6% in the fish fed Diet 6 to 51.6% in the fish fed Diet 2, but was not significant (P=0.06).

Discussion

Fish fed Diets 1 and 2 tended to perform better over the course of the trial despite the general lack of significant responses among treatments for growth parameters and health indices. Conversely, Diet 7 consistently appeared to be the poorest performing diet over the course of the trial when various growth parameters were examined. These results suggest that when 46.3% of a FM ingredient is replaced with bioprocessed soy products, growth performance and overall health of the fish is

not negatively affected. The results from this study are consistent with previous work that has suggested that FM can be partially or fully replaced in the diets of rainbow trout without compromising culture performance (Barnes et al. 2014; Bruce et al. 2017; Yamamoto et al. 2010).

RG and SGR were both significantly different among treatments at the 9-week sampling. The significant response was detected due to the general poor performance of Diets 6 and 7, relative to the other diets. During the 12-week sampling RG was the only growth parameter to offer a significant response and neither RG nor SGR were significantly different among treatments when they were analyzed at the final sampling period (Figure 3-1).

The lack of performance separation towards the end of the trial may be due to system limitation. Towards the end of the trial, especially between the 12-week and 15-week sampling periods, water quality began to deteriorate as NO_2 and NH_3 levels began to increase and dissolved oxygen concentrations became lower. Generally, minimum dissolved oxygen levels are recommended in the range of 5 to 9 mg L^{-1} and unionized ammonia levels should not exceed 0.0125 to 0.04 mg L^{-1} for rainbow trout culture (Ellis et al. 2002). However, it is difficult to define water quality requirements because they change across culture systems, especially high intensity water reuse systems (Colt 2006).

Increased fish density may also have a negative effect on FCR, nutritional condition, and growth but stocking density requirements are difficult to define due to varying system designs and water quality (Ellis et al. 2002; Person-Le Ruyet et al.

2008). However, density of fish should not have limited growth as the average density of fish fed Diet 2, the treatment with the highest mean tank density upon completion of the trial, was only 25.31 g L^{-1}.

FCR was the only growth metric that provided a significant response upon completion of the trial. The significant result was primarily due to the high FCR of fish fed Diet 7, the FM control diet, relative to Diets 2, 3, 4, and 5. The high FCR of fish fed Diet 7 is expected as this same group of fish also had the highest mean total consumption and the highest mean consumption as a percentage of fish biomass compared to all other treatment groups. The crude fat content in Diets 2, 3, 4, and 5 were all higher than the crude fat content in Diet 7. It has been noted that fish consume to meet their energetic demands (Lee 2015; NRC 2011), therefore the elevated consumption of Diet 7 may be due to the fact that it had a slightly lower crude fat concentration than Diets 2, 3, 4, and 5. Consequently, a high FCR in the fish fed Diet 7 may be due to the fact that the crude fat level was lower in Diet 7 relative to the other diets. Despite the higher FCR observed in the fish fed Diet 7, the fish in this treatment group, as well as fish fed the other experimental diets, still exhibited similar FCRs as rainbow trout in other similar studies (Barnes et al. 2014; Caballero et al. 2002; Oliva-Teles et al. 1994).

HSI was the only health index that significantly differed among treatments at the end of the trial. The fish fed Diet 7 had a significantly higher HSI than the fish fed Diet 2. An increased HSI in fish may be indicative of an increased energy reserve in the liver relative to the fish in other treatment groups (Nunes et al. 2011). However,

the fish that were fed five of the other diets (Diets 1, 2, 3, 4, and 6) had a higher average VFI than fish fed Diet 7 so this may not be the reason for an increased HSI in fish fed Diet 7.

The experimental diets containing BP-SBM that were formulated in this study not only replaced a portion of FM but also replaced a portion of other animal protein sources as well. There has been recent consumer interest in not only replacing fishmeal in the diets of livestock, but completely replacing all animal protein sources to produce a 100% plant-based diet (FAO 2016). The experimental soy diets containing BP-SBM replaced 46.3% of FM, 78.3% of poultry meal, and 6.3% of feather meal as compared to Diet 7, the FM-based reference diet containing no BP-SBM. Also, Diet 7 contained astaxanthin in order to closely replicate other commercial rainbow trout FM based diets.

Table 3-1. Diet formulations and nutrient compositions. BP-SBM ingredient fractions (Diets 1, 2, and 3), a BP-SBM ingredient with an enzyme inclusion during fermentation (Diet 4), the base BP-SBM diet with an extra wash step (Diet 5), the base BP-SBM diet (Diet 6), and the FM control diet (Diet 7). NFE=Nitrogen free extract. Values reported as g 100 g^{-1} dm.

	Diet						
Diet Composition	1	2	3	4	5	6	7
Total Ash	5.61	5.71	5.65	5.61	5.47	5.85	8.84
Crude Fat	14.72	14.83	14.83	14.93	15.50	14.47	14.70
Crude Fiber	4.43	4.28	4.59	4.65	2.50	4.72	3.45
Crude Protein	46.37	46.35	46.83	46.73	45.97	46.58	47.46
NFE	28.87	28.82	28.10	28.08	31.61	28.22	25.02
Ingredient							
BP-SBM Fraction #1[a]	25.00	0.00	0.00	0.00	0.00	0.00	0.00
BP-SBM Fraction #2[a]	0.00	25.00	0.00	0.00	0.00	0.00	0.00
BP-SBM Fraction #3[a]	0.00	0.00	25.00	0.00	0.00	0.00	0.00
BP-SBM + Enzyme[a]	0.00	0.00	0.00	25.00	0.00	0.00	0.00
BP-SBM Base + Wash[a]	0.00	0.00	0.00	0.00	26.00	0.00	0.00
BP-SBM Base[a]	0.00	0.00	0.00	0.00	0.00	25.00	0.00
Blood Meal[b]	5.04	5.04	5.04	5.04	5.00	5.00	5.00
Wheat Midds[c]	13.60	13.60	13.60	13.60	12.60	13.60	16.85
Whole Cleaned Wheat[d]	16.62	16.62	16.62	16.62	16.62	16.62	16.00
Poultry Meal[e]	5.04	5.04	5.04	5.04	5.00	5.00	23.00
Feather Meal[e]	7.56	7.56	7.56	7.56	7.50	7.50	8.00
Fish Meal[f]	8.06	8.06	8.06	8.06	8.00	8.00	15.00
Vitamin Premix[g]	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Lysine[h]	2.02	2.02	2.02	2.02	2.00	2.00	1.80
Methionine[h]	0.76	0.76	0.76	0.76	0.75	0.75	0.50
Choline Chloride[i]	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Mineral Premix[j]	0.76	0.76	0.76	0.76	0.75	0.75	0.75
Stay C[k]	0.20	0.20	0.20	0.20	0.20	0.20	0.25
Soy Oil[l]	6.65	6.65	6.65	6.65	6.65	6.65	5.25
Fish Oil[m]	6.65	6.65	6.65	6.65	6.65	6.65	5.25
Astaxanthin[n]	0.00	0.00	0.00	0.00	0.00	0.00	0.05
Calcium diphosphate[o]	0.00	0.00	0.00	0.00	0.00	0.00	0.50
Totals	100	100	100	100	100	100	100

[a]South Dakota State University, Brookings, SD, [b]Mason City By-Products, Mason City, IA, [c]Consumer Supply Distributing, Sioux City, IA, [d]Ag First Farmer's Cooperative, Brookings, South Dakota, [e]Tyson Foods, Springdale, AR, [f]Special Select, Omega Protein, Houston, TX, [g]ARS 702 premix, Nelson and Sons, Murray, UT, [h]Pure Bulk, Roseburg, OR, [i]BalChem Corporation, New Hampton, NJ, [j]ARS 640 trace mix, Nelson and Sons, Murray, UT, kDSM Nutritional Products, Parsippany, NJ, [l]South Dakota Soybean Processors, Volga, SD, [m]Viginia Prime Gold, Omega Protein, Houson, TX, [n]ChemSol, Minnetonka, MN, [o]Feed Products Inc., St. Louis, MO.

Table 3-2. Proximate composition and amino acid profile of main protein ingredients. Values are g/100 g dm unless noted. EAA=essential amino acid; NEAA= non-essential amino acid; Total AA= total amino acids.

	Main Protein Ingredient						
Analyte	1	2	3	4	5	6	7
Ash	3.70	4.03	3.74	3.53	3.25	4.65	21.48
Fat	0.98	1.01	0.86	0.78	1.00	0.32	9.47
Fiber	10.91	5.15	11.69	5.58	4.44	8.46	0.76
Protein	61.05	60.70	61.14	62.43	64.12	67.51	69.16
Moisture (wb)	8.34	10.72	6.72	10.38	7.98	5.49	7.31
EAA							
Arginine	4.66	4.57	4.56	4.50	4.31	4.57	3.95
Histidine	1.95	1.67	1.64	1.93	1.66	1.69	1.92
Isoleucine	3.21	3.36	3.18	3.31	3.43	3.56	2.77
Leucine	5.48	5.49	5.45	5.55	5.66	5.86	4.90
Lysine	4.22	4.09	4.03	4.22	3.58	3.45	5.19
Methionine	0.91	0.92	0.94	0.93	1.09	1.07	1.91
Phenylalanine	3.58	3.56	3.56	3.64	3.41	3.54	2.71
Taurine	0.09	0.07	0.10	0.08	0.00	0.00	0.54
Threonine	2.61	2.59	2.57	2.59	3.01	2.86	2.85
Valine	3.32	3.44	3.30	3.43	3.58	3.63	3.27
NEAA							
Alanine	2.89	2.91	2.89	2.97	3.23	3.29	4.53
Aspartic Acid	7.38	7.40	7.26	7.45	7.84	0.00	6.26
Glutamic Acid	12.00	11.98	11.79	11.94	10.76	12.02	9.28
Glycine	2.77	2.79	2.78	2.85	2.95	2.93	5.18
Proline	3.28	3.28	3.22	3.26	3.36	3.52	3.56
Serine	3.44	3.29	3.27	3.15	3.43	3.25	2.70
Tyrosine	2.45	2.18	2.39	2.34	2.58	2.72	2.20
Total AA	64.14	63.53	62.83	64.04	63.87	57.96	63.17

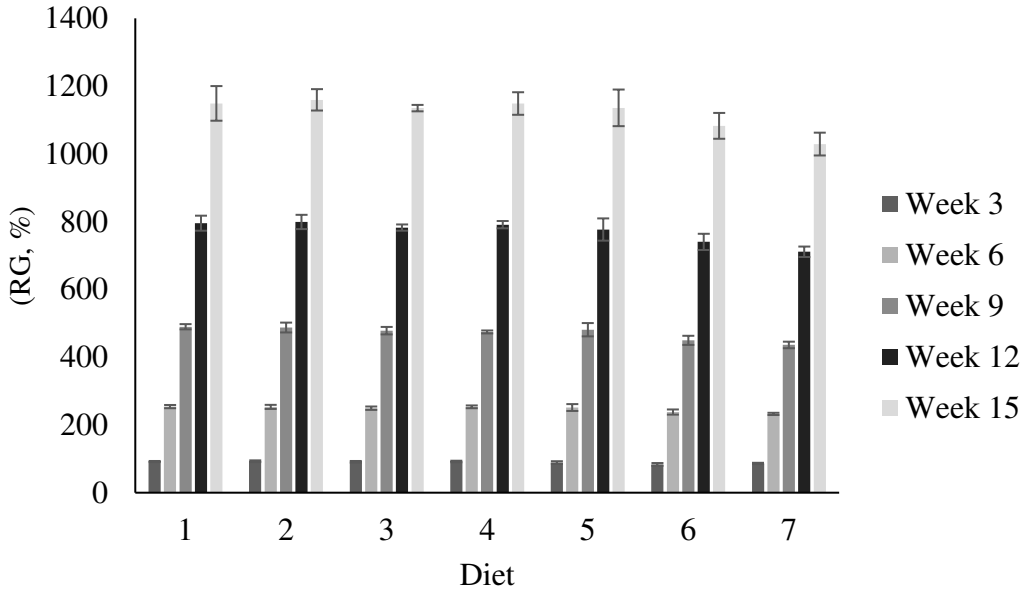
Table 3-3. Growth performance indices. Values given are treatment means±SE. Significant differences (P<0.05) are indicated by different letters within a given column. Average biomass gain per tank (Gain Tank^{-1}, g, wet), average biomass gain per fish (Gain Fish^{-1}, g, wet), average total consumption per tank (Consumption, g, dry), relative growth (RG, %), specific growth rate (SGR), feed conversion ratio (FCR), and Fulton-type condition factor (K).

	Performance Characteristic						
Diet	Gain Tank^{-1}	Gain Fish^{-1}	Consumption	RG	SGR	FCR	K
1	4083.2±126.8	138.8±4.3	4777.7±194.7	1149.0±51.1	2.42±0.04	1.17±0.06^{abc}	1.511±0.013
2	4057.3±107.1	143.5±4.7	4667.0±160.9	1159.5±31.6	2.43±0.02	1.15±0.02^{bc}	1.499±0.012
3	4012.1±103.9	138.0±2.8	4535.4±45.0	1135.0±9.5	2.42±0.01	1.13±0.03^{bc}	1.509±0.014
4	4008.6±119.6	138.7±3.9	4547.8±123.8	1148.6±33.3	2.43±0.03	1.13±0.00^{bc}	1.507±0.013
5	3901.6±139.2	139.2±5.5	4320.2±154.0	1135.8±54.0	2.41±0.04	1.11±0.02^{c}	1.497±0.014
6	3832.2±116.2	132.6±10.0	4615.3±80.9	1082.7±38.1	2.37±0.03	1.21±0.04^{ab}	1.513±0.013
7	3778.3±57.9	128.2±2.9	4803.4±136.7	1029.0±33.7	2.33±0.03	1.27±0.04^{a}	1.504±0.012

Table 3-4. Necropsy variables for rainbow trout. Values given are treatment means±SE. Significant differences (P<0.05) are indicated by different letters within a given column. Hematocrit (%), HSI=hepatasomatic index, SSI=splenosomatic index, VSI=viscerosomatic index, VFI=visceral fat index, and Fillet Yeild (%).

	Performance Characteristic					
Diet	Hematocrit	HSI	SSI	VSI	VFI	Fillet Yield
1	51.93±1.48	1.10±0.05^{ab}	0.094±0.007	13.76±0.45	4.86±0.41	51.14±0.86
2	46.43±1.05	1.08±0.05^{b}	0.095±0.007	13.40±0.53	4.17±0.30	51.62±1.26
3	49.47±1.03	1.10±0.04^{ab}	0.083±0.008	13.70±0.78	4.48±0.23	51.31±0.96
4	49.64±1.20	1.14±0.06^{ab}	0.091±0.006	13.64±0.50	3.97±0.31	49.71±0.90
5	47.36±0.95	1.17±0.06^{ab}	0.100±0.011	14.59±0.57	3.71±0.40	49.83±1.20
6	49.29±1.63	1.19±0.05^{ab}	0.107±0.009	13.88±0.58	4.21±0.52	47.56±1.14
7	48.13±1.38	1.33±0.07^{a}	0.078±0.007	13.76±0.51	3.84±0.29	49.64±0.56

Figure 3-1. Average relative growth (RG, %) of treatments at each 3-week sampling interval for the duration of the trial.



CHAPTER 4. PERFORMANCE OF HYBRID STRIPED BASS *Morone chrysops x M. saxatilis* FED ENHANCED SOY PRODUCTS

Introduction

The global human population has been increasing at a rapid rate and there is no indication that this exponential growth and need for increased food supply will slow any time in the near future. In addition to the ever increasing population is a recent increase in per capita consumption of seafood. Per capita seafood consumption was at 9.9 kilograms (kg) in the 1960's and preliminary estimates suggest that consumption is currently over 20 kg (FAO 2016).

The combination of these factors has been the main driver for the increased demand of seafood and this has resulted in overexploitation of wild marine fish stocks. Currently, 89.5% of marine fisheries are fully fished or overfished at biologically unsustainable levels (FAO 2016). As a result, aquaculture has become a common practice in recent years in an effort to supply seafood for a demand that wild marine resources can no longer sustain. The aquaculture industry has grown considerably in recent years, accounting for 44.1% of total fish production in 2014 and now exceeds wild fish harvest for food fish (FAO 2016).

Farm-raised fish are usually fed a pelleted feed. Traditionally, the main protein ingredient in aquafeeds has been fish meal (FM). FM is a de-oiled fine powder that is produced from the processing of marine fish species. However, the supply of FM is unstable which results in a variable and unfavorable market for producers. Therefore,

the recent use of FM in the diets of cultured fishes has decreased and the continued future use of FM is uncertain (Tacon and Metian 2008).

Increased interest in identifying plant-based sustainable protein sources for use in aquafeeds is attributable to the uncertain supply and market of FM. Soybean meal (SBM), typically defatted meal, has emerged as a favorable candidate for potential replacement of FM in the diets of cultured fishes. SBM has a well-balanced amino acid profile, favorable protein content, and lesser amounts of antinutrients relative to other plant-based protein sources (Dersjant-Li 2002; Gatlin et al. 2007). Nonetheless, SBM does have restrictions in its use, especially in the diets of carnivorous species. Antinutritional factors (ANF) including non-starch polysaccharides (NSP), protease inhibitors, lectins, saponins, phytic acid, phytoestrogens, and allergens are all present in SBM and may inhibit palatability, digestibility, and growth of cultured fishes (Francis et al. 2001). SBM is also often limiting in lysine and methionine, therefore diet formulations containing of SBM will likely need to supplemented to ensure proper growth (Gatlin et al. 2007).

One method of enhancing the nutritional profile of SBM is fermentation or bioprocessing. Bioprocessing is a procedure in which microorganisms such as algae, fungi, or bacteria are combined with a substrate to produce single cell protein (SCP). The SCP produced during bioprocessing is highly digestible, high in protein, and contains fats, carbohydrates, nucleic acids, vitamins, minerals, and increased levels of lysine and methionine relative to SBM (Suman et al. 2015). Bioprocessing of SBM may be a viable option when attempting to reduce ANFs and improve the nutritional

profile of SBM in order to increase the use of soy products in aquafeeds without inhibiting growth performance of cultured fishes.

This research focused on the digestibility, growth performance, and overall health of hybrid striped bass *Morone chrysops x M. Saxatilis* fed various bioprocessed soybean meal (BP-SBM) ingredients compared to positive (FM) and negative (SBM) control diets. Two separate experiments were conducted; a 45-day digestibility trial followed by a 105-day growth study. Apparent digestibility coefficient of protein (ADC-P) was analyzed among hybrid striped bass fed various BP-SBM treatments and two control diets in the first study. Health indices and growth performance of hybrid striped bass were analyzed among BP-SBM treatments and a FM control diet in the subsequent growth study.

Methods and Materials

Experimental Design

Two experiments were completed to fully assess the performance of hybrid striped bass fed various BP-SBM products against a FM reference diet and a SBM control diet. The first experiment, a digestibility trial, was designed to assess the protein digestibility of experimental ingredients in seven total diets. The reference diet was formulated using a documented formulation from the Agricultural Research Service digestibility database (Barrows et al. 2012) (Table 4-1). The remaining six experimental diets were manufactured by blending 72% of the bulk reference diet (dm) with 28% of the experimental BP-SBM ingredient or defatted SBM (dm). Chromic oxide (Cr_2O_3) was included at 1% across all diets.

The experimental ingredients used in this study included three fractions of a base BP-SBM ingredient (Diets 1, 2, and 3), a BP-SBM ingredient that was processed with an enzyme during fermentation (Diet 4), and a washed base BP-SBM ingredient (Diet 5). The main protein ingredient in the reference diet was FM (Table 4-1).

The subsequent 105-day growth experiment was designed to analyze growth performance and health indices of hybrid striped bass. Palatability was also assessed by comparing consumption of treatment diets during the first phase of the growth trial. The manufactured diets were formulated with the same five experimental BP-SBM ingredients (Diets 1, 2, 3, 4, and 5) and the FM ingredient (Diet 7) that were used in the digestibility study (Table 4-2). The SBM ingredient used in the digestibility experiment was replaced with a base BP-SBM ingredient for use in this trial (Diet 6). The decision to replace SBM with an additional BP-SBM test ingredient was based on results from the digestibility trial. The six experimental diets were manufactured with a 25% inclusion of their respective BP-SBM ingredients. All diets, including the control diet (Diet 7) were manufactured with a 10% inclusion of FM (Table 4-2).

Dry ingredients were ground using a Fitzpatrick Comminutator (Elmhurst, IL) with a 1.27 mm screen prior to blending. Milled ingredients were then transferred to a ribbon mixer (Patterson Equipment, Toronto, ON) and blended for five minutes. The homogenous feedstuff was then extruded using an Extru-Tech E325 single-screw extruder (Sabetha, KS).The extruder was equipped with 4-mm die inserts to produce 5-mm floating pellets for the digestibility trial and 2.5-mm die inserts to produce 3.2-mm diameter floating pellets for the growth trial. Extruded pellets were dried with a

conveyor oven drier (Colorado Mill Equipment, Canon City, CO), screen sifted with a Rotex screener (Rotex Inc., Cincinnati, OH) and then lipid coated using a Phlauer vacuum coater (A&J Mixing, Oakville, Ontario). The diets were then bagged and stored at room temperature until use.

Feeding Trials

Digestibility Trial

Naïve hybrid striped bass (~700 g) were randomly stocked (n=175) at a density of 35 fish per tank. Three separate trial runs were completed to provide duplicate tanks per experimental treatment and one FM control tank for each trial run. The FM control diet was fed to all tanks for five days before being switched to their respective experimental diets which they were fed twice per day to apparent satiation for ten days.

On the tenth day, fecal samples were collected from anesthetized fish (80 ppm tricaine methanesulfonate, MS-222) seven hours post-prandial by abdominal palpation stripping of distal digesta. Individual fecal samples were pooled for each tank and were stored at -20°C. Individual fish were then randomly reassigned to new tanks and switched back to the reference diet to begin the next trial run. Identical methods of feeding and fecal collection were used for each trial run.

Samples were freeze dried for 72 hours using a FreeZone 2.5 Liter freeze dry system (Labconco, Kansas City, MO). Dry samples were analyzed for crude protein (*%N x 6.25,* AOAC 2006, method 990.03) and chromic oxide (Cr_2O_3) (acid digestion and spectrophotometry) concentrations by a certified laboratory. All calculations of

digestibility were based on the dry matter composition of nutrient and tracer in feeds and feces. Apparent digestibility coefficients were calculated using the formulas described by Kleiber (1961) and Forster (1999):

$$ADC_{ref\ and\ diet} = \frac{100 - 100(\%marker\ in\ diet\ \times\ \%nutrient\ in\ feces)}{(\%marker\ in\ feces\ \times\ \%nutrient\ in\ diet)}$$

$$ADC_{ing} = \frac{[(a+\ b)\ \times\ ADC_{diet} - (a)\ \times\ ADC_{ref}]}{b}$$

where a = nutrient contribution of the reference diet to the test diet = (level of nutrient in reference diet) * (100-i); b = nutrient contribution of test ingredient to nutrient content of test diet = (level of nutrient in test ingredient) * i, i = level of test ingredient in combined diet (%); and (a + b) = level of nutrient in combined diet (%).

Experimental ingredients were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to determine the effect that bioprocessing and enzyme pretreatment had on molecular protein size. Samples were extracted in duplicate in SDS extraction buffer (2% SDS, 25% glycerol, 63 mM TrisHCl, pH 6.8) at a ratio of 20 mg to 1 ml. After weighing dry samples into Eppendorf tubes, SDS extraction buffer was added and the samples were briefly vortexed. Samples were heated at 95°C in a water bath for 30 minutes and afterwards agitated on a plate shaker at room temperature for an additional 30 minutes. Samples were spun at 10,000 g for 10 minutes and then aliquots of the supernatants were transferred to fresh tubes. Aliquots of the duplicate samples were combined into one tube per sample and bromophenol blue (0.33 mg ml^{-1} final concentration) as well as dithiothreitol (DTT) (67 mM final concentration) were added. Samples were heated at 95°C in a water bath for 10 minutes

and aliquots of the denatured and reduced samples (20 µl or 5 µl) were loaded onto gels (Novex™ WedgeWell™ Tris-Glycine 4%-20% gradient mini gels) and proteins were separated by running a constant voltage of 200 in Novex™ Tris-Glycine SDS Running Buffer for 45 minutes. Gels were washed three times in distilled water for 5 minutes and then fixed for 15 minutes (25% isopropyl alcohol/5% acetic acid) before staining them in PAGE-Blue Protein Staining Solution (ThermoFisher Scientific, Waltham, MA) for one hour. Several changes of distilled water were used until clear banding was observed. Gels were than de-stained in distilled water for several days before using the Pierce Silver Stain Kit (ThermoFisher Scientific, Waltham, MA) as a second more sensitive stain. Quantitative protein concentrations were measured in the individual supernatants with the Pierce BCA Protein Assay Kit using Bovine Serum Albumin (BSA) as standard protein.

Growth Experiment

Naïve, juvenile hybrid striped bass (mean±SE, 17.83±0.11 g) were randomly distributed (n=560) at a density of 20 fish per tank. Each treatment was randomly assigned to 4 replicate tanks using a random number generator in Microsoft Excel. All fish were fed the same ration of a FM-based holding diet prior to the start of the trial.

Upon the start of the trial each tank of fish was switched from the holding diet to their respective experimental diets. The experimental diets were offered three times per day (0800, 1200, and 1600 hr) for 105 days. A known amount of feed was offered to each tank at the beginning of each feeding event. After 10 minutes tanks were checked for remaining floating pellets. If pellets were left floating in the tank, the fish

in that tank were assumed to have reached apparent satiation. If no pellets remained in the tank, the fish in that tank were again fed a predetermined ration and allowed 10 minutes to consume the feed before they were checked again. Feeding continued in this manner until all tanks had feed floating on the surface of the water and it was therefore determined that all fish had reached apparent satiation. Care was taken to avoid feeding in extreme excess of satiation to avoid any detrimental effects on water quality or any feed waste.

Upon completion of the final feeding each day, feed containers were gathered and remaining feed was weighed in order to monitor daily consumption over the course of the trial. Feed consumption values were used to calculate feed conversion ratio (FCR). FCR was calculated as follows:

$$FCR = \frac{mass\ of\ feed\ consumed\ (dry, g)}{fish\ biomass\ gain\ (wet, g)}$$

Mass measurements were recorded when stocking of fish occurred (Day 0) and again at 3-week intervals until completion of the trial. Mass measurements included total tank biomass of all tanks in the system during each sampling and individual weights and lengths of all fish in 1 tank of each treatment on Day 0 and all fish in 3 replicate tanks of each treatment for all remaining 3-week sampling intervals and upon completion of the trial.

Total tank biomass measurements were sampled to calculate relative growth (RG), specific growth rate (SGR), and biomass gain over the course of the trial. Weights and lengths of individual fish were sampled to calculate Fulton's condition

factor (K) and to monitor any separation in growth among individuals within tanks. RG, SGR, and K were calculated as follows:

$$RG = \frac{\Delta\, mass\ (wet, g)}{initial\ mass\ (wet, g)} \times 100\%$$

$$SGR = \frac{[\ln(final\ wt\ (g)) - \ln(initial\ wt\ (g))]\ \times 100}{n\ (days)}$$

$$K = \frac{weight\ (wet,g)}{length\ (mm)^3} \times 100{,}000.$$

Three fish per tank were euthanized at the end of the study using lethal levels of buffered MS-222 according to Institutional Animal Care and Use Committee (IACUC) protocol (IACUC Approval Number 16-089A). Samples including blood, liver, spleen, viscera, fat, and muscle tissue were gathered from fish to obtain hematocrit values, hepatosomatic index (HSI), splenosomatic index (SSI), viscerosomatic index (VSI), visceral fat index (VFI), and fillet yield, respectively. Organosomatic indices were calculated as follows:

$$HSI = \frac{Liver\ weight}{Body\ weight} \times 100$$

$$SSI = \frac{Spleen\ weight}{Body\ weight} \times 100$$

$$VSI = \frac{Viscera\ weight}{Body\ weight} \times 100$$

$$VFI = \frac{Visceral\ fat\ weight}{Body\ weight} \times 100$$

$$Fillet\ Yield = \frac{Weight\ of\ 1\ fillet\ (skin\ attached) \times 2}{Body\ weight} \times 100.$$

Distal intestinal tissues were excised during the final necropsy and fixed in phosphate buffered formalin for storage. The intestinal tissue samples were later sectioned, stained with hematoxylin and eosin, and mounted to slides for histological analysis. A total of 20 slides were prepared, 4 replicate slides from fish fed Diets 1, 4, 5, 6, and 7. Treatment selection for histological sampling was based on examination of growth performance and health indices response of the treatments. Slides were examined using 10x magnification (Nikon E-200, Melville, New York) and scored using previously reported methods (Barnes et al. 2014) (Table 4-3). Two separate reviewers independently analyzed all slides at random and assigned a ranking to each slide, based on appearance of lamina propria, connective tissue, and vacuolization of intestinal samples. Any discrepancies among ranks were discussed by reviewers and adjusted accordingly and final ranks were averaged for overall gut scoring.

Culture Systems

The recirculating aquaculture systems (RAS) used for the two experiments were similar in design but differed in tank volume and quantity. The digestibility experiment was conducted in a 5,510 liter (L) recirculating aquaculture system equipped with 5, 760 L circular tanks. The 4,682 L RAS used for the growth experiment contained 28, 114 L circular tanks.

The tanks in both systems were each equipped with a “recirculating” drain which withdrew water from the subsurface and a “sludge” drain which was affixed to

the lowest point in the bottom at the center of the tank. Each tank also contained forced air diffusers fed by a blower and half covers to minimize disturbance. The RASs were also equipped with a pump, bead filter, bag filter, UV filter, biofilter, solids settling sump, clarifying sump, water inlet float valve, and heater/chiller unit.

The RAS used in the digestibility trial was supplied with a municipal water source that had been dechlorinated with sodium thiosulfate and stored in head tanks for later use. The water source that was used for the growth study was sourced from a shallow well. Water quality parameters were similar for both studies. Water temperature was maintained between 25 and 27°C, dissolved oxygen was held at levels greater than 5 mg L^{-1}, and pH ranged from 7 to 8. Temperature, dissolved oxygen, and pH were monitored daily while ammonia (NH_3) and nitrite (NO_2) were monitored less frequently, on average 3 times per week.

Statistical Analysis

Statistical analyses were conducted with JMP, Version 12 (SAS Institute Inc., Cary, North Carolina). All data were first checked for normality with a Shapiro-Wilk test and then for equal variances with a Levene's test. Analysis of Variance (ANOVA) was used on all normally distributed data with equal variances and a Kruskal-Wallis test was used on non-normal data. A Kruskal-Wallis test was also used on the rank data for the hindgut histological analysis. Any significant outcomes were further investigated with a Tukey's HSD post hoc test for multiple comparisons on normal data or a Steel-Dwass post hoc test for multiple comparisons on non-normal data and rank data. An *a priori* alpha value of $\alpha=0.05$ was used for all statistical analyses.

Results

The same experimental ingredients were used in both experiments for this research with the exception of the SBM ingredient that was used in the negative control diet in the digestibility trial. A few differences were noted among the ingredients when proximate composition was examined. The ingredient with the highest crude protein, ash, fat, methionine, and lysine concentrations was the FM ingredient used in the control diet in both studies (Table 4-4). FM was also the ingredient with the lowest fiber content among all other ingredients. The SBM ingredient that was used in the digestibility trial contained the lowest protein, lysine, and methionine concentrations of all experimental ingredients. The ingredient with the lowest crude protein content that was used in the growth study was the BP-SBM ingredient used in Diet 2 (Table 4-4).

Digestibility Experiment

Nutritional composition and amino acid profile were well balanced among diets when proximate composition was analyzed in the digestibility experiment (Table 4-5). Diet 2 (48.89%) had the highest protein concentration while the lowest protein concentration was found in Diet 4 (44.34%). Fat concentrations also had little variation among diet with the reference diet, Diet 7, containing the highest fat content (17.43%). Analysis also revealed that final chromic oxide concentration among diets ranged from 0.88% in Diet 5 to 1.03% in Diet 1 (Table 4-5).

A significant response was revealed when ADC-P was analyzed (P=0.043). The highest digestibility of protein was found in Diet 4 (87.8 %), significantly higher

than Diet 6 (76.9 %). No significant differences were detected among any of the remaining diets when ADC-P was analyzed. Diet 5 and Diet 1 performed similarly to Diet 4, exhibiting ADC-Ps of 86.7 % and 86.3 %, respectively. The FM reference diet, Diet 7, provided intermediate (81.4 %) performance compared to the experimental diets. Three diets exhibited higher ADC-Ps and three diets had lower ADC-Ps relative to Diet 7 (Figure 4-1).

Growth Experiment

The diets in the growth experiment were formulated on an isonitrogenous and isocaloric basis, therefore proximate composition of nutrients varied much less among diets than the experimental ingredients. However, there were a few evident differences among the experimental diets that are worth noting (Table 4-6). The FM control diet, Diet 7, contained the lowest crude protein concentration of all diets (45.49). The other diets contained very similar concentrations of protein ranging from 47.02 to 47.92%. It is also worth noting that Diet 7 had the highest nitrogen free extract (NFE) (32.69%). Crude fat concentrations were also very similar among diets ranging from 8.13% in Diet 6 to 8.99% in Diet 4 (Table 4-6).

Significant growth performance responses were revealed as early as the first 3-week sampling interval. Mean consumption per tank was one of the parameters that produced a significant result at the 3-week sampling (P=0.03). The fish fed Diet 7 (556.5 g) consumed significantly more feed per tank than the fish that were fed Diets 3 (413.9 g) and 4 (412.3 g). This same significant result was found at the next two samplings (6 and 9-weeks). The highest mean consumption per tank was still found in

Diet 7 at the 12-week sampling, however it was only significantly greater than Diet 4 (P=0.01). Analysis of mean consumption per tank at the final sampling (15-week) also produced a significant response (P=0.01). At this sampling it was found that an average of 4,224 g was consumed per tank in treatment 7, significantly greater than treatments 2, 3, and 4 (Table 4-7).

Consumption was also assessed as a mean percentage of bodyweight consumed per day to correct for variations in biomass. When this metric was analyzed at the 3, 6, 12, and 15-week samplings it produced a significant result, however no significant differences were found at the 9-week sampling (P=0.06). At the 3-week sampling event Diet 7 was consumed significantly more than Diets 3 and 4; at the 6-week sampling Diet 7 consumption was observed to be significantly higher than Diets 1, 4, and 5; and at the 12-week sampling Diet 7 consumption was significantly higher than Diets 1, 2, and 5 (P<0.05). At the 15-week sampling, consumption (% BWPD) of diets 7 (2.31%) and 3 (2.08%) was significantly higher than Diet 1 (1.86%) (P=0.04) (Table 4-7).

Fulton's condition factor (K) was significant at each sampling except for the 6-week sampling. The fish fed Diet 1 had the highest K among treatments at every sampling throughout the trial. At the final sampling, the Diet 1 fish exhibited a K of 1.43, significantly higher than the fish fed Diets 4 (1.37) and 7 (1.32) (P<0.001). Condition factor was similar in the fish fed Diet 3 with the second highest final value of 1.41. Diet 7 K was the lowest of all treatments upon completion of the experiment, significantly lower than Diets 1, 3, 5, and 6 (P<0.001) (Table 4-7).

Feed conversion ratio (FCR) did not show a significant response among treatments until the 6-week sampling but then produced a significant result at every sampling interval for the remainder of the trial. The lowest FCR was observed in Diet 1 at every sampling except the 12-week interval when Diet 5 had a slightly lower FCR. At the final sampling Diet 1 exhibited a FCR of 1.44 while Diet 5 produced a similar result of 1.45. The primary reason for the repeated significant responses over the course of the experiment was due to the relatively high FCR found in the fish fed Diet 7. The highest FCR was observed in Diet 7 at every sampling during the study and was significantly higher than every other diet at the final sampling when a value of 1.93 was produced ($P<0.01$). Diet 1 (1.44) also had a significantly lower FCR than Diets 3 (1.62) and 4 (1.55) (Table 4-7).

Relative growth (RG) and specific growth rate (SGR) did not produce a significant result until the final sampling at 15 weeks. Diet 1 (RG=783.3, SGR=2.07) performed significantly better than Diet 4 (RG=622.1, SGR=1.88) when both RG ($P=0.02$) and SGR ($P=0.02$) were analyzed at the 15-week sampling. Diet 5 produced similar results to Diet 1 when RG and SGR were examined, indicating that it was also a top performer (Table 4-7).

Mean biomass gain per tank did not produce a significant result throughout the experiment ($P>0.05$). However, it should be noted that Diet 1 (2,563 g) and Diet 5 (2,532 g) had the highest average growth per tank among all treatments in this study. Also, the fish fed Diet 4 had the lowest growth among all treatments, only growing 2,113 g over the 15-week experiment (Table 4-7).

A final necropsy occurred to obtain blood for hematocrit and weights of fat, tissue, liver, spleen, and viscera to calculate visceral fat index (VFI), fillet yield, hepatosomatic index (HSI), splenosomatic index (SSI), and viscerosomatic index (VSI), respectively. Distal intestinal tissues were also collected in order to complete a hindgut histological analysis.

A significant difference in hematocrit levels was found among treatments in this study ($P<0.01$). The fish that were fed Diets 1 (54.5%) and 7 (54.7%) had significantly higher hematocrit than fish fed Diet 4 (49.8%) (Table 4-8). HSI also produced a significant response among treatments when analyzed ($P<0.001$). The fish that were fed Diet 7 (1.76), the treatment with the highest HSI, had a significantly greater HSI than the fish fed Diets 1, 2, 3, and 5. Diet 6 (1.64) also had a significantly greater HSI than Diet 3 (1.40) which exhibited the lowest HSI among treatments.

The remaining performance characteristics (SSI, VSI, VFI, and Fillet Yield) did not produce a significant response among treatments ($P>0.05$). SSI only varied from 0.052 in Diet 7 to 0.059 in Diet 4 (Table 4-8). VSI also was similar among treatments, only varying from 8.57 in the lowest treatment (Diet 5) to 9.32 in Diet 6, the treatments with the greatest VSI. The highest VFI was found in the fish fed Diet 3 (5.12) and the highest fillet yield was observed in the Diet 6 fish (52.4%). The lowest VFI was 4.25 (Diet 7) and the lowest fillet yield was observed in Diet 4 (50.9%) (Table 4-8).

Hindgut histological analysis also did not produce any significant differences among treatments ($P=0.15$). Treatments 1, 4, 5, 6, and 7 were the only treatments

analyzed based on findings from health indices and growth parameters. Even though the treatments did not significantly differ, it should be noted that the mean score of the fish fed Diet 1 was increased relative to the other analyzed tissue samples. The mean histological score of treatments 4, 5, 6, and 7 ranged from 1.71 to 1.75 while Diet 1 exhibited a mean score of 2.33.

Discussion

The ADC-Ps that were found in the digestibility study were similar to the results reported by Barrows et al. (2012) when the high quality FM ingredient, SBM ingredient, and enhanced soy ingredients were analyzed. The digestibility study revealed that Diet 4, the BP-SBM + Enzyme ingredient, was the most digestible ingredient among all other experimental ingredients that were fed to hybrid striped bass. This indicates that the enzyme pretreatment was the most effective strategy in producing a highly digestible protein ingredient as compared to the other experimental ingredients. The results of this study are in agreement with previous findings that have found enzyme pretreated soy products to have a higher digestibility of protein, improved absorption of minerals, and a higher bioavailability of phosphorus as compared to other soy based diets without enzyme pretreatments (Cain and Garling 1995; Storebakken et al. 2000; Wang et al. 2009). It can also be noted that fermentation of soy resulted in increased digestibility of protein as all diets containing BP-SBM had improved ADC-Ps as compared to Diet 6, the diet with the SBM inclusion that was not fermented.

Further investigation of the experimental ingredients through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed that the enzyme pretreatment effectively reduced the average molecular size of proteins in the BP-SBM + Enzyme ingredient (Lane 5) relative to the other experimental ingredients (Figure 4-2). Bands greater than 40,000 Daltons were greatly reduced and an increase in staining intensity can be noted at 10,000 Daltons in this enzyme treated ingredient. It can also be noted that the three BP-SBM fractions (Lanes 2, 3, and 4) are very similar in the overall distribution and intensity of protein branding patterns which may give insight to the similar performance of the three ingredients in both the digestibility and growth experiments. Finally, Lane 10 shows that the SBM ingredient contains characteristic major protein bands reflecting its glycinin and beta conglycinin protein constituents that had a negative effect on digestibility and resulted in the SBM ingredient having the lowest ADC-P of all treatments in this research (Figure 4-2).

Diets 1 and 5 tended to stand out as the performance leaders among all treatments in the 105-day growth experiment while Diet 4 appeared to be a low performer when many of the performance characteristics were analyzed. The FM reference diet, Diet 7, performed intermediately among all diets in the growth study, however it appeared to be the highest consumed diet and in turn was the diet with the highest FCR among treatments for the latter part of the trial. The increased nature of consumption and FCR may however be due to pellet characteristics and feeding biases.

All diets were extruded to produce floating pellets so feeding could be monitored by remaining floating pellets after each feeding event and fish could be fed

to apparent satiation. This method of monitoring consumption assumes that all pellet characteristics are similar across treatments and that no feed is lost down the drain (Helland et al. 1996). However, Diet 7 may have had an average pellet density that was higher than the rest of the diets in the study resulting in some pellets that would sink to the bottom of the tank and later be lost down the drain. Consequently, feeding may have been biased in the tanks of fish that were fed Diet 7 and overfeeding may have occurred resulting in biased estimates for consumption and FCR. The lowest expansion ratio (ER) of all the diets fed in the growth study was found in the pellets of Diet 7. This may give insight to the reason that the pellets of Diet 7 did not float as well, resulting in sinking feed and the increased possibility of feed being lost down the drain.

Hindgut histological analysis revealed a higher average score for fish fed Diet 1 (2.33), indicating an overall unhealthier hindgut compared to fish fed other diets in the growth study. Though this was not a significant response, it is interesting considering that Diet 1 was considered a top performing diet when fish growth response and other health indices were analyzed. Digestion of protein also appeared to be improved in this diet over five other diets including the FM reference diet when ADC-P was analyzed. Some ANFs that are present in soy may cause inflammation and reduced absorption of nutrients however rainbow trout have been known to be able to adapt to inclusions of plant-based protein sources by increasing endogenous enzyme levels (Krogdahl et al. 2005).

The experimental diets containing BP-SBM that were formulated in this study replaced a portion of animal protein sources including poultry by-product meal and feather meal. There has been recent consumer interest in replacing all animal protein in the diets of livestock to produce a 100% plant-based diet (FAO 2016). The experimental soy diets containing BP-SBM in the growth study replaced 47.8% of poultry meal and 66.7% of feather meal as compared to Diet 7, the reference diet containing no BP-SBM. Also, the BP-SBM experimental ingredients replaced 54.4% of wheat middling in the experimental diets as compared to the FM-based reference diet.

Overall, all fish were healthy and grew well regardless of experimental treatment resulting in 100% survival of the fish in the growth experiment. These experiments provide results that are consistent with other research that has found success in including enhanced or fermented soy as the main protein ingredient in the diets of hybrid striped bass (Rombenso et al. 2013). Continued research on the inclusion of enhanced soy products and its effects on the digestibility and growth of hybrid striped bass will further the development of sustainable plant-based protein ingredients and their potential use in the diets of hybrid striped bass.

Table 4-1. Bulk reference diet formulation used in digestibility study. Formulation obtained from Agricultural Research Service digestibility database (Barrows et al. 2012). Values reported as g 100 g^{-1} dm.

Ingredient Composition	g 100 g^{-1}
Squid meal[a]	25
Soy protein concentrate[b]	17.14
Corn gluten meal[c]	8.34
Soybean meal[d]	4.3
Wheat flour[e]	28.33
Taurine[f]	0.5
Menhaden fish oil[g]	13.39
Vitamin premix[h]	1
Choline Chloride[i]	0.6
Vitamin C[j]	0.2
Chromic oxide[f]	1
Yttrium oxide[f]	0.1
Mineral Premix[f]	0.1

[a]Wilbur-Ellis, Portland, OR, [b]Solae Inc., Pro-fine VF, St. Louis, MO, [c]Cargill Inc., Minneapolis, MN, [d]Archer Daniels Midland Company, Decatur, IL, [e]Nelson and Sons Inc., Murray, UT, [f]Sigma-Aldrich Company, St. Louis, MO, [g]Omega Protein Corporation, Hammond, LA, [h]DSM Nutritional Products, Basel, Switzerland, [i]NB Group Co. LTD., Shangdong, China, [j]Ascorbic Acid Stay-C 35, DSM Nutritional Products, Basel, Switzerland.

Table 4-2. Experimental diet formulations used in the 105-day growth trial. BP-SBM ingredient fractions (Diets 1, 2, and 3), a BP-SBM ingredient with an enzyme inclusion during fermentation (Diet 4), the base BP-SBM diet with an extra wash step (Diet 5), the base BP-SBM diet (Diet 6), and the FM control diet (Diet 7). Values reported as g 100 g^{-1} dm.

	Diet						
Ingredient	1	2	3	4	5	6	7
BP-SBM Fraction #1[a]	25.00	0.00	0.00	0.00	0.00	0.00	0.00
BP-SBM Fraction #2[a]	0.00	25.00	0.00	0.00	0.00	0.00	0.00
BP-SBM Fraction #3[a]	0.00	0.00	25.00	0.00	0.00	0.00	0.00
BP-SBM + Enzyme[a]	0.00	0.00	0.00	25.00	0.00	0.00	0.00
BP-SBM Base + Wash[a]	0.00	0.00	0.00	0.00	25.00	0.00	0.00
BP-SBM Base[a]	0.00	0.00	0.00	0.00	0.00	25.00	0.00
Blood Meal[b]	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Wheat Midds[c]	10.00	10.00	10.00	10.00	10.00	10.00	21.92
Whole Cleaned Wheat[d]	16.67	16.67	16.67	16.67	16.67	16.67	15.00
Poultry Meal[e]	12.00	12.00	12.00	12.00	12.00	12.00	23.00
Feather Meal[e]	2.50	2.50	2.50	2.50	2.50	2.50	7.50
Fish Meal[f]	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Vitamin Premix[g]	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Lysine[h]	1.00	1.00	1.00	1.00	1.00	1.00	1.75
Methionine[h]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline Chloride[i]	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Mineral Premix[j]	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Stay C[k]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Fish Oil[l]	6.50	6.50	6.50	6.50	6.50	6.50	4.50
Dicalcium phosphate[m]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Defatted SBM[n]	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Totals	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a]South Dakota State University, Brookings, SD, [b]Mason City By-Products, Mason City, IA, [c]Consumer Supply Distributing, Sioux City, IA, [d]Ag First Farmer's Cooperative, Brookings, South Dakota, [e]Tyson Foods, Springdale, AR, [f]Special Select, Omega Protein, Houston, TX, [g]ARS 702 premix, Nelson and Sons, Murray, UT, [h]Pure Bulk, Roseburg, OR, [i]BalChem Corporation, New Hampton, NJ, [j]ARS 640 trace mix, Nelson and Sons, Murray, UT, [k]DSM Nutritional Products, Parsippany, NJ, [l]Viginia Prime Gold, Omega Protein, Houson, TX, [m]Feed Products Inc., St. Louis, MO, [n]South Dakota Soybean Processors, Volga, South Dakota.

Table 4-3. Histological scoring system for hybrid striped bass distal intestines in the growth study (Barnes et al. 2014).

Score	Appearance
	Lamina propria of simple folds
1	Thin and delicate core of connective tissue in all simple folds
2	Lamina propria slightly more distinct and robust in some folds
3	Clear increase in lamina propria in most simple folds
4	Thick lamina propria in many folds
5	Very thick lamina propria in many folds
	Connective tissue between base of folds and stratum compactum
1	Very thin layer of connective tissue between base of folds and stratum compactum
2	Slightly increased amount of connective tissue beneath some mucosal folds
3	Clear increase of connective tissue beneath most mucosal folds
4	Thick layer of connective tissue beneath many folds
5	Extremely thick layer of connective tissue beneath some folds
	Vacuolization
1	Large vacuoles abundant and present in most epithelial cells
2	Large vacuoles numerous
3	Increased number of large vacuoles
4	Very few large vacuoles present
5	Large vacuoles absent

Table 4-4. Proximate composition and amino acid profile of main protein ingredients used in digestibility and growth experiments. Values are g/100 g dm unless noted. EAA=essential amino acid; NEAA= non-essential amino acid; Total AA= total amino acids.

	Ingredient							
Analyte	1	2	3	4	5	6	7	SBM
Ash	3.70	4.03	3.74	3.53	3.25	4.65	21.48	6.56
Fat	0.98	1.01	0.86	0.78	1.00	0.32	9.47	1.56
Fiber	10.91	5.15	11.69	5.58	4.44	8.46	0.76	2.89
Protein	61.05	60.70	61.14	62.43	64.12	67.51	69.16	48.39
Moisture (wb)	8.34	10.72	6.72	10.38	7.98	5.49	7.31	10.15
EAA								
Arginine	4.66	4.57	4.56	4.50	4.31	4.57	3.95	3.65
Histidine	1.95	1.67	1.64	1.93	1.66	1.69	1.92	1.51
Isoleucine	3.21	3.36	3.18	3.31	3.43	3.56	2.77	2.40
Leucine	5.48	5.49	5.45	5.55	5.66	5.86	4.90	4.02
Lysine	4.22	4.09	4.03	4.22	3.58	3.45	5.19	3.43
Methionine	0.91	0.92	0.94	0.93	1.09	1.07	1.91	0.70
Phenylalanine	3.58	3.56	3.56	3.64	3.41	3.54	2.71	2.67
Taurine	0.09	0.07	0.10	0.08	0.00	0.00	0.54	0.12
Threonine	2.61	2.59	2.57	2.59	3.01	2.86	2.85	1.99
Valine	3.32	3.44	3.30	3.43	3.58	3.63	3.27	2.50
NEAA								
Alanine	2.89	2.91	2.89	2.97	3.23	3.29	4.53	2.24
Aspartic Acid	7.38	7.40	7.26	7.45	7.84	0.00	6.26	5.73
Glutamic Acid	12.00	11.98	11.79	11.94	10.76	12.02	9.28	0.53
Glycine	2.77	2.79	2.78	2.85	2.95	2.93	5.18	2.19
Proline	3.28	3.28	3.22	3.26	3.36	3.52	3.56	2.48
Serine	3.44	3.29	3.27	3.15	3.43	3.25	2.70	2.38
Tyrosine	2.45	2.18	2.39	2.34	2.58	2.72	2.20	1.88
Total AA	64.14	63.53	62.83	64.04	63.87	57.96	63.17	40.32

Table 4-5. Proximate composition and amino acid profile of diets used in the digestibility experiment. Values are g/100 g dm unless noted. EAA=essential amino acid; NEAA= non-essential amino acid; Total AA= total amino acids.

	Diet						
Analyte	1	2	3	4	5	6	7
Ash	5.79	5.66	5.79	6.67	5.42	6.08	6.11
Fat	15.22	14.32	14.77	14.11	16.30	13.50	17.43
Fiber	3.63	3.85	4.04	4.01	4.89	4.54	3.40
Protein	48.33	48.89	48.48	44.34	46.56	48.81	44.41
Moisture (wb)	4.26	3.82	5.26	4.69	6.31	4.77	6.15
Chromic oxide	1.03	1.00	1.01	1.02	0.88	1.02	0.97
EAA							
Arginine	3.03	3.08	3.02	2.68	2.85	2.76	2.47
Histidine	1.11	1.13	1.12	1.01	1.06	0.99	0.91
Isoleucine	2.31	2.32	2.31	2.04	2.22	2.06	1.81
Leucine	4.26	4.30	4.27	3.87	4.17	3.91	3.72
Lysine	2.65	2.68	2.73	2.54	2.51	2.36	2.21
Methionine	0.84	0.84	0.89	0.81	0.86	0.79	0.85
Phenylalanine	2.48	2.48	2.47	2.21	2.28	2.17	1.95
Taurine	0.60	0.61	0.61	0.55	0.60	0.58	0.74
Threonine	1.75	1.79	1.77	1.58	1.75	1.65	1.56
Valine	2.38	2.36	2.34	2.08	2.32	2.16	1.99
NEAA							
Alanine	2.42	2.45	2.45	2.22	2.45	2.29	2.27
Aspartic Acid	4.51	4.60	4.54	4.00	4.34	4.15	3.68
Glutamic Acid	8.39	8.52	8.37	7.56	7.87	7.48	7.14
Glycine	2.14	2.20	2.20	1.96	2.18	2.05	1.96
Proline	2.76	2.79	2.79	2.51	2.68	2.52	2.41
Serine	1.91	1.97	1.93	1.58	1.92	1.82	1.66
Tyrosine	1.71	1.77	1.75	1.58	1.61	1.56	1.40
Total AA	44.65	45.28	44.95	40.24	43.08	40.71	37.98

Table 4-6. Proximate composition of diets used in growth study. All values reported as g/100 g dm. NFE= nitrogen free extract.

	Constituent				
Diet	Ash	Fat	Fiber	Protein	NFE
1	7.71	8.79	4.65	47.32	31.53
2	7.89	8.53	3.86	47.92	31.80
3	7.63	8.97	4.47	47.25	31.68
4	7.71	8.99	5.33	47.32	30.64
5	7.77	8.49	5.62	47.02	31.10
6	8.07	8.13	5.27	47.65	30.88
7	8.88	8.42	4.52	45.49	32.69

Table 4-7. Growth performance indices. Values given are treatment means±SE. Significant differences (P<0.05) are indicated by different letters within a given column. Average biomass gain per tank (Gain Tank^{-1}, g, wet), average total consumption per tank (Consumption, g, dry), average consumption per day as a percentage of bodyweight per day (% BWPD), relative growth (RG, %), specific growth rate (SGR), feed conversion ratio (FCR), and Fulton-type condition factor (K).

	Performance Characteristic						
Diet	Gain Tank^{-1}	Consumption	% BWPD	RG	SGR	FCR	K
1	2563.5±131.3^{a}	3699.6±163.5^{ab}	1.86±0.05^{b}	783.3±34.4^{a}	2.07±0.04^{a}	1.44±0.012^{c}	1.43±0.01^{a}
2	2398.6±147.3^{a}	3570.0±175.6^{b}	1.88±0.08^{ab}	722.8±38.8^{ab}	2.00±0.05^{ab}	1.49±0.031^{bc}	1.38±0.02^{abc}
3	2216.7±55.3^{a}	3592.9±89.7^{b}	2.08±0.05^{a}	656.7±16.5^{ab}	1.93±0.02^{ab}	1.62±0.028^{b}	1.41±0.02^{ab}
4	2112.8±119.5^{a}	3270.8±153.2^{b}	1.93±0.06^{ab}	622.1±45.6^{b}	1.88±0.06^{b}	1.55±0.023^{b}	1.37±0.02^{bc}
5	2531.8±90.9^{a}	3676.0±121.8^{ab}	1.92±0.05^{ab}	750.3±30.5^{ab}	2.04±0.03^{ab}	1.45±0.009^{bc}	1.40±0.01^{ab}
6	2394.8±127.5^{a}	3680.1±142.1^{ab}	2.01±0.06^{ab}	704.3±32.2^{ab}	1.98±0.04^{ab}	1.54±0.051^{bc}	1.40±0.01^{ab}
7	2195.5±65.8^{a}	4224.1±65.1^{a}	2.31±0.14^{a}	647.9±15.7^{ab}	1.92±0.02^{ab}	1.93±0.044^{a}	1.33±0.01^{c}

Table 4-8. Necropsy variables for hybrid striped bass. Values given are treatment means±SE. Significant differences (P<0.05) are indicated by different letters within a given column. Hematocrit (%), HSI=hepatosomatic index, SSI=splenosomatic index, VSI=viscerosomatic index, VFI=visceral fat index, and Fillet Yield (%).

	Performance Characteristic					
Diet	Hematocrit	HSI	SSI	VSI	VFI	Fillet Yield
1	54.5±0.7^{a}	1.49±0.06^{bc}	0.055±0.004^{a}	8.85±0.31^{a}	4.87±0.21^{a}	51.95±0.44^{a}
2	52.5±1.1^{ab}	1.44±0.06^{bc}	0.056±0.004^{a}	8.61±0.26^{a}	4.84±0.20^{a}	52.03±0.55^{a}
3	53.3±0.7^{ab}	1.40±0.03^{c}	0.057±0.004^{a}	9.09±0.29^{a}	5.12±0.26^{a}	51.27±0.71^{a}
4	49.8±1.4^{b}	1.56±0.05^{abc}	0.059±0.004^{a}	9.28±0.21^{a}	4.94±0.29^{a}	50.91±0.72^{a}
5	51.2±1.1^{ab}	1.44±0.06^{bc}	0.053±0.002^{a}	8.57±0.25^{a}	4.73±0.20^{a}	51.04±0.59^{a}
6	50.5±0.8^{ab}	1.64±0.04^{ab}	0.058±0.003^{a}	9.32±0.15^{a}	4.55±0.19^{a}	52.42±0.43^{a}
7	54.7±0.9^{a}	1.76±0.06^{a}	0.052±0.006^{a}	8.60±0.19^{a}	4.25±0.16^{a}	50.55±0.65^{a}

Figure 4-1. Change (%) of apparent digestible coefficient of protein (ADC-P) of experimental diets relative to the FM reference diet (81.4 %).

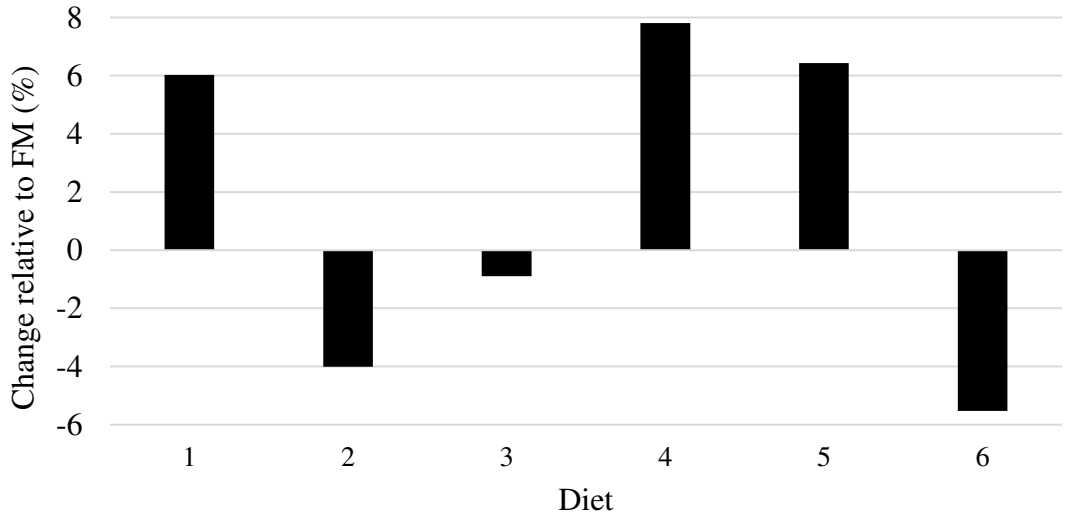
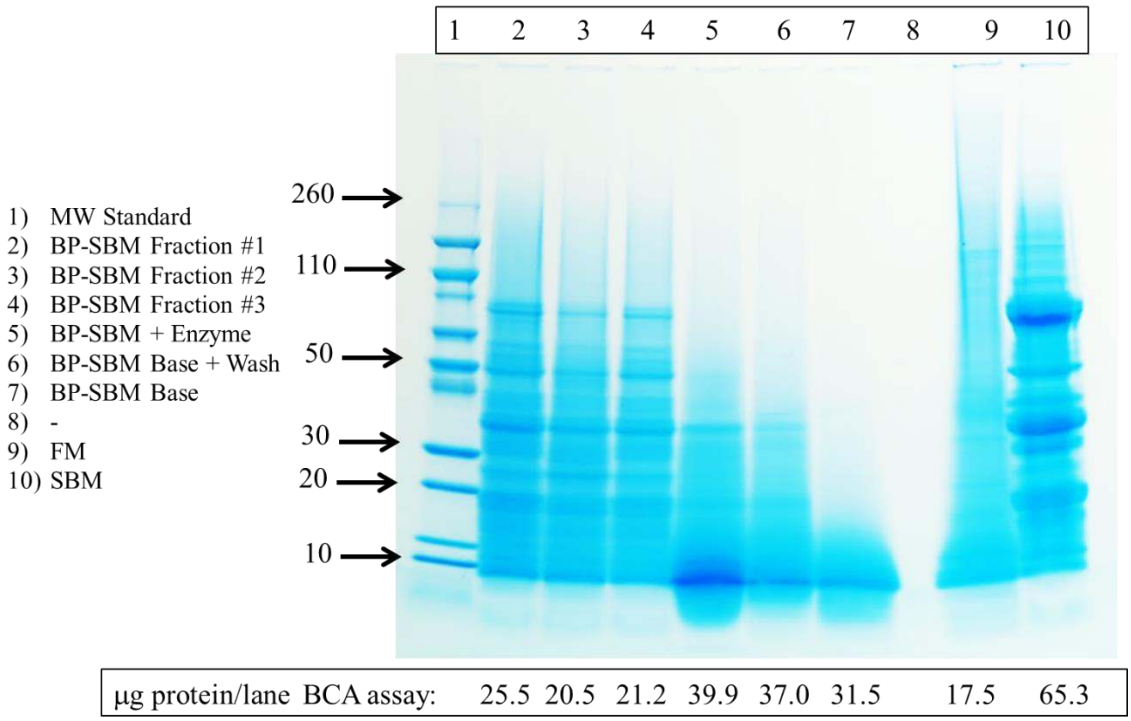


Figure 4-2. SDS-PAGE analysis of various experimental ingredients used in hybrid striped bass digestibility and growth trials. Lane 1= MW Standard, Lane 2= BP-SBM Fraction #1, Lane 3= BP-SBM Fraction #2, Lane 4= BP-SBM Fraction #3, Lane 5= BP-SBM + Enzyme, Lane 6= BP-SBM Base + Wash, Lane 7= BP-SBM Base, Lane 8= blank, Lane 9= FM (Control), Lane 10= SBM (Control).



References

AOAC. 2006. Official Methods of Analysis. Association of Official Analytical Chemists (AOAC), Arlington, VA.

Asche, F., A. Oglend, and S. Tveteras. 2013. Regime shifts in the fish meal/soybean meal price ratio. Journal of Agricultural Economics 64(1):97-111.

Barnes, M. E., M. L. Brown, T. Bruce, S. Sindelar, and R. Neiger. 2014. Rainbow trout rearing performance, intestinal morphology, and immune response after long-term feeding of high levels of fermented soybean meal. North American Journal of Aquaculture 76(4):333-345.

Barnes, M. E., M. L. Brown, K. A. Rosentrater, and J. R. Sewell. 2012. An initial investigation replacing fish meal with a commercial fermented soybean meal product in the diets of juvenile rainbow trout. Open Journal of Animal Sciences 2(4):234.

Barrows, F. T., T. G. Gaylord, W. M. Sealey, L. Porter, and C. E. Smith. 2008. The effect of vitamin premix in extruded plant-based and fish meal based diets on growth efficiency and health of rainbow trout, Oncorhynchus mykiss. Aquaculture 283(1-4):148-155.

Barrows, F. T., T. G. Gaylord, W. M. Sealey, and S. D. Rawles. 2012. Database of nutrient digestibility's of traditional and novel feed ingredients for trout and hybrid striped bass.

Bruce, T., S. Sindelar, J. Voorhees, M. Brown, and M. Barnes. 2017. Performance and immunological responses of rainbow trout (Oncorhynchus mykiss) fed bioprocessed plant-based proteins. Aquaculture Nutrition.

Caballero, M., and coauthors. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, Oncorhynchus mykiss. Aquaculture 214(1):253-271.

Cain, K. D., and D. L. Garling. 1995. Pretreatment of soybean meal with phytase for salmonid diets to reduce phosphorus concentrations in hatchery effluents. The Progressive Fish-Culturist 57(2):114-119.

Cao, L., and coauthors. 2008. Effects of pretreatment with microbial phytase on phosphorous utilization and growth performance of Nile tilapia (Oreochromis niloticus). Aquaculture Nutrition 14(2):99-109.

Colt, J. 2006. Water quality requirements for reuse systems. Aquacultural Engineering 34(3):143-156.

Dersjant-Li, Y. 2002. The use of soy protein in aquafeeds. L. E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M. G. Gaxiola-Cortés, and N. Simoes, editors. Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola. 3 al 6 de Septiembre del 2002., Cancún, Quintana Roo, México.

Drew, M. D., T. L. Borgeson, and D. L. Thiessen. 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Animal Feed Science and Technology 138(2):118-136.

Ellis, T., and coauthors. 2002. The relationships between stocking density and welfare in farmed rainbow trout. Journal of Fish Biology 61(3):493-531.

Engle, C., and P. Sapkota. 2012. A comparative analysis of the economic risk of hybrid striped bass fingerling production in ponds and indoor tanks. North American Journal of Aquaculture 74(4):477-484.

FAO. 2005. Cultured Aquatic Species Information Programme. Oncorhynchus mykiss. FAO Fisheries and Aquaculture Department [online].

FAO. 2014. The State of World Fisheries and Aquaculture. Food and Agriculture Oranization of the United Nations, Rome, Italy.

FAO. 2016. The State of World Fisheries and Aquaculture. Food and Agricultural Orginization of the United Nations, Rome, Italy.

Forster, I. 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquaculture Nutrition 5(2):143-145.

Forster, I. 2002. Use of soybean meal in the diets of non-salmonid marine fish. United Soybean Board and American Soybean Association.

Francis, G., H. P. S. Makkar, and K. Becker. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199(3-4):197-227.

Gatlin, D. M. 2002. Use of soybean meal in the diets of omnivorous freshwater fish. Texas A&M University System.

Gatlin, D. M., and coauthors. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38(6):551-579.

Glencross, B. D., M. Booth, and G. L. Allan. 2007. A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition 13(1):17-34.

Guner, Y., V. Kizak, M. Altunok, and I. Celik. 2016. Spawning and larval rearing in hybrid striped bass (Morone chrysops♀ X Morone saxatilis♂) in Turkey. Ekoloji Dergisi 25(98).

Hardy, R. W. 2002. Rainbow trout, Oncorhynchus mykiss. Nutrient Requirements and Feeding of Finfish for Aquaculture:184-202.

Helland, S. J., B. Grisdale-Helland, and S. Nerland. 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture 139(1-2):157-163.

Hertrampf, J. W., and F. Piedad-Pascual. 2000. Handbook on Ingredients for Aquaculture Feeds. Dordrecht, The Natherlands.: Kluwer academics publishers.

Hodson, R. G. 1989. Hybrid Striped Bass: Biology and Life History. Southern Regional Aquaculture Center publication (USA).

Kamalam, B. S., F. Medale, and S. Panserat. 2017. Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies. Aquaculture 467:3-27.

Kleiber, M. 1961. The Fire of Life: An Introduction to Animal Energetics. John Wiley & Sons, New York & London.

Krogdahl, A., G. I. Hemre, and T. P. Mommsen. 2005. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. Aquaculture Nutrition 11(2):103-122.

Lee, C.-S. 2015. Dietary Nutrients, Additives and Fish Health. John Wiley & Sons.

Lincoln, R. F., and A. P. Scott. 1983. Production of all-female triploid rainbow trout. Aquaculture 30(1-4):375-380.

Ludwig, G. M. 2003. Tank culture of larval sunshine bass, Morone chrysops (Rafinesque) x M. saxatilis, at three feeding levels. Aquaculture Research 34(14):1277-1285.

Ludwig, G. M. 2004. Hybrid Striped Bass: Fingerling Production in Ponds. Southern Regional Aquaculture Center. SRAC Publication No. 302.

McEntire, M., S. Snyder, and D. Freeman. 2015. Comparison of growth between Morone hybrids (palmetto and sunshine) in earthen ponds. Journal of the World Aquaculture Society 46(5):557-563.

Miles, R., and F. Chapman. 2006. The benefits of fish meal in aquaculture diets. IFAS Extension, University of Florida.

NRC. 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press.

Nunes, C., A. Silva, E. Soares, and K. Ganias. 2011. The use of hepatic and somatic indices and histological information to characterize the reproductive dynamics of Atlantic sardine Sardina pilchardus from the Portuguese coast. Marine and Coastal Fisheries 3(1):127-144.

Oliva-Teles, A., A. Gouveia, E. Gomes, and P. Rema. 1994. The effect of different processing treatments on soybean meal utilization by rainbow trout, Oncorhynchus mykiss. Aquaculture 124(1):343-349.

Person-Le Ruyet, J., and coauthors. 2008. Combined effects of water quality and stocking density on welfare and growth of rainbow trout (Oncorhynchus mykiss). Aquatic Living Resources 21(2):185-195.

Ravindra, P. 2000. Value-added food:: Single cell protein. Biotechnology Advances 18(6):459-479.

Read, E. S., and coauthors. 2014. Investigation of the effects of dietary protein source on copper and zinc bioavailability in fishmeal and plant-based diets for rainbow trout. Aquaculture 432:97-105.

Rombenso, A., C. Crouse, and J. Trushenski. 2013. Comparison of traditional and fermented soybean meals as alternatives to fish meal in hybrid striped bass feeds. North American Journal of Aquaculture 75(2):197-204.

Storebakken, T., S. Refstie, and B. Ruyter. 2000. Soy products as fat and protein sources in fish feeds for intensive aquaculture. Pages 127-170 in J. Drackley, editor. Soy in Animal Nutrition. Federation of Animal Science Societies, Savoy, IL.

Storebakken, T., K. D. Shearer, and A. J. Roem. 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, Salmo salar. Aquaculture 161(1-4):365-379.

Suman, G., M. Nupur, S. Anuradha, and B. Pradeep. 2015. Single cell protein production: a review. International Journal of Current Microbiology and Applied Sciences 4(9):11.

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(1-4):146-158.

USDA. 2012. Census of Agriculture. United States Department of Agriculture.

Wang, F., and coauthors. 2009. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and

nutrient excretion of rainbow trout (Oncorhynchus mykiss). Aquaculture International 17(2):143.

Webster, C. D., C. Webster, and C. Lim. 2002. Hybrid striped bass. Nutrient Requirements and Feeding of Finfish for Aquaculture:327-343.

Welker, T., F. Barrows, K. Overturf, G. Gaylord, and W. Sealey. 2016. Optimizing zinc supplementation levels of rainbow trout (Oncorhynchus mykiss) fed practical type fishmeal-and plant-based diets. Aquaculture Nutrition 22(1):91-108.

World Bank. 2017. Global Economic Monitor. Available: http://data.worldbank.org/data-catalog/global-economic-monitor (April, 2017).

Yamamoto, T., and coauthors. 2010. Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout Oncorhynchus mykiss. Aquaculture 309(1-4):173-180.