5-1-2013

ESTIMATION OF OPTIMAL PROTEIN TO ENERGY RATIO AND PERCENT SOYBEAN MEAL REPLACEMENT OF FISH MEAL IN JUVENILE SCAPHIRHYNCHUS STURGEON DIETS

Elliott C. Kittel
Southern Illinois University Carbondale, elliott.kittel@siu.edu

ESTIMATION OF OPTIMAL PROTEIN TO ENERGY RATIO AND PERCENT SOYBEAN MEAL REPLACEMENT OF FISH MEAL IN JUVENILE *SCAPHIRHYNCHUS* STURGEON DIETS

By

Elliott C. Kittel
B.S., University of Arkansas Pine Bluff, 2010

A Thesis
Submitted in Partial Fulfillment of the
Requirements for the
Masters of Science Degree

Department of Animal Science, Food and Nutrition
In the Graduate School
Southern Illinois University Carbondale
May 2013

THESIS APPROVAL

ESTIMATION OF OPTIMAL PROTEIN TO ENERGY RATIO AND PERCENT SOYBEAN MEAL REPLACEMENT OF FISH MEAL IN JUVENILE *SCAPHIRHYNCHUS* STURGEON DIETS

By

Elliott C. Kittel

A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of
Master of Science Degree
In the Department of
Animal Science, Food and Nutrition

Approved by:

Dr. Brian C. Small, chair

Dr. Gary Apgar

Dr. Jesse Trushenski

Graduate School
Southern Illinois University Carbondale
8 February 2013

AN ABSTRACT OF THE THESIS OF

Elliott C. Kittel, for the Masters of Science degree at Southern Illinois University Carbondale

TITLE: Estimation of optimal protein to energy ratio and percent soybean meal replacement of fish meal in juvenile *Scaphirhynchus* sturgeon diets.

MAJOR PROFESSOR: BRIAN C. SMALL

Research developing artificial propagation, husbandry, and nutrition of sturgeon species has developed greatly since the collapse of the Caspian Sea fisheries in the 1980s. Sturgeon species are commercially prized for their unfertilized roe which is marketed as the delicacy, caviar. Sturgeon production units commonly utilize commercial salmonid feeds, which contain large proportions of fish meal (FM) and fish oil (FO). Concerns regarding economics and sustainability have put pressure on aquafeed manufactures to efficiently utilize FM and FO, and to incorporate alternative protein sources, such as soybean meal (SBM). Therefore, the present studies estimated the optimal protein:energy ratio of juvenile Pallid Sturgeon *Scaphirhynchus albus* and evaluated the effects of increasing SBM composition on growth, feed efficiency, body and liver composition, and intestinal morphology of juvenile Shovelnose Sturgeon *Scaphirhynchus platorhynchus*, two species of *Scaphirhynchus* sturgeon of regional commercial importance.

Protein:energy ratios were investigated using casein and dextrose based, semi-purified diets. Results indicated that *Scaphirhynchus* sturgeon are able to perform similarly across a wide range of protein:energy ratios (79 – 147 mg protein $kcal^{-1}$), so long as adequate dietary energy (3,800 kcal kg^{-1} gross energy) is provided and essential amino acids are not limiting. Soybean meal was evaluated utilizing practical, isocaloric, isolipidic, isoenergetic test diets, designed to replace FM with increasing SBM. Juvenile *Scaphirhynchus* sturgeon were found to perform

similarly on diets with up to 50% of FM replaced with SBM (336 g kg^{-1} diet). Evaluation of liver composition revealed that juvenile Shovelnose Sturgeon store less glycogen and crude lipid than other fish species, although no dietary differences were observed. Histological evaluation of the distal intestine revealed indications of SBM induced enteritis, though no statistical differences in measures were detected between treatments. This research is the first to describe optimal dietary formulation for the culture of *Scaphirhynchus* sturgeon.

ACKNOWLEDGEMENTS

First, I would like to thank my graduate advisor, Dr. Brian Small, for giving me the opportunity to pursue my M.S. in fish physiology and nutrition at Southern Illinois University Carbondale. I greatly appreciate the personal attention and endless patience I have received and look forward to his continuing mentorship. I also extend my thanks to Dr. Gary Apgar and Dr. Jesse Trushenski, for serving on my graduate committee and providing additional commentary, guidance, and support. I also wish to express my appreciation to my lab-mates, Luke Nelson and Carlin Fenn, as well as our under-graduate technicians, Jentzi Schumm, Julie Schroeter, Cheyenne Adams, and Laura Archdale, for always being happy to assist. I also wish to express my appreciation to the entirety of the Fisheries and Illinois Aquaculture Center and the Department of Animal Science, Food and Nutrition, and I would particularly like to thank Mary Janello, Andy Coursey, Bill Hintz, Artur Rombenso, Dr. Rebecca Atkinson, and Dr. David Glover for their regular assistance and advice. I would also like to thank the Illinois Soybean Association for funding my research and education. Additionally, I would like to thank Dr. Wendy Sealey and the U.S. Fish and Wildlife Service Bozeman Fish Technology Center for collaboration, manufacturing of diets, and providing sturgeon. I would also like to thank my previous instructors from the University of Arkansas Pine Bluff, for continuing to offer their support and advice. I would like to express my appreciation to Arzanah Opon, for always supporting me, double checking my math and writing, and motivating me to do my best. I would also like to thank my family and church, for continually encouraging me. Finally, I wish to express my gratitude to my father, Carl Kittel, for setting me on the path of fisheries science and natural resource management, my mother, Ana Lisa Puro-Kittel, for always having time to listen, and my sisters Penelope and Desiree, for whom I hope to have set a positive precedent.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Sturgeon species are included in the family Acipenseridae and encompass the four genera *Acipenser*, *Huso*, *Scaphirhynchus* and *Pseudoscaphirhynchus*. Sturgeon are characterized by their unique physical appearance, which is defined by whisker-like barbels, exterior scutes, and a heterocercal caudal fin. Generally, commercially valuable sturgeon species are anadromous, long lived, and delay sexual development for up to a decade or longer (Conte et al. 1988, Doroshov et al. 1997). The commercial value of sturgeon lies mostly in their unfertilized roe, which is sold as the prized delicacy, caviar (Catarci 2004).

Because of its value, caviar is the major commercial product of sturgeon culture and capture fisheries. An official definition for caviar was adopted by the Codex Alimentarius Commission of the FAO and WHO, describing the product as fish eggs of the Acipenseridae family, treated with food-grade salt and removed from the connective tissue of ovaries (Bronzi et al. 2011). Prices for the most rare Beluga Sturgeon *Huso huso* caviar have reportedly retailed for 14,780 USD kg^{-1}, while prices of farmed sturgeon caviar have averaged 300 USD kg^{-1} in North America and approach 900 USD kg^{-1} in Europe (Bronzi et al. 2011). Sturgeon are also reared and harvested for meat (Wei et al. 2004, Moghim et al. 2006) and occasionally, swim bladder tissue (Catarci 2004). The dried swim bladder tissue of sturgeon is used to produce isinglass, which in turn is used to clarify wine and beer. Additionally, some Acipenseriformes are made available for sale in the ornamental trade (Catarci 2004).

Sturgeon culture is a relatively new practice, having developed greatly since the 1950s (Hung and Deng 1991, Logan et. al 1994, Wei et al. 2004, Moghim et al. 2006, Bronzi et al. 2011). These advancements can be tied to the collapse of the Caspian Sea capture fisheries in the

late 1980s, which prefaced marked declines in nearly every sturgeon species due to anthropogenic activities (Keenlyne 1997, Catarci 2004, Moghim et al. 2006, Seibert 2011). Continued pressure from impoundment, pollution, commercial fishing, and illegal poaching, has led to the near-extinction of many Russian, Chinese, and North American Acipenseriformes (Logan et al. 1995, Quist and Guy et al. 2002, Wei et al. 2004, Moghim et al. 2006, Tripp et al. 2009a, Bronzi et al. 2011). As a result, worldwide landings of Acipenseriformes declined from a high of 32,000 MT in 1977 to 5,000 MT in 1999 (Catarci 2004, Bronzi et al 2011).

Modern sturgeon aquaculture originated from nationally operated stock restoration programs proximal to the Caspian Sea. As worldwide populations declined, the national governments of Russia, the United States, Iran, and China, invested in the development of sturgeon production techniques for the purpose of wild stock restoration (Conte 1988, Catarci et al. 2004, Wei et al. 2004, Abdolhay and Tahori 2006, Moghim et al. 2006, Bronzi et al. 2011). Throughout the past 30 years, a multitude of sturgeon species and hybrids have been investigated for aquaculture production. Currently, the most well understood species include the White *Acipenser transmontanus* (Conte et al. 1988, Hung and Deng 2002, Garcia-Gallego et al. 2009), Siberian *Acipenser baerii* (Dabrowski et al. 1985, Medal et al. 1991, Hung and Deng 2002, Garcia-Gallego et al. 2009, Liu et al. 2009), Atlantic *Acipenser oxyrinchus* (Mohler 2003, King et al. 2004), and Persian Sturgeon *Acipenser persicus* (Moghim et al. 2006, Mohseni et al. 2007, Molla and Amirkolaie 2011). Sturgeon aquaculture production has grown considerably, from 328 MT in 1990, to nearly 20,000 MT in 2005, with 15,000 MT produced in China alone (Nierentz 2007). Although most sturgeon production has focused on the larger species belonging to the genera *Acipenser* and *Huso*, the *Scaphirhynchus* genera of sturgeon possess many unique attributes conducive to aquaculture.

The three members of the *Scaphirhynchus* genera, the Pallid *Scaphirhynchus albus*, Alabama *Scaphirhynchus suttkusi,* and Shovelnose Sturgeon *Scaphirhynchus platorynchus*, are endemic to the large river systems of North America (Keenlyne 1997, Tripp et al. 2009a). The largest member of the genus, the Pallid Sturgeon, is currently listed as an endangered species due to chronic declines in wild populations and limited recruitment (USFWS 1990). Similarly, the Alabama Sturgeon is listed as a critically endangered species due to continuing population decline (Mayden and Kuhajda 1996). The Shovelnose Sturgeon is the only member of the genera not directly protected and currently, wild Shovelnose Sturgeon are commercially harvested for caviar. However, new legislation has restricted harvest of Shovelnose Sturgeon in regions where it cohabitates with Pallid Sturgeon, due to similarity of appearance (USFWS 2010).

Shovelnose Sturgeon reach sexual maturity at a substantially smaller size and younger age than currently cultured sturgeon species (Conte et al. 1988, Kennedy et al. 2005, Tripp et al. 2009b). Of greatest importance, Shovelnose Sturgeon can produce substantial volumes of desirable, high quality caviar (Keenlyne 1997, Seibert et al. 2011). Tripp et al. (2009a) found that caviar from wild Shovelnose Sturgeon could fetch approximately 400 USD kg^{-1}. The population surveys of Kennedy et al. (2005) reported an average weight of gravid females of 1.52 kg and an average absolute fecundity of 26,720 eggs. Additionally, the data of Kennedy et al. (2005) indicated that up to 25% of the weight of gravid females was egg mass. In contrast to most anadromous sturgeons, the potamodromous Shovelnose Sturgeon can benefit from simplified rearing and breeding techniques while also presenting opportunity for inland culture. As one of the smallest sturgeon species, size of broodstock, rearing tanks, and capital investment would also be lower for Shovelnose Sturgeon. Tripp et al. (2009b) reported that that this species may have shorter inter-spawning intervals than other Acipenseriformes. Considering availability,

environmental preferences, size at sexual maturity, and rate of reproduction, the Shovelnose Sturgeon, among other sturgeon species, demonstrates great potential as an aquaculture species.

Unfortunately, information regarding husbandry, physiology, and nutrition of sturgeon, and specifically *Scaphirhynchus* sturgeon, is limiting. Lack of refined, species-specific diets is a foremost challenge in the culture of sturgeon (Moore et al. 1988, Liu et al. 2009). Culture operations commonly utilize readily available commercial salmonid diets for production of meat and caviar (Conte et al. 1988, Garcia-Gallego et al. 2009, Liu et al. 2009). These diets are protein and lipid dense, and incorporate large proportions of crude protein (CP) from fish meal (FM) and crude lipid (CL) from fish oil (FO). As FM and FO are increasingly valuable resources, one of the most urgent advancements needed in sturgeon culture is the development of cost effective, nutritionally complete aquafeeds.

Characterization of the optimal protein:energy ratio is the first step in developing economically and nutritionally efficient aquafeeds. This is particularly important in the development of juvenile culture diets, as fish consume the most diet during the grow-out phase of production. As the primary product of sturgeon production is caviar, this need is exacerbated due to delayed sexual maturity. Estimation of the optimal protein:energy ratio facilitates efficient inclusion of CP and energy, which in turn minimizes catabolism of protein, unwanted deposition of lipid, and feed cost while maintaining production efficiency (NRC 2011). Limited information is available regarding optimal protein:energy ratios in sturgeon species. Molla and Amirkolaie (2011) investigated the effects of differing protein:energy ratios in Persian Sturgeon fry, reporting that only dietary CP affected performance and suggested a minimum of 45% dietary CP and 3,900 kcal kg^{-1} dry matter. Hung and Deng (1991) and Mohseni et al. (2007) both recommended dietary CP inclusion of 40% in juvenile Siberian Sturgeon and sub-yearling

Persian Sturgeon, respectively. Additional nutritional studies have shown that CP requirements decline with life stage and that the protein and energy provided in commercial salmonid diets may be higher than required for juvenile and adult sturgeons.

Traditionally, FM is the primary protein source used in commercial aquafeeds (Naylor et al. 2009). Recent market volatility, a trend of increasing commodity prices, and growing concerns regarding sustainability, has placed pressure on aquaculture to increase the efficiency of FM usage and to incorporate alternative sources of protein (Trushenski et al 2006, Gatlin et al. 2007, Naylor et al. 2009). Though FM has many properties that make it an ideal protein source, including high digestibility, palatability, and a complete amino acid profile (NRC 2011), FM is not without drawbacks. Particularly, the dependence of FM production on marine capture fisheries is a foremost concern. The FAO (2010) reported that approximately 82% of marine fish stocks are considered fully exploited, over exploited, depleted, or are recovering from overexploitation. As a result, the cost of FM increased from approximately 600 USD MT^{-1} to nearly 1,700 USD MT^{-1} between august of 2003 and 2012 (FAO 2012). Furthermore, Naylor et al. (2009) reported that 68% of FM production goes to aquaculture, double the amount from the decade prior.

The next step in improving aquafeed efficiency is the incorporation of alternative protein sources in culture diets. Investigations evaluating protein digestibility have found that sturgeon are capable of efficiently utilizing a variety of proteins sources (Stuart and Hung 1989, Degani 2002, Liu et al. 2009). De-hulled, solvent extracted soybean meal (SBM), is an attractive plant-derived alternative protein, with potential to alleviate pressure on static FM resources (Trushenski et al 2006, Gatlin et al. 2007, Naylor et al. 2009). Soybean meal, which generally contains 48% CP, has been found to be highly digestible and utilizable in many fish species

(Gatlin 2002, Watanabe 2002, NRC 2011). These results were corroborated in sturgeon by Degani (2002) and Liu et al. (2009), who evaluated the digestibility of SBM in hybrid sturgeon *Acipenser gueldenstaedtii* × *Acipenser bester* and Siberian Sturgeon, respectively, and found SBM digestibility comparable to FM. In addition, SBM is currently much less expensive than FM. According to the FAO (2012), the average annual cost of SBM was 481 USD MT^{-1}, as of August, 2012, while, the average annual cost of FM was 1,462 USD MT^{-1}. As such, there is great value in assessing the capacity of *Scaphirhynchus* sturgeon to utilize SBM as a means of lowering the expense of production and increasing the sustainability of aquaculture.

Until recently, almost no information was available describing the capacity of sturgeon species to utilize SBM. New research has addressed various aspects of dietary SBM inclusion. In addition to the afore mentioned studies of Degani (2002) and Liu et al. (2009), Imanpoor et al. (2010a) compared blood serum indices of Persian Sturgeon fed either a FM control diet or a 40% SBM replacement of FM diet, and reported that a lack of available phosphorus limited growth in the SBM treatment. Imanpoor et al. (2010b) also evaluated the potential of added phytase to improve the 40% SBM formulation and found that that addition of phytase significantly improved growth. Khajepour and Hosseini (2010) found similar results when using citric acid supplementation to increase bioavailability of minerals in SBM diets fed to juvenile Beluga Sturgeon. Zhang et al. (2011) evaluated the effects of production setting, handling, and diets containing SBM or soy protein, on caviar yield and quality in White Sturgeon and found that differing dietary SBM inclusion did not influence caviar quality or production.

As there are no commercially available diets formulated specifically for the cost effective culture of sturgeon species and as no research has been conducted on the nutritional requirements of *Scaphirhynchus* sturgeon, the objectives of the present research are;

(1) To estimate the optimal dietary protein:energy ratio for maximum growth and condition in juvenile Pallid Sturgeon, as a model for *Scaphirhynchus* sturgeon culture.

(2) To assess the performance of juvenile Shovelnose Sturgeon fed diets containing increasing amounts of SBM, with amino acid supplementation, at the estimated optimal protein:energy ratio determined in Objective 1.

The dual purpose of the present research is to first, introduce the potential of the *Scaphirhynchus* sturgeon as candidate aquaculture species, and second, provide information that facilitates the design of nutritionally complete, cost effective diets for the grow out of juvenile sturgeon species. Furne et al. (2005), compared the enzymatic digestive capacities of Adriatic Sturgeon *Acipenser narccarii* and Rainbow Trout *Oncorhynchus mykiss* and reported that Adriatic Sturgeon were able to not only utilize protein and lipids as a carnivore, but also carbohydrates in a manner similar to an omnivore. Considering the findings of Stuart and Hung 1989, Degani 2002, Furne et al. 2005, and Liu et al. 2009, it is hypothesized that juvenile *Scaphirhynchus* sturgeon will perform more similarly to cultured omnivorous species, than highly carnivorous species, in regards to optimal protein:energy ratio and dietary SBM inclusion. This research is valuable, as limited information is available regarding the nutritional requirements of sturgeon species. Additionally, this information is of growing importance, as global production of Acipenseriformes has increased substantially throughout the past 30 years (Wei et al. 2004, Nierentz 2007).

CHAPTER 2

GROWTH PERFORMANCE AND BODY COMPOSITION OF JUVENILE PALLID STURGEON, *SCAPHIRHYNCHUS ALBUS*, FED DIETS CONTAINING DIFFERING PROTEIN:ENERGY RATIOS

INTRODUCTION

Research developing the artificial propagation, husbandry, and nutrition of sturgeon species has developed greatly since the 1950s (Conte et al. 1988, Celikkale et al. 2005). This increase in research activity can be correlated with the sharp worldwide decline of Acipenseriformes throughout 1980s, caused in part by habitat alteration and overharvest of wild stocks (Keenlyne 1997, Catarci 2004, Moghim et al. 2006, Seibert 2011). Throughout the northern hemisphere, sturgeon species are prized for their meat and unfertilized roe which is processed and marketed as the delicacy, caviar (Catarci 2004). Currently, many sturgeon species are cultured in captivity for both stock enhancement and commercial purposes (Conte et al. 1988, Fajfer et al. 1999, Ballestrazzi and Garavello 2003, Wei et al. 2004, Celikkale et al. 2005, Mohensi 2006).

The success of culture operations depends on optimized financial management and a developed understanding of the target organism's biology and specifically, nutritional requirements. Sturgeon culture operations commonly utilize readily available commercial salmonid diets for the husbandry of Acipenseriformes (Conte et al. 1988, Garcia-Gallego et al. 2009, Liu et al. 2009). Feeds formulated for commercial salmonid production often contain great proportions of crude protein (CP) from fish meal (FM) and crude lipid (CL) from fish oil (FO). Although both ingredients have been found to be highly digestible in sturgeon species (Degani

2002, Liu et al. 2009), concerns regarding sustainability and cost have arisen (Naylor et al. 2009). According to the FAO (2012), the cost of FM rose from approximately 650 USD MT^{-1} in July of 2002 to over 1,700 USD MT^{-1} ten years later. Naylor et al. (2009) reported that aquaculture now consumes 68% of FM production. Similarly, the FAO (2012) reported that aquaculture currently utilizes 85% of the world's annual FO supply and that the price of FO had increased from just under 1,000 USD MT^{-1} in May of 2010, to approximately 1,650 USD MT^{-1} in March of 2012.

Literature describing the biology and nutritional requirements of Acipenseriformes is incomplete. Singer and Ballantyne (2004) stated that the study of metabolic and hormonal regulatory systems in Acipenseriformes provides valuable information regarding vertebrate evolution and developing this understanding is increasingly imperative given the decline of wild populations. Fortunately, a sizeable body of research has been accumulated regarding the anatomy, physiology, and nutrition of sturgeon species (Dabrowski et al. 1987, Doroshov et al. 1997, Hung and Deng 2002, Singer and Ballantyne 2004, Garcia-Gallego et al. 2009). For example, essential amino acid requirements were investigated by evaluating whole body, specific tissue, and egg composition of White Sturgeon *Acipenser transmontanus* (Ng and Hung 1994, Ng and Hung 1995) and also via whole body analysis of Siberian Sturgeon *Acipenser baerii* Brandt (Kaushik et al. 1991). Additional research has focused on feedstuff digestibility (Kaushik et al. 1989, Stuart and Hung 1989, Medale et al. 1991, Herold et al. 1995, Liu et al. 2009), dietary CP requirements (Moore et al. 1988, Mohseni et al. 2007), digestive enzyme activities (Buddington and Doroshov 1986a, Buddington and Doroshov 1986b, Lin et al. 1997, Furne et al. 2005, Babaei et al. 2011), thermal regimes (Hung et al. 1989a, Mayfield and Cech Jr. 2004, Kappenman et al. 2009,), feeding rate and frequency (Hung et al. 1993, Cui et al. 1997, Deng et

al. 2003, Mohenseni et al. 2006), as well as the effects of culture densities (Fajfer et al. 1999, Celikkale et al. 2005). It is important to note, however, that these previous examples utilized many different sturgeon species, including White Sturgeon (Buddington and Doroshov 1986a, Buddington and Doroshov 1986b, Hung et al. 1989a, Hung et al. 1989b, Hung et al. 1993, Cui et al. 1997, Lin et al. 1997, Ballestrazzi and Garavello 2003, Deng et al. 2003), Siberian Sturgeon (Medale et al. 1991, Liu et al. 2009), Atlantic Sturgeon *Acipenser oxyrinchus* (Mohler et al. 1996, King et al. 2004), Adriatic Sturgeon *Acipenser* naccarii (Furne et al. 2005), Chinese Sturgeon *Acipenser sinensis* (Xiao et al. 1999), Russian Sturgeon *Acipenser gueldenstaedtii* (Celikkale et al. 2005), Persian Sturgeon *Acipenser persicus* (Mohseni et al. 2007, Babaei et al. 2011), Lake Sturgeon *Acipenser fulvescens* (Fajfer et al. 1999), Green Sturgeon *Acipenser medirostris* (Mayfield and Chech Jr. 2004), Beluga Sturgeon *Huso huso* (Mohseni et al. 2006), and Shovelnose Sturgeon *Scaphirhynchus platorynchus* (Kappenman et al. 2009). Despite the breadth of previous research objectives, limited information is available regarding optimal protein:energy ratios for the grow-out of juvenile sturgeon species. Additionally, no literature is available describing specific nutritional requirements of the uniquely freshwater *Scaphirhynchus* genus of sturgeon.

The *Scaphirhynchus* genus is comprised of the Pallid *Scaphirhynchus albus*, Alabama *Scaphirhynchus suttkusi*, and Shovelnose Sturgeon. These fish are endemic to the large, freshwater, river systems of North America (Keenlyn 1997, Tripp et al. 2009a). As with other sturgeon species, the Pallid and Shovelnose Sturgeon are prized for their meat and roe (Tripp et al. 2009b, Seibert et al. 2011). In addition to commercial harvest pressure, lack of recruitment due to impoundment and channelization, led to the listing of the Pallid Sturgeon as an endangered species in 1990 (USFWS 1990). Subsequently, in 2010, the Shovelnose Sturgeon

was granted limited protection as a threatened species under the Endangered Species Act of 1973, in regions where it cohabitates with the Pallid sturgeon (USFWS 2010). As a result, interest in sturgeon culture has developed to supplement and replace harvest of dwindling wild stocks protected by increasingly tight harvest restrictions. Estimation of optimal dietary protein:energy ratio, using the Pallid Sturgeon as a model species, will aid in the development of sustainable sturgeon culture diets, which minimize waste and maximize growth and condition. The present research sets the groundwork for developing juvenile *Scaphirhynchus* sturgeon diets by providing the first estimation of the optimal protein:energy ratio.

METHODS

Dietary Formulations

Twelve semi-purified diets were formulated using a four by three factorial design. Four CP levels (32%, 39%, 46%, and 53%) were assigned to three gross energy (GE) levels (3,600, 4,200, and 4,800 kcal kg^{-1}). Limited information was available regarding digestible energy of feedstuffs in juvenile sturgeon, therefore diets were assayed for GE which was used to calculate protein:energy ratios. As each CP increment was associated with each GE increment, the resulting protein:energy ratios overlapped between treatments (Table 2.1).

Feeds were produced by homogenizing ingredients in a cutter-mixer (Model CM450, Hobart Corp., Troy, OH), formed into pellets using a meat grinder (1.5 hp electric grinder, Cabela's, Sydney, NE), and then air dried at 100°C, to approximately 10% moisture using a commercial food dehydrator (Harvest Saver R-5A, Commercial Dehydrator Systems Inc., Eugene, OR). CP and GE levels, as well as dietary ingredients, were based upon previous studies conducted in other sturgeon species (Moore et al. 1988, Hung et al. 1990, Herold et al. 1995,

Mohler et al. 1996, Cui et al. 1997), as well as the commercial diets, Aquamax© fry starter 100 and 200 (PMI Nutrition International, LCC, Brentwood, MO), fed to the fish for the five months prior to the study. Amino acid profiles were maintained at or above the requirements reported by Ng and Hung (1995) for White Sturgeon by the addition of feed grade arginine, which was first limiting. All experimental diets were designed to incorporate an identical basal formulation of FM, blood meal, brewer's yeast, dehydrated fish solubles, crystallized arginine, betaine hydrochloride, vitamins, and minerals (Table 2.2). CP and GE content were varied by increasing or decreasing casein, wheat feed flour, FO, dextrose, and cellulose. The semi-purified diets included casein and dextrose to allow for the formulation of a wide range of CP and GE.

Growth Trial

An 18 week feeding trial was conducted using 180 juvenile Pallid Sturgeon with an average weight of 73.3 ± 1.2 g (mean ± SE). Fingerlings originated from wild parents spawned at Gavin's Point National Fish Hatchery, South Dakota. Prior to the initiation of the experiment, individuals were stocked into 36, 150 L aquaria and allowed to acclimate to the experimental environment for three months. The experimental system incorporated two sets of tanks. One set of 24 tanks was linked to large mechanical and biological filters and another set of 12 tanks was linked to smaller filters, and both systems were linked together. The system as a whole was treated as a randomized complete block design such that former block contained two replicates of all treatments and latter contained one replicate of all treatments.

Water was supplied by treating municipal water with sodium thiosulfate ($Na_2S_2O_3$), sodium bicarbonate ($NaHCO_3$), crushed limestone, and rock salt (NaCl), for dechlorination and maintenance of alkalinity and salinity, respectively. Dissolved oxygen (DO) and water temperature were checked daily using a YSI Model 550A Oxygen Meter (Yellow Springs, OH).

Water temperature was maintained at 21.7 ± 0.3°C throughout the experiment, following the suggestions of Kappenman et al. (2009), which estimated the optimum temperature for growth in the closely related Shovelnose Sturgeon at approximately 22°C**.** DO was maintained at 6.81 ± 0.04 mg L^{-1}. Total alkalinity, total unionized ammonia (NH_3), nitrite, and pH were monitored weekly using a LaMotte Smart3© Colorimeter (La Motte Co., Chestertown, MD) and a S20 SevenEasy pH meter (Mettler Toledo, Columbus, OH), and averaged 122 ppm, 0.57 ppm, 0.01 ppm, and 7.84 respectively. Salinity was maintained at approximately 1 ppt and was monitored using a salinity refractometer. All fish were maintained on a 12-h light:dark cycle.

At the start of the growth trial, all fish were captured and sedated with 100 mg L^{-1} tricaine methanesulfonate (MS-222; Western Chemicals Inc., Ferndale WA). Fish were weighed and fin clipped for individual identification, then five fish were randomly distributed to each aquaria. Dietary treatments were subsequently randomized in triplicate following the complete block design. Feeding to apparent satiation was a logistical difficulty and as such, feed was offered at 2% body weight day^{-1}, split into two equivalent feedings, following the recommendations of Hung et al. (1989a) and Mohseni et al. (2006).

Every three weeks, fish were sedated and sampled to adjust feed amounts. Fish were fasted for 24-h prior to each sampling. Performance was evaluated by assessing differences in total weight gain and proximate composition of fish carcasses between dietary treatments.

After the 18^{th} week, all fish in the experiment were euthanized, weighed, and stored at -20°C for proximate analysis. All fish sacrificed in the study were euthanized by overdose of MS-222 in a neutral pH buffered solution at a concentration above 250 mg L^{-1} and remained in the solution for 10-min after the cessation of opercular movement, as suggested by the American

Veterinary Medical Association (AVMA 2007). This experimental protocol was approved by the Institutional Animal Care and use Committee (IACUC) of Southern Illinois University, Carbondale (Protocol number 10-035).

Proximate Analysis

Diet and fish carcass composition were analyzed using the following standard methodology. Moisture was determined by weight lost upon lyophilization (Labconco Freezone 6, Labconco Corp., Kansas City, MO.). After dehydration, samples were ground prior to further analytical procedures. Percent CP was determined using a LECO model FP-528 nitrogen determinator (LECO Corp., St. Joseph, MI) following AOAC Official Method 992.15. Percent CL was determined using an ANKOM XT10 (ANKOM Technology, Macedon, NY), following AOCS official procedure AM 5-04. Percent ash was determined via combustion in a muffle furnace at 600°C following AOAC protocol number 942.05. GE was determined using a Parr 1425 Semimicro bomb calorimeter (Parr Instrument Company, Moline Il).

Statistical Analysis

Data were analyzed by two-way analysis of variance (ANOVA) for a randomized complete block. Assumptions for homogeneity of variance and normality of the data were tested by examination of correlation between absolute residuals and predicted values and the Shapiro-Wilkes test for normality. All data met the assumptions. If the ANOVA was significant, pair-wise contrasts using Fisher's LSD test were performed to identify significant differences between treatments at the alpha = 0.05 level. All statistical analyses were performed using SAS 9.2 software (SAS Institute Inc., Cary, NC)

RESULTS

Mean weight gain was significantly affected by the GE content of the dietary treatment (P<0.05; Figure 2.1). The most energetically dense diets, assayed to contain an average of 4,321 kcal kg^{-1}, yielded significantly larger fish than the least energetically dense diets, which contained 3,524 kcal kg^{-1}. The intermediate diets, 3,842 kcal kg^{-1}, were not significantly different from either of the other two. Mean weight gain was comparable between fish fed protein:energy ratios between 79 to 147 mg protein $kcal^{-1}$, so long as energy met or exceeded 3,842 kcal kg^{-1}. Growth performance was not significantly (P >0.05) affected by dietary CP or protein:energy interactions. The coefficient of variation of fish weight increased over time, from 22.3% at initiation to 56.5% at 18 weeks. Initially, fish ranged in weight from 44.5 g to 105.4 g. At the end of the study, fish ranged in weight from 35.2 g to 313.7 g. Survival was high (95%) with mortalities occurring randomly in all three GE levels.

The proximate composition of the fish carcasses was also affected (P <0.05) by GE content of the diet. Carcass CP was significantly higher in the lowest energy treatment, while the intermediate and high energy treatments were not different from one another (Table 2.3). Carcass moisture, CL, and ash were not significantly affected (P >0.05) by dietary CP, GE, or protein:energy interactions.

DISCUSSION

The results of this study emphasize the importance of dietary energy in formulating Pallid Sturgeon feeds. It is important to note that all diets were formulated to meet the estimated amino acid requirements reported by Ng and Hung (1995) for White Sturgeon. As such, it is not surprising that dietary CP content did not significantly affect weight gain or whole body composition of fish carcasses. Dietary protein:energy ratios have been evaluated for many fish

species (NRC 2011), with optimal values estimated particular to both taxon and life stage. Most fish species' optima range between 84 and 105 mg digestible protein $kcal^{-1}$ digestible energy (NRC 2011).

Using practical FM and FO formulations, Mohseni et al. (2007) estimated the CP requirement of 140 g juvenile Persian Sturgeon at 40% of the diet. Mohseni et al. (2007) reported an ideal protein:energy ratio between 75.2 and 83.6 mg protein $kcal^{-1}$. Kaushik et al. (1989) used practical ingredients to estimate the dietary CP requirement of 90 and 150 g juvenile Siberian Sturgeon between 36% and 42%, and predicted an optimal protein:energy ratio of 75.2 mg digestible protein $kcal^{-1}$. The study of Moore et al. (1988) used casein and dextrose based formulations to estimate the dietary CP requirement of 145 g juvenile White Sturgeon at 40.5%. Applying the standard energetic values of 4, 4, and 9 cal g^{-1} for protein, carbohydrate, and lipid, respectively, the protein:energy ratio of the optimal diet in Moore et al. (1988) was approximately 106 mg protein $kcal^{-1}$.

Data from the present study suggest Pallid Sturgeon grow similarly across a wide range of protein:energy ratios. Growth was not different between fish fed diets ranging from 79 to 147 mg protein $kcal^{-1}$ when energy approached or exceeded 3,842 kcal kg^{-1}. Other studies have found that some fish species perform equivalently across a wide range of protein:energy ratios. Bright et al. (2005) found that Largemouth Bass *Micropterus salmoides* utilized diets with protein:energy ratios between 86 and 137 mg protein $kcal^{-1}$ without relative detriment to growth. Also, Nematipour et al. (1992) reported that hybrid Striped Bass *Morone chrysops* ♀ × *M. saxatilis* ♂ grew equally well on protein:energy ratios ranging from 111.1 to 166 mg protein $kcal^{-1}$.

Dietary composition was diverse in the present study in order to formulate the wide range of protein:energy ratios. Even so, purified ingredients, e.g. casein and dextrose, were maintained at near constant levels within energy treatments across protein levels. Since dietary energy, and not protein, had significant effects on growth and body composition, neither casein or dextrose appears to have impacted performance. Utilization of these ingredients appears to differ between sturgeon species. Stuart and Hung (1989) found that White Sturgeon performed comparably when fed experimental diets formulated with casein and dextrose or a herring meal and dextrose control. Hung et al. (1989b) reported that White Sturgeon were capable of using simple monosaccharides and disaccharides, including dextrose, though other carbohydrates, e.g. lactose and fructose, were found to be poor sources of energy and significantly reduced lipid utilization. Furthermore, Kaushik et al. (1989) found that Siberian Sturgeon did not efficiently utilize complex carbohydrates. These studies, together with the present study, support the use of dextrose in experimental feeds for sturgeon. An exception is Xiao et al. (1999), who reported that 3.8 g Chinese Sturgeon performed relatively poorly on a casein and dextrose formulation. The small size of those fish further suggests life stage may be a factor.

Our findings suggest that the relatively high dietary CL levels of the 4,321 kcal kg^{-1} treatments (26.4 ± 0.7 %) are not necessarily optimal for efficient growth when considering cost and sustainability. Commercial salmonid diets often contain CL above 20%, which may be excessive in sturgeon feeds, as the middle energy treatments contained 16.2 ± 1.8 % CL and provided statistically comparable weight gain. It is important to note that this study was conducted under a restricted feeding regime of 2% body weight day^{-1}, split into two equivalent feedings. Though Einen and Roem (1997) recommended that studies evaluating protein:energy

ratios be conducted only with fish fed to satiation, the described methodology was chosen due to the slow, benthic browsing patterns typical of sturgeon species.

The Pallid Sturgeon used in this study demonstrated a high level of variability, with the coefficient of variation for fish weight increasing from 22.3% at initiation to 56.5% at 18 weeks. Monaco et al. (1981) reported that coefficients of variation for total length of White Sturgeon fed artificial diets increased from 29% at five months post hatch, to over 80% at ten months post hatch. A dominance hierarchy appeared to develop within the tanks during the present 18 week feeding trial as size variability increased. As fish grew, the largest fish demonstrated aggressive behavior and appeared to out-compete smaller fish for space and feed. High variability in growth was confounding in the present study and may be related to the interactions of dominance, genetics, and culture stress. In general, juvenile Pallid Sturgeon appear to be able to utilize a wide range of protein:energy ratios, as long as amino acids are not limiting and the diet contains GE approaching or above 3,842 kcal kg^{-1}. Additional research evaluating nutrient requirements and ingredient utilization, as well as a better understanding of sturgeon feeding behavior and husbandry, will help lead to a more complete understanding of sturgeon culture requirements.

CHAPTER 3

GROWTH PERFORMANCE, BODY COMPOSITION, AND INTESTINAL HISTOLOGY OF SHOVELNOSE STURGEON, *SCAPHIRHYNCHUS PLATORYNCHUS*, FED PRACTICAL DIETS CONTAINING INCREASING INCLUSION OF SOYBEAN MEAL

INTRODUCTION

The Shovelnose Sturgeon *Scaphirhynchus platorynchus* is a new candidate species for aquaculture production. Currently, many sturgeon species are cultured for meat and roe, the latter of which is processed and sold as the delicacy, caviar (Catarci 2004, Wei et al. 2004, Celikkale et al. 2005, Mohensi 2006, Liu et al. 2009). The *Scaphirhynchus* genera of sturgeon have several attributes that uniquely benefit aquaculture production. The three members of the genera, the Pallid *Scaphirhynchus albus*, Alabama *Scaphirhynchus suttkusi*, and Shovelnose Sturgeon, naturally complete their entire lifecycles in freshwater, a trait unique among currently cultured species (Keenlyne 1997). The Shovelnose Sturgeon reaches sexual maturity at a substantially smaller size and younger age than currently cultured sturgeon species (Conte et al. 1988, Doroshov et al. 1997). Most importantly, the Shovelnose Sturgeon has the capacity to produce high quality caviar (Keenlyne 1997, Seibert et al. 2011). Kennedy et al. (2005) reported that wild female Shovelnose Sturgeon reach maturity between ages seven and nine years and are thought to spawn every two to three years. Kennedy et al. (2005) also reported a mean weight of wild female Shovelnose Sturgeon of approximately 1.52 kg and a mean absolute fecundity of 26,720 eggs. Additionally, the data of Kennedy et al. (2005) indicated that up to 25% of the weight of wild gravid females was egg mass.

Sturgeon species are commonly cultured using readily available commercial salmonid diets (Conte et al. 1988, Celikkale et al. 2005, Garcia-Gallego et al. 2009, Liu et al. 2009). These feeds often contain high proportions of crude protein (CP) from fish meal (FM) and crude lipid (CL) from fish oil (FO). Reliance on marine capture fisheries for aquafeeds has resulted in increased scrutiny over the past 60 years regarding the sustainability of aquaculture. Currently, 82% of marine fish stocks are considered fully exploited, over exploited, depleted, or are recovering from overexploitation (FAO 2010). Current market volatility and rapid increases in FM and FO commodity prices through the last decade have made consistent aquafeed manufacturing difficult for commercial enterprises (Naylor et al. 2009). Furthermore, pressure from environmentally conscious consumers to improve the fish in:fish out ratio and waste management practices of aquaculture, has led aquafeed manufactures to seek alternative sources of CP and CL (Naylor et al. 2009).

As feed is often one the greatest operating costs of aquaculture operations, exploring the potential utilization of plant meals in sturgeon feeds provides both economic and environmental benefits. Many plant derived protein sources have been investigated for the effective large-scale reduction of FM in aquafeeds. In order for an ingredient to effectually reduce dependence on FM, it must contain high amounts of digestible protein, possess a compatible amino acid profile, retain the capacity to remain an economically and environmentally viable alternative, and have potential for increased production to meet growing demand (Gatlin et al. 2007, Naylor et al. 2009). De-hulled solvent extracted soybean meal (SBM), which contains 48% CP, has been found to be an effective source of dietary protein for use in aquafeeds. Previous research has established that SBM possesses a compatible amino acid profile and is highly digestible in many fish species (Gatlin 2002, Watanabe 2002, Gatlin et al. 2007). Additionally, SBM is currently

much less expensive than FM and as the most cultured oil seed crop worldwide, has far more opportunity for production growth than the marine capture fisheries that supply FM (Gatlin et al. 2007). According to the FAO (2012), the average annual cost of SBM was 481 USD MT^{-1}, as of August, 2012. In comparison, the annual average cost of FM was 1,462 USD MT^{-1}.

The goal of the present study was to investigate the potential of Shovelnose Sturgeon to utilize increasing amounts of SBM in a practical culture diet. Such information is valuable as very little research has been conducted evaluating the performance of sturgeon fed diets with increasing SBM composition. In addition, no such studies have been previously conducted on the relatively small, albeit commercially important, freshwater genus of *Scaphirhynchus* sturgeon.

METHODS

Dietary Formulations

Four isonitrogenous, isocaloric, and isolipidic diets were formulated utilizing practical feed ingredients, while increasing percentages of FM with SBM (Table 3.1). A high FM diet containing no SBM (100% FM) was used as a control and three subsequent diets were formulated to replace FM with SBM at 25% (75% FM), 50% (50% FM), and 75% (25% FM) FM with SBM. Feeds were produced at the USFWS Bozeman Fish Technology Center, MT, and analyzed for proximate composition at Southern Illinois University Carbondale (SIUC). Prior to mixing, all ingredients were ground using an air-swept pulverizer (Jacobsen 18H, Minneapolis Minnesota, USA) for 100% pass through a screen with 250 µm openings. Dry ingredients were mixed in a horizontal paddle mixer (Marion Mixers, Marion, Iowa) where approximately 30% w/w moisture was added to the mix. All diets were formed using a water-cooled extrusion system (Italgi Model No. P35A**,** Carasco, Italy). Particle sizes of 1.5 mm diameter were

produced and dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) with air discharge temperature remaining below 102^{o}C to obtain a final moisture content of less than 8%.

Ingredient selection, CP, CL, and gross energy (GE) inclusion were extrapolated from a previous study evaluating protein:energy ratios in the closely related Pallid Sturgeon (Chapter 2). All experimental diets were designed to incorporate an identical basal formulation of poultry by-product meal, brewer's yeast, fish solubles, betaine hydrochloride, mineral premix, and vitamin premix. Wheat gluten meal and wheat starch were fluctuated to maintain CP, CL, and GE levels. Amino acid supplementation was provided by adding feed grade threonine and lysine, in order to maintain the amino acid profiles at or above the requirements reported for White Sturgeon (Ng and Hung 1995).

Growth Trial

An eight week feeding trial was conducted using 204 juvenile Shovelnose Sturgeon with a mean weight of 174.5 ± 4.8 (Mean ± SE) grams. Larvae were provided by the USFWS Bozeman Fish Technology Center, MT, and reared at SIUC for one year. Prior to initiation, fish were stocked, 17 fish per tank, into a recirculating aquaculture system comprised of 12, 1.8 m diameter tanks, containing a volume of 1100 L. Water was supplied by treating municipal water with sodium thiosulfate ($Na_2S_2O_3$), sodium bicarbonate ($NaHCO_3$), calcium chloride ($CaCl_2$), and crystal salt (NaCl) for dechlorination and maintenance of alkalinity, calcium hardness, and salinity, respectively. Dissolved oxygen (DO) and water temperature were checked daily using a YSI Model 550A Oxygen Meter (Yellow Springs, OH). DO was maintained above 7.0 mg L^{-1} throughout the study. Water temperature was maintained at 21.7 ± 0.1°C throughout the experiment, following the suggestions of Kappenman et al. (2009), which reported an optimum temperature for growth in juvenile Shovelnose Sturgeon at approximately 22°C. Calcium

hardness was maintained above 100 ppm and was monitored using a LaMotte Calcium Hardness Fresh and Salt Water Test Kit 3609 (LaMotte Co., Chestertown, MD). Total alkalinity, total unionized ammonia (NH_3), nitrite, and total hardness were monitored weekly, using a LaMotte Smart3© Colorimeter (La Motte Co., Chestertown, MD) and averaged 86.8 ± 5.7 ppm, 0.26 ± 0.09 ppm, 0.07 ± 0.0 ppm, 194.2 ± 7.5 ppm, respectively. Salinity was maintained at approximately 1 ppt and was monitored using a salinity refractometer. System pH was measured using a S20 Seveneasy pH meter (Mettler-Toledo, Columbus, OH) and averaged 8.04 ± 0.03. All fish were maintained on a 12-h light:dark cycle.

The FM control diet was offered to the all fish during a three week acclimation period. Upon initiation all fish were captured, sedated with 100 mg L^{-1} tricaine methanesulfonate (MS-222; Western Chemical Inc., Ferndale, WA.) in a pH buffered solution, their weights recorded, and experimental treatments randomly assigned, three tanks per dietary treatment. Experimental feed were offered in excess three times daily on weekdays and twice daily on weekends. After 1-h, uneaten feed was removed by siphoning, collected, dried, and quantified. Performance was evaluated by determining weight gain, cumulative feed intake, feed efficiency (FE = (total weight gain/feed intake × 100), specific growth rate (SGR = (ln $weight_{(final)}$ – ln $weight_{(initial)}$)/days × 100), proximate composition of fish carcasses, hepatosomatic index (HSI = (liver weight/total body weight × 100), liver moisture, CL, and glycogen content, and histology of the distal intestine.

After eight weeks of feeding, all fish were fasted for 24-h, captured, sedated with MS-222 and weighed. Nine fish per tank were euthanized by overdose of MS-222 in a neutral pH buffered solution at a concentration above 250 mg L^{-1} and remained in the solution for 10-min after the cessation of opercular movement, as suggested by the American Veterinary Medical

Association (AVMA 2007). Of the nine fish euthanized, five were randomly selected for whole body proximate analysis and stored at -20°C. The other four fish were used for determination of liver indices and intestinal histology. Whole livers were excised and weighed for calculation of HSI. Livers were then wrapped in aluminum foil, flash frozen in liquid nitrogen, and stored at -80°C for later determination of moisture, CL, and glycogen content. Whole intestinal tracts were excised, rinsed, the distal sections trimmed, and stored directly in 10% neutral buffered formalin. This experimental protocol was approved by the Institutional Animal Care and use Committee of SIUC (Protocol number 10-037).

Analysis of Diets and Fish Tissues

Whole-Body and Diet Proximate Composition

The five fish sacrificed for whole-body proximate analysis were combined into one homogenous sample per tank. Frozen whole fish were cut into two inch cubes and ground wet using a Grindomix GM 300 knife mill (Retsch, Haan, Germany). Fish carcass and diet proximate composition were analyzed following standard methods. Moisture was determined via weight lost upon lyophilization, using a Labconco Freezone 6 (Labconco Corp., Kansas City, MO). After dehydration, samples were pulverized prior to further analytical procedures. CP content was determined using a LECO model FP-528 nitrogen determinator (LECO Corp., St. Joseph, MI) and a CE Elantec model 1112 combustion element analyzer (CE Elantec Inc., Lakewood, NJ) following AOAC Official Method 992.15. CL content was determined using an ANKOM XT10, following AOCS official procedure AM 5-04 (ANKOM Technology, Macedon, NY). Ash content was determined via combustion in a muffle furnace at 600ºC following AOAC protocol number 942.05.

Liver Glycogen and CL content

Livers were assayed for glycogen following the methodology of Murat and Serfaty (1974). Glucose liberated was measured using a FLUOstar Omega microplate reader (BMG Labtech, Ortenberg, Germany). Liver sections were homogenized in 2 ml tubes, containing 0.1M ice-cold citrate buffer using a Mixer Mill MM 400 (Retsch, Haan, Germany). Immediately after homogenization, free glucose content was determined following Pointe Scientific Inc. glucose oxidase methodology (Catalog no. G7521; Pointe Scientific Inc., Canton, MI). Amyloglucosidase (exo-1,4-∝-glucosidase EC# 10115-1G-F**;** Sigma-Aldrich, St. Louis, MO) was then added to the homogenate at 1 mg ml^{-1} citrate buffer. The solution was then incubated at room temperature for 24-h before final determination of total glucose. Liver CL was determined following the methodology previously described for whole body CL analysis.

Histology

Histological slides were prepared using standard methodology. After fixation in 10% neutral buffered formalin for 24-h at room temperature, samples were dehydrated, cleared, embedded in paraffin, and serially sectioned at a five µm thickness using a rotary microtome. The resulting transverse sections were mounted onto glass slides and stained with hematoxylin and eosin (H&E) to observe the effects of dietary composition on morphology of the distal intestine. Stained sections were cover-slipped and photographed using a Leica DM750 combination microscope and camera (Leica Microsystems Inc., Buffalo Grove, IL). Photographs were taken at 400X magnification and two images per fish were blindly evaluated following the semi-quantitative scoring system of Uran et al. (2008b; Table 3.2). Mucosal folds (MF), sub-epithelial mucosa (SM), and supranuclear vacuoles (SNV) were semi-quantitatively scored on a

scale of one to five, with lower scores indicating superior tissue condition, and higher scores suggesting the development of intestinal enteritis. Goblet cells (GC) and eosinophilic granulocytes (EG) were individually identified and counted and the width of the lamina propria (LP) was measured using NIS Elements imaging software (Table 3.3, Nikon Instruments Inc., Melville, NY).

Statistical Analysis

Growth parameters, as well as diet and body composition, were analyzed by one-way analysis of variance (ANOVA). Assumptions for homogeneity of variance and normality of the data were tested by examination of correlation between absolute residuals and predicted values and the Shapiro-Wilkes test for normality. All data met the assumptions. If the ANOVA was significant, pair-wise contrasts using Fisher's LSD test were performed to identify significant differences between treatments at the alpha = 0.05 level. Histology was scored by ranking aspects of intestinal morphology as previously described. Histological scores were treated as nonparametric data and were analyzed using the Kruskal-Wallis one-way analysis of variance at the alpha = 0.05 level. All statistical analyses were conducted using SAS 9.2 software (SAS Institute Inc., Cary, NC).

RESULTS

No mortalities were recorded during the present trial. Weight gain, FE, and SGR were significantly affected by dietary treatment ($P \leq 0.05$; Table 3.4). Feed intake, HSI, and both carcass and liver composition, were not significantly affected by dietary treatment ($P > 0.05$; Table 2). Fish fed diets 100% FM, 75% FM, and 50% FM, which were formulated to replaced 0%, 25%, and 50% of FM with SBM, gained significantly more weight and had superior FE and

SGRs than fish fed diet 25% FM. Fish fed diet 25% FM did not gain any weight, had a FE of -5.8%, and a SGR of -0.03% day^{-1}. In contrast, fish fed diet 100% FM gained an average of 20.7 g, had a FE of 29.3%, and a SGR of 0.19% day^{-1}. The coefficient of variance for fish weight increased in every treatment over time. At the start of the experiment, the coefficient of variance was 39.5% and increased to an average of 52.7% across all treatments after eight weeks.

Histological evaluation of the distal intestine revealed characteristic symptoms of SBM-induced intestinal enteritis, including disruption of the MFs, widening of the LP, reduced numbers of SNVs, and increased abundance of GCs and EGs (Figure 3.1). However, due to variation between individual fish, statistical evaluation detected no significant differences between dietary treatments.

DISCUSSION

Evaluation of weight gain, FE, and SGR, suggests that Shovelnose Sturgeon are able to effectively utilize diets with up to 50% of FM replaced with SBM. Weight gain, FE, and SGR were negatively impacted when 75% of FM was replaced with SBM. The FE of diets 100% FM, 75% FM, and 50% FM, are comparable to the values reported by Kappenman et al. (2009), in which 22.9 g Shovelnose Sturgeon, which were subjected to various thermal regimes for 87 days, achieved a mean FE of 15% and a maximum FE of 24.5%. However, the maximum SGR achieved in the study of Kappenman et al. (2009) was 1.09 % day^{-1}, higher than the values observed in the present study. It is possible differences in fish age, initial size, and study duration, could account for differences between the SGRs observed.

The relatively modest weight gains in the present study may be related to the advanced life stage of the fish utilized. Shovelnose Sturgeon reach adulthood at a substantially smaller size

than other sturgeons (Conte et al. 1988 Keenlyne 1997, Doroshov et al. 1997). In cultured fishes, growth rate declines with size and age. Hung et al. (1995) reported that during an eight week feeding trial, yearling White Sturgeon ranging in weight from 581 to 928 g, gained between 35.1 and 59.2% body weight on average, when fed at least 0.9% body weight day^{-1}. In comparison, the eight week trials of Stuart and Hung (1989) and Hung et al. (1993), which utilized sub-yearling White Sturgeon with a mean weight below 30 g, reported that fish gained 222% and 375% body weight, respectively. There are few direct comparisons between *Scaphirhynchus* and *Acipenser* sturgeon in culture related literature. Kennedy et al. (2005) evaluated wild populations of Shovelnose Sturgeon and reported a mean weight of 1.5 kg, in reproductive females. In comparison, Doroshov and Lutes (1984) stated that the size of mature wild female White Sturgeon ranged from 14 to 52 kg. The dramatic differences reported in weight gain between age-0 and sub-adult White Sturgeon (Stuart and Hung 1989, Hung et al. 1993, Hung et al. 1995) is suggestive of the differences observed between the age-0 Shovelnose Sturgeon in the Kappenman et al. (2009) study and the sub-adult Shovelnose Sturgeon in the present study. It is unlikely that feed restriction limited growth, as excess was observed and removed after every feeding in each treatment.

Under the present conditions, juvenile Shovelnose Sturgeon were found to have body composition consistent with juvenile White (Stuart and Hung 1989, Fynn-Aikins et al. 1992) and Atlantic Sturgeon (King 2004). Shovelnose Sturgeon were observed to have less glycogen and CL in the liver compared to reports for White sturgeon. The study of Fynn-Aikins (1992) reported liver glycogen between 20 and 60 mg g^{-1} and CL between 296 and 372 mg g^{-1}. The present Shovelnose Sturgeon were found to store between 9 and 22 mg g^{-1} glycogen and 30 to 60 mg g^{-1} CL. Liver glycogen in the range of 20 to 90 mg g^{-1} have been reported for other fish

species including Striped Bass *Morone saxatilis* (Small and Soares 1999), Gilthead Seabream *Sparus aurata* (Venou et al. 2008), and Cobia *Rachycentron canadum* (Webb et al. 2010).

SBM has been found to be an effective protein source for many cultured fishes. The review of Gatlin (2002) reported that Chinese and Indian carp species, *Ictalurid* and *Clarius* catfish, and several Tilapia species, are able to utilize SBM effectively in intensive culture feeds. Viola et al. (1982) reported that, in comparison to a FM reference diet, SBM could be utilized as the sole protein source in diets of Common Carp *Cyprinus carpio* while providing equivalent performance so long as methionine, lysine, and CL were supplemented. Additionally, El-Saidy and Gaber (2002) reported that using SBM, FM could be completely removed from the diets of fingerling Nile Tilapia *Oreochromis niloticus*, so long as dietary lysine was supplemented. Although SBM can be limiting in regards to the essential amino acids lysine, methionine, and threonine, these deficiencies can be addressed with the strategic usage of other protein sources and crystallized amino acids (Gatlin et al. 2007).

Though SBM has demonstrated great promise, disadvantages have been documented in some fish species, including high carbohydrate composition and low mineral availability (Gatlin et al 2007). Non-starch polysaccharides, which comprise approximately 20% of a raw soybean, have been identified as antinutritional factors which, along with antigenic proteins, protease inhibiters, and lectins, limit digestibility and negatively affect intestinal morphology, particularly in highly carnivorous fishes (Gatlin et al. 2007). Studies in Atlantic Salmon *Salmo salar* have associated transient intestinal enteritis with dietary SBM inclusion (Baeverfjord and Krogdahl 1996, Uran et al. 2008b). The study of Knudsen et al. (2007) identified naturally occurring saponins, found in SBM, as a cause of intestinal enteritis. Further studies in 35 g Common Carp found that when offered a diet with 20% of protein replaced with SBM, enteritis developed and

subsequently regressed over the span of three weeks as the animals physiologically adapted (Uran et al. 2008a).

Histological evaluation demonstrated symptoms associated with intestinal enteritis, as described by Baeverfjord and Krogdahl (1996), Uran et al. (2008b), and Raskovic et al. (2011). In particular, increased GC presence was noted in treatments fed SBM diets. Pronounced widening of the LP, presence of EG, shortening and disruption of the MF, and loss of SNV were noted in every SBM treatment. The fish fed diet 25% FM were found to have highly variable intestinal morphology, possibly due to reduced feed intake. Fish fed diet 25% FM lost weight on average, indicating tissue wasting and influencing scoring and statistical analysis. Differences in individual feeding behavior and physiological tolerance of SBM may have impacted statistical analysis and no significant differences were found between dietary treatments. The review of Merrifield et al. (2011), describes several species that do not develop intestinal enteritis when fed SBM containing diets, including Atlantic Cod *Gadus morhua*, Atlantic Halibut *Hippoglossus hippoglossus*, Cobia, Egyptian Sole *Solea aegyptiaca*, European Sea Bass *Dicentrarchus labrax*, and Gilthead Seabream.

Until recently, almost no information was available regarding the capacity of sturgeon species to utilize SBM. Furne et al. (2005), compared the enzymatic digestive capacity of Rainbow Trout *Oncorhynchus mykiss* and Adriatic Sturgeon *Acipenser naccarii* and found that the sturgeon were able to digest protein and lipids like a carnivore and carbohydrates like an omnivore. Degani (2002) evaluated the apparent digestibility of SBM using an experimental diet containing 595 g kg^{-1} SBM in 250 to 400 g hybrid sturgeon *Acipenser guldenstadtii* × *A. bester* and reported an apparent digestibility coefficient of 81.9%. Similarly, Liu et al. (2009) reported the apparent digestibility coefficient of SBM at 76.9% in 8.4 g Siberian Sturgeon *Acipenser*

baerii, when fed an experimental diet containing 435 g kg^{-1} SBM. Liu et al. (2009) also found that protein and amino acid digestibility was comparable to FM. However, Imanpoor et al. (2010a) compared blood serum indices of Persian Sturgeon *Acipenser persicus* fed either a FM control diet or a 40% SBM replacement diet and reported that a lack of available phosphorus limited growth in the SBM treatment. Imanpoor et al. (2010b) subsequently evaluated the potential of added phytase to improve the 40% SBM formulation and found that the addition of phytase significantly improved growth. Similarly, Khajepour and Hosseini (2010) reported that citric acid supplementation increased bioavailability of minerals in SBM diets fed to juvenile Beluga Sturgeon *Huso huso*. Zhang et al. (2011) evaluated the effects of production setting, handling, and diets containing SBM or soy protein, on caviar yield and quality in White Sturgeon and found that differing dietary SBM inclusion did not influence caviar quality or production.

The present results are supported by the findings of Degani (2002), Liu et al. (2009), and Zhang et al. (2011), as 174.5 g Shovelnose Sturgeon appear able to utilize diets containing approximately 336 g kg^{-1} SBM without statistically significant detriment, when compared to the FM control diet. As a model for freshwater *Scaphirhynchus* sturgeon, the Shovelnose Sturgeon used in the present study appeared to utilize SBM diets in a comparable fashion to *Acipenser* sturgeon. Further study is recommended to evaluate SBM replacement of FM between 40 and 60%, to evaluate the potential for Shovelnose Sturgeon to utilize refined soy proteins, and to assess potential SBM utilization at both earlier and later life stages.

CHAPTER 4

DISCUSSION

Objective 1 highlighted the importance of dietary energy in formulating feeds for juvenile *Scaphirhynchus* sturgeon. Once a minimum gross energy (GE) inclusion (3,800 kcal kg^{-1}) was met, all subsequent crude protein (CP) inclusions (34 - 57%) resulted in statistically equivalent weight gain. GE was used, as information regarding digestible energy (DE) of feedstuffs in sturgeon was incomplete. Dietary CP and protein:energy interactions were not found to impact growth performance. During the 18 week feeding trial, juvenile Pallid Sturgeon performed equivalently on diets containing protein:energy ratios between 79 and 147 mg protein $kcal^{-1}$. As such, juvenile Pallid Sturgeon were observed to effectively utilize a wide range of protein:energy ratios without relative detriment, so long as essential amino acid and energetic requirements were met.

The GE levels evaluated in Objective 1 were based upon common inclusions found in salmonid and omnivorous fish feeds. These inclusions were similar to levels used at the University of California Davis during early investigations of nutritional requirements of White Sturgeon (Garcia-Gallego et al. 2009). According to the NRC (2011), most studies evaluating digestible protein (DP):DE ratios have reported optima between 84 and 105 mg protein $kcal^{-1}$. Mohseni et al. (2007), estimated the optimal protein:energy ratio of larval Persian Sturgeon between 75 and 85 mg protein $kcal^{-1}$, values similar to the lower end of what was observed in Objective 1.

Growth in Objective 1 was lower than anticipated. No previous studies have been conducted evaluating nutritional requirements of juvenile *Scaphirhynchus* sturgeon. Early studies assessing the husbandry and nutritional requirements of *Acipenser* sturgeon often utilized flow-

through culture systems with direct access to large water bodies. Objective 1 was conducted in a recirculating system comprised of 36, 150 L aquaria, which lacked horizontally flowing water and a large footprint. The Pallid Sturgeon utilized were spawned from wild parents with no previous selection for the culture environment. It is possible that variability between fish in initial weight, genetics, tolerance of environmental and handling stressors, and social hierarchy, affected performance.

Objective 2 characterized the potential of Shovelnose Sturgeon to effectively utilize diets with high SBM composition (336 g kg^{-1}). Isocaloric, isoenergetic, isolipidic diets (46% CP, 15% CL, 9% ash) formulated to replace 25% and 50% of fishmeal (FM) with SBM performed equivalently to the 100% FM control. After eight weeks of feeding, fish composition was unaffected by dietary treatment. Hepatosomatic index (HSI), liver composition, and histological scoring of the distal intestine were also similar among dietary treatments. However, fish in SBM treatments displayed morphological characteristics consistent with the development of SBM induced enteritis. Again, weight gain and growth rates were lower than expected. The Shovelnose Sturgeon utilized were also spawned from wild parents and lacked domestication. It is possible that the larger initial starting weight of the fish, genetics, environmental stressors, and social hierarchy, affected performance. A longer duration of study may have elicited more pronounced changes in body and liver composition, and intestinal morphology.

The results of Objective 1 and 2 reflect the variability and plasticity of juvenile *Scaphirhynchus* sturgeon. A large amount of variability in growth was documented between fish within the same dietary treatments. In Objectives 1 and 2, the coefficient of variance of weight increased from 22.3 to 56.5% and from 39.5 to 52.7%, respectively. A proportion of the observed variability may be attributable to behavior and social hierarchy. In both experiments,

some individuals adjusted to different feeds and grew more quickly than others. Subsequently, these fish were observed to out-compete smaller fish for room and feed, occasionally demonstrating aggressive behavior, and appeared to recover more quickly from hatchery stressors, such as sedation and sampling. It was for the latter reason that Objective 2 was conducted without intermediate samplings.

Information detailing ideal sturgeon husbandry parameters is limited and much was learned during the course of these studies. *Acipenser* culture operations historically have utilized flow through culture systems with direct access to large water bodies and thus, information describing ammonia, nitrate, and nitrite tolerance is limited. A study conducted by Nelson and Small (unpublished) described no cortisol or blood glucose response in Pallid Sturgeon exposed to 0.60 ppm un-ionized ammonia (NH_3) for 24-h, suggesting an inherent biological tolerance to elevated ammonia concentrations. Description of minimum dissolved oxygen (DO) levels (USGS 2011) and optimal pH, alkalinity, and hardness in the culture settings are incomplete. Most literature describes maintenance of DO above 5 mg L^{-1}, pH between 7.4 and 8.2, and alkalinity between 100 and 200 ppm (Conte 1988, Fynne-Aikins et al. 1992, Hung et al. 1993). Nelson and Small (unpublished) observed a marked cortisol stress response in juvenile Pallid Sturgeon exposed to DO below 2 mg L^{-1} for 30-min, and did not observe mortalities. No detailed information regarding optimal water hardness is available. Additionally, no information is available describing salinity tolerance in *Scaphirhynchus* species under culture conditions. Pallid Sturgeon have been documented in brackish-water environments in the wild (USGS 2011), but little is known about their ability to adapt rapidly or perform during long-term exposure to elevated salinity.

It is well established that post-larval *Acipenser* sturgeon can tolerate temperatures ranging from above freezing to 25°C, though specific ranges are particular to each species (USGS 2011). *Scaphirhynchus* sturgeon have a thermal optima similar to cultured White Sturgeon. The study of Kappenman et al. (2009), determined that Shovelnose Sturgeon reached optimum growth performance at 22°C while under an unrestricted feeding regime. Hung et al. (1993) estimated the optimum temperature for performance in White Sturgeon at 23°C. In contrast, Mayfield and Cech Jr. (2004) reported an optimal temperature for performance in Green Sturgeon between 15°C and 19°C and the USGS (2011) stated that most *Acipenser* thermal optima are below 25°C, with mortality occurring above 30°C.

It appears that *Scaphirhynchus* sturgeon are particularly sensitive to low DO and rapid changes in water quality parameters. Relatively large changes in temperature, alkalinity, and pH outside of study periods qualitatively impacted performance and mortality. Such observations were noted during husbandry, when large water exchanges were conducted during back-flushing of mechanical filters, siphoning to remove settled solids, or when replacing evaporative loss. This was exacerbated in culture systems with relatively small volumes. All culture systems were subject to ambient building temperatures, which sometimes resulted in fluctuations of over 5°C within a day. This was a cause of concern, as sturgeon naturally inhabit large water bodies which permit thermal regulation by locomotion. From a qualitative perspective, smaller fish appeared more sensitive to fluctuating parameters than larger fish and Pallid Sturgeon appeared more sensitive than Shovelnose Sturgeon. Based on personal observation, *Scaphirhynchus* sturgeon prefer tanks with large footprints and horizontally flowing water.

Growth rate appeared superior when fish were fed continuously with autofeeders. However, due to the prohibitive cost of autofeeders and the necessity to quantify feed

consumption, hand feeding was performed two to three times daily and excess feed was recovered via siphon. During Objective 1, feeding a fixed amount appeared to benefit the biggest, most aggressive fish. The slow, benthic, browsing, habits of sturgeon made feeding to satiation a logistical difficulty. In Objective 2, fish were fed in excess in order to minimize competition. By lowering the height of standpipes, fish appeared to feed more efficiently, cleaning of tanks was easier and likely less stressful to the fish and mortality due to jumping was minimized.

The life history and biological attributes of *Scaphirhynchus* sturgeon, and particularly the Shovelnose Sturgeon, demonstrate great potential as an aquaculture species, though there are still challenges to overcome. The importance of the conservation of these species is described by Ballestrazzi and Garavello (2003), citing biological and phylogenetic significance. In particular, the Shovelnose Sturgeon demonstrate great potential as an efficient source of caviar and meat. Tripp et al. (2009a) reported that Shovelnose Sturgeon caviar fetches 400 USD kg^{-1}. The Shovelnose Sturgeon sexually matures faster and at a smaller body size than currently cultured sturgeon, while producing a similar proportion of high quality, salable caviar. These fish may also be easier to domesticate, due to faster reproductive cycles. Domesticated Shovelnose Sturgeon may be selected for growth rate, egg production, SBM tolerance, and the culture setting. The future for cultured sturgeon is bright, as Asian markets have demonstrated great interest not just in caviar, but also sturgeon meat (Wei et al. 2004), and global production of sturgeon has increased from 300 MT to 20,000 MT in just 15 years (Nierentz 2007).

Future investigations of the biology and nutrition of *Scaphirhynchus* sturgeon are needed. A domesticated line of Shovelnose Sturgeon should be established as soon as possible to produce fish acclimated to the culture environment. Evaluations of general husbandry parameters,

including photoperiod and light intensity, pH, salinity, and feeding rate would improve understanding of the animals in the production setting. Further evaluation of SBM inclusion is recommended, specifically assessing liver and intestinal responses, and the capacity of the fish to adapt to SBM over time, perhaps in a manner similar to Common Carp in the study of Uran et al. (2008a). Evaluation of the potential of feed additives such as phytase and citiric acid, as well as refined soybean products, which contain more CP and less carbohydrates and anti-nutritional factors, is also recommended.

Table 2.1. Determined protein:energy ratios (P:E; mg protein $kcal^{-1}$) of experimental diets fed to Pallid Sturgeon calculated using analyzed mean dietary crude protein and gross energy.

Crude Protein (mg g^{-1})	P:E (mg $kcal^{-1}$)		
341	96.8	88.8	78.9
400	113.5	104.1	92.6
489	138.8	127.3	113.2
566	160.6	147.3	131.0
Gross Energy (kcal kg^{-1}):	3,524	3,842	4,321

Table 2.2. Dietary formulations (g kg^{-1}) and determined proximate composition (g 100 g^{-1} dry matter) of experimental diets for Pallid Sturgeon.

	Experimental Diets											
Ingredients (g kg^{-1})	34:3524	40:3524	49:3524	57:3524	34:3842	40:3842	49:3842	57: 3842	34:4321	40:4321	49:4321	57:4321
Fish meal[1]	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245
Casein	70.8	152.2	234.0	315.0	70.8	152.2	234.0	315.0	77.6	159.0	240.4	321.7
Blood meal	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6
Yeast, brewers	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Fish solubles[1]	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6
Fish oil[1]	154.0	144.8	135.5	126.3	154.0	144.8	135.5	126.3	237.8	219.3	210.1	200.8
Dextrose	219.0	146.8	75.0	2.5	219.0	146.8	75.0	2.5	215.6	152.7	80.6	8.4
Wheat flour	88.2	88.2	88.2	88.2	88.2	88.2	88.2	88.2	39.2	39.2	39.2	39.2
Cellulose	39.2	39.2	39.2	39.2	39.2	39.2	39.2	39.2	1.0	1.0	1.0	1.0
Alginate HV[2]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20
Crystallized Arginine	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Betaine hydrochloride	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Dicalcium phosphate	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7
Sodium phosphate	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7
Choline	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
Mineral premix[3]	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Vitamin premix[4]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay C[5]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Proximate composition (g 100 g^{-1} dry matter):												
Crude Protein	33.1	39.4	45.9	55.8	33.6	40.0	50.9	57.0	35.5	40.6	49.9	57
Crude Lipid	13.6	10.3	9.4	9.1	19.0	11.1	17.9	16.9	27.6	26.3	27.4	24.4
Ash	9.7	10.1	10.1	10.2	9.1	9.6	8.6	9.7	8.5	8.9	9.4	9.3
Gross Energy (kcal kg^{-1})	3372	3442	3563	3718	3838	3655	4032	3842	4242	4568	4187	4289
Protein: Energy (mg protein $kcal^{-1}$)	98.3	114.5	128.7	150.0	87.5	109.4	126.2	148.3	83.7	89.0	119.1	132.9

[1]Omega Protein, Houston, TX.

[2]Manucol high viscosity alginate, FMC Corp., Philadelphia, PA.

[3]Contribution, mg kg^{-1} of diet: zinc, 215.36; iron, 38.06; manganese, 24.57; copper, 6.67; iodine, 2.40; selenium, 0.30; cobalt, 0.17; potassium, 0.000072.

[4]Contribution per kg of diet: vitamin A, 5,000 IU; vitamin D-3, 50 IU; vitamin E, 84 IU; vitamin K (as menadione), 8.432 mg; thiamin hydrochloride, 11.488 mg; riboflavin, 75.000 mg; niacin, 125.000 mg; pantothenic acid, 57.506; folic acid, 4.500 mg; pyridoxine, 20.575 mg; biotin, 1.250 mg; vitamin B-12, 50.000 mg; ascorbic acid, 87.500 mg.

[5]DSM Nutritional products, Basel, Switzerland.

Table 2.3. ANOVA results of Pallid Sturgeon carcass proximate composition after 18 weeks.

Values having different superscripts are significantly different at $P \leq 0.05$ within a main effect.

Main Effect			Composition (g 100 g^{-1} dry matter)		
	Dietary treatment	% Moisture	Protein	Lipid	Ash
Energy:					
	3524	77.65	61.73^{Z}	14.06	15.91
	3842	75.22	53.76^{Y}	17.53	12.91
	4321	75.27	53.38^{Y}	18.01	11.88
	P-value	0.18	0.01	0.39	0.11
	PSE[1]	1.05	1.80	2.17	1.33
Crude Protein:					
	34	76.65	55.88	14.86	13.42
	40	76.67	57.34	14.71	12.83
	49	77.03	58.91	14.31	16.14
	57	73.85	53.02	21.26	11.87
	P-value	0.22	0.25	0.21	0.26
	PSE	1.20	2.08	2.50	1.54
Protein × Energy:					
	34:3524	76.87	57.47	17.23	12.77
	40:3524	77.37	62.97	11.77	14.83
	49:3524	79.51	65.60	11.27	20.01
	57:3524	76.84	60.90	15.97	16.03
	34:3842	76.91	56.70	9.67	15.63
	40:3842	75.64	53.83	16.23	11.73
	49:3842	75.24	54.10	19.60	14.23
	57:3842	73.11	50.40	24.63	10.03
	34:4321	76.17	53.47	17.67	11.87
	40:4321	76.97	55.23	16.13	11.93
	49:4321	76.34	57.03	15.07	14.17
	57:4321	71.61	47.77	23.17	9.53
	P-value	0.85	0.73	0.56	0.70
	PSE	2.06	3.60	4.34	2.67

[1]Pooled standard error.

Table 3.1. Dietary formulations (g kg^{-1}) and determined proximate composition (g 100 g^{-1} dry matter) of experimental diets for Shovelnose Sturgeon.

	Experimental Diets			
Ingredients (g kg^{-1})	100%FM	75%FM:25%SBM	50%FM:50%SBM	25%FM:75%SBM
Fish meal[1]	403.0	303.0	202.0	101.0
Soybean meal[2]	0.0	174.2	336.2	512.3
Wheat starch[3]	168.1	114.5	62.3	13.6
Wheat gluten meal[1]	144.6	118.7	99.5	68.4
Poultry by-product meal[4]	100.0	100.0	100.0	100.0
Yeast, brewers	20.0	20.0	20.0	20.0
Fish solubles[5]	20.0	20.0	20.0	20.0
Fish oil[5]	99.4	105.0	114.6	117.2
Threonine[6]	1.1	0.7	0.4	0.0
L-lysine (95%)	0.0	0.0	1.2	3.7
Betaine hydrochloride[7]	2.0	2.0	2.0	2.0
Dicalcium phosphate[1]	15.0	15.0	15.0	15.0
Choline[1]	6.0	6.0	6.0	6.0
Mineral premix[8]	8.9	8.9	8.9	8.9
Vitamin premix[9]	10.0	10.0	10.0	10.0
Vitamin C-DSM[7]	2.0	2.0	2.0	2.0
Proximate composition (g 100 g^{-1})				
Crude Protein	46.6	46.0	46.1	45.2
Crude Lipid	14.2	14.8	15.5	15.7
Ash	10.1	9.6	8.7	7.8

[1]Skretting, Toole, UT.

[2]ADM, Decatur, IL.

[3]Manildra Milling, Shawnee Mission, KN.

[4]Tyson, Sedalia, MO.

[5]Omega Protein, Houston, TX.

[6]Evonik, Essen, Germany.

[7]Sigma Aldrich, St. Louis, MO.

[8]Contributed in mg kg^{-1} of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3.

[9]Contributed per kg of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D_3, 720 IU; vitamin E (as DL-%-tocopheryl-acetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulfate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B_{12}, 30 ug.

Table 3.2. Semi-quantitative scoring criteria used to assess development of enteritis in Shovelnose Sturgeon fed SBM diets, adapted from the methodology of Uran et al. (2008b).

Score	Parameter
Mucosal folds (MF)	
1	Normal height and shape
2	Mild reduction height, change in shape
3	Reduced height, altered shape, some tissue disruption
4	Increased tissue disruption
5	Prevalent tissue disruption, altered appearance
Sub-epithelial mucosa (SM)	
1	Normal appearance
2	Increased size SM
3	Medium size SM
4	Large SM
5	Very large SM
Supranuclear vacuoles (SNV)	
1	Normal size
2	Some size reduction
3	Diffuse size reduction
4	Size reduction and few SNV present
5	No SNV present

Table 3.3. Histological scores (mean ± SE) of Shovelnose Sturgeon fed diets with increasing SBM inclusion.

	Experimental Diets				
Condition Indices	100% FM	75%FM:25%SBM	50%FM:50%SBM	25%FM:75%SBM	*P*-value
Semi-quantitative scores:[1]					
Mucosal Folds (MF)	2.64 ± 0.19	3.05 ± 0.20	2.83 ± 0.18	2.67 ± 0.18	0.38
Sub-epithelial mucosa (SM)	2.23 ± 0.17	2.45 ± 0.18	2.54 ± 0.17	2.17 ± 0.17	0.64
Supranuclear vacuoles (SNV)	3.05 ± 0.21	3.15 ± 0.23	3.33 ± 0.21	2.79 ± 0.21	0.22
Quantitative scores:[2]					
Goblet Cells (GC)	18.96 ± 2.82	27.65 ± 2.96	28.25 ± 2.7	24.08 ± 2.7	0.11
Eosinophilic granulocytes (EG)	11.73 ± 3.39	19.05 ± 3.56	9.88 ± 3.25	9.17 ± 3.25	0.69
Lamina Propria (LP)	2.07 ± 0.46	3.34 ± 0.49	1.89 ± 0.44	1.86 ± 0.44	0.92

[1]MF, SM, and SNV were semi-quantitatively scored on a scale of one to five, with lower scores indicating superior tissue condition, and higher scores suggesting the development of intestinal enteritis.

[2]GC and EG were individually identified and counted, and the width of the LP was measured (µm).

Table 3.4. Performance indices of Shovelnose Sturgeon fed diets with increasing SBM inclusion. Values with different superscripts are significantly different at $P \leq 0.05$.

	Experimental Diets				
Performance Indices	100% FM	75%FM:25%SBM	50%FM:50%SBM	25%FM:75%SBM	PSE[1]
Mean weight gain (g)	20.3^{Z}	11.3^{Z}	12.0^{Z}	-2.5^{Y}	4.2
Total feed consumed (g)	1169.9	1139.7	1082.6	993.7	71.9
FE (%)	29.3^{Z}	16.8^{Z}	18.3^{Z}	-5.8^{Y}	6.6
SGR (% day $^{-1}$)	0.19^{Z}	0.11^{Z}	0.12^{Z}	-0.03^{Y}	0.04
HSI	1.05	0.96	0.99	0.94	0.20
Body composition (g 100 g^{-1} dry matter)					
Crude protein	54.0	49.4	50.0	55.7	2.3
Crude lipid	32.1	35.1	35.3	29.3	2.4
Ash	10.6	10.6	10.3	11.6	0.8
Liver composition (g 100 g^{-1})					
Moisture	28.9	29.1	28.2	37.3	5.5
Glycogen	2.24	0.93	0.96	0.95	0.42
CL	6.68	6.25	3.95	3.61	1.12

[1]Pooled standard error.

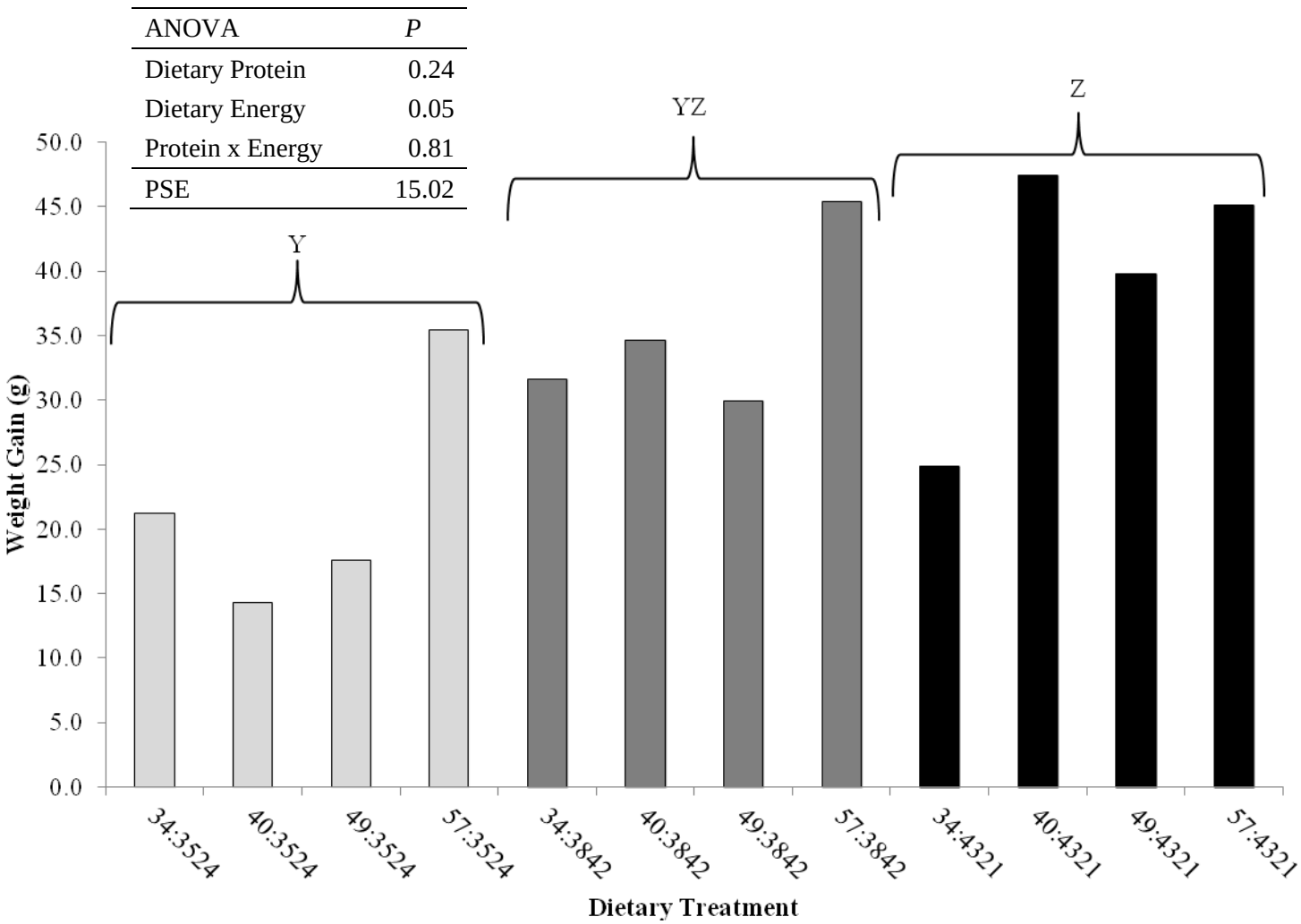

Figure 2.1. Mean weight gain (g) of Pallid Sturgeon fed diets varying in crude protein and gross energy content. Energy levels (indicated by bar color and brackets) having different letters are significantly different at $P \leq 0.05$.

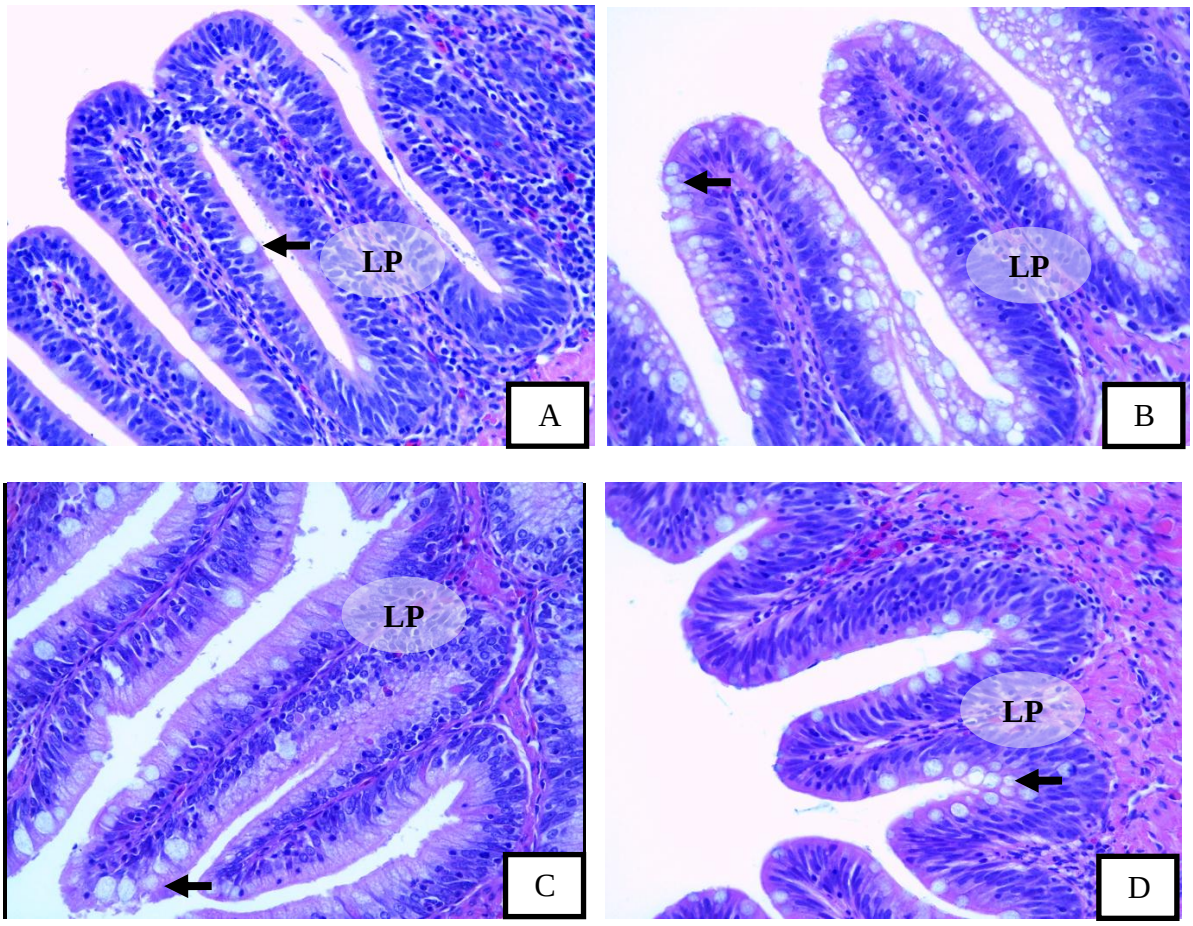

Figure 3.1. Histology of the distal intestine of Shovelnose Sturgeon fed diets with decreasing fish meal and increasing SBM (100% FM (A), 75%FM:25%SBM (B), 50%FM:50%SBM (C), 25%FM:75%SBM (D)) composition (400X). Increased presence of goblet cells (black arrows) were noted in fish fed SBM diets.

LITERATURE CITED

Abdolhay, H. A. and H. B. Tahori. 2006. Fingerling production and release for stock enhancement of sturgeon in the southern Caspian sea: an overview. Journal of Applied Ichthyology 22:125-131.

AVMA. 2007. AVMA guidelines on euthanasia. American Veterinary Medical Association. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf.

Babaei, S. S., A. A. Kenari, R. Nazari, E. Gisbert. 2011. Developmental changes of digestive enzymes in Persian Sturgeon (*Acipenser persicus*) during larval ontogeny. Aquaculture 318:138-144.

Baeverfjord, G., A. Krogdahl. 1996. Development and regression of soybean meal induced enteritis in Atlantic Salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. Journal of Fish Diseases 19(5):375-387.

Ballestrazzi R. and S. Garavello. 2003. The effects of commercial diets on performance, muscle proximate composition and waste loads of White Sturgeon *Acipenser transmontanus*. Italian Journal of Animal Science 2:631-633.

Bright, L. A., S. D. Coyle, J. H. Tidwell. 2005. Effect of dietary level on protein energy ratio on growth and body composition of Largemouth Bass (*Micropterus salmoides*). Journal of the World Aquaculture Society 36:129-134.

Buddington, R. K. and S. I. Doroshov. 1986a. Development of digestive secretions in White Sturgeon juveniles (*Acipenser transmontanus*). Comparative Biochemistry and Physiology 83A:223-238.

Buddington, R. K. and S. I. Doroshov. 1986b. Digestive enzyme complement of White Sturgeon (*Acipenser transmontanus*). Comparative Biochemistry and Physiology 83A:561-567.

Catarci, C., 2004. World markets and industry of selected commercially-exploitedaquatic species with an international conservation profile. FAO Fisheries Circular No.990.

Celikkale, M. S., E. E. Memis, F. Cagiltay. 2005. Growth performance of juvenile Russian Sturgeon (*Acipenser gueldenstaedtii* Brandt and Ratzenburg, 1833) at two stocking densities in net cages. Journal of Applied Ichthyology 21:14-18.

Conte, F. S, S. I. Doroshov, P. B. Lutes, E. M. Strange. 1988. Hatchery manual for the White Sturgeon *Acipenser transmontanus* Richardson with application to other North American Acipenseridae. Cooperative Extension University of California Division of Agriculture and Natural Resources: Publication 3322.

Cui, Y., S. S. O. Hung, D. F. Deng, Y. Yang. 1997. Growth performance of juvenile White Sturgeon as affected by feeding regimen. The Progressive Fish-Culturist 59:31-35.

Dabrowski, K., S. J. Kaushik, B. Fauconneau. 1987. Rearing of (*Acipenser baeri* Bradnt) larvae: III. Nitrogen and energy metabolism and amino acid absorption. Aquaculture 65:31-41.

Degani, G. 2002. Availability of protein and energy from three protein sources in hybrid sturgeon *Acipenser guldenstadtii* × *A. bester*. Aquaculture Research 33:725-727.

Deng, D. F., S. Koshio, S. Yokoyama, S. C. Bai, Q. Shao, Y. Cui, S. S. O. Hung. 2003. Effects of feeding rate on growth performance of White Sturgeon (*Acipenser transmontanus*) larvae. Aquaculture 217:589-598.

Doroshov, S. I. and P. B. Lutes. 1984. Preliminary data on the induction of ovulation in White Sturgeon (*Acipenser transmontanus* Richardson). Aquaculture 38:221-227.

Doroshov, S. I., G. P. Moberg, J. P. Van Eenennaam. 1997. Observations on the reproductive cycle of cultured White Sturgeon, *Acipenser transmontanus*. Environmental Biology of Fishes 48:265-278.

Einen, O. and A. J. Roem. 1997. Dietary protein/energy ratios for Atlantic Salmon in relation to fish size: growth, feed utilization and slaughter quality. Aquaculture Nutrition 3:115-126.

El-Saidy, D. M. S. D., Gaber, M. M. A. 2002. Complete replacement of fish meal by soybean meal with dietary supplementation for Nile Tilapia *Oreochromis niloticus* (L.) fingerlings. Journal of the World Aquaculture Society 33(3):297-306.

FAO. 2010. The state of world fisheries and aquaculture 2010. FAO Fisheries and Aquaculture Department, Rome, Italy. http://www.fao.org/docrep/013/i1820e/i1820e.pdf. Last accessed 9/23/2012.

FAO. 2012. Online database for commodity prices. http://www.fao.org/economic/est/prices. Last accessed 9/18/2012.

Fajfer, S., L. Meyers, G. Willman, T. Carpenter, M. J. Hansen. 1999. Growth of juvenile Lake Sturgeon reared in tanks at three densities. North American Journal of Aquaculture 61:331-35.

Furne, M., M. C. Hidalgo, A. Lopez, M. Garcia-Gallego, A. E. Morales, A. Domezain, J. Domezaine, A. Sanz. 2005. Digestive enzyme activities in Adriatic Sturgeon *Acipenser naccarii* and Rainbow Trout *Oncorhynchus mykiss*. A comparative study. Aquaculture 250:391-398.

Fynn-Aikins, K., S. S. O. Hung, W. Liu, H. Li. 1992. Growth, lipogenesis and liver composition of juvenile White Sturgeon fed different levels of D-glucose. Aquaculture 105:61-72.

Garcia-Gallego, M., A. Domezain, M. de la Higuera, M. C. Hidalgo, M. Furne, A. E. Morales, A. Sanz. 2009. On nutrition and feeding studies as the basis for the culture of different sturgeon species. Chapter 13. Pages 215-234 *in* Biology, Conservation, and Sustainable Development of Sturgeons. Springer, Netherlands.

Gatlin, D. M. 2002. Use of soybean meal in the diets of omnivorous freshwater fish. Department of Wildlife and Fisheries Sciences, Faculty of Nutrition, Texas A&M University System.

Gatlin, D, M, F. T. Barrows, P. Brown, K. Dabrowski, T. G. Gaylord, R. W. Hardy E. Herman, G. Hu, A. Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E. J. Souza, D. Stone, R. Wilson. E. Wurtele. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38:551-579.

Herold M. A., S. S. O. Hung, K. Fynn-Aikins. 1995. Apparent digestibility coefficients of carbohydrates for White Sturgeon. The Progressive Fish-Culturist 57:137-140.

Hung, S. S. O., P. B. Lutes, F. S. Conte, T. Storebakken. 1989a. Growth and feed efficiency of White Sturgeon (*Acipenser transmontanus*) sub-yearlings at different feeding rates. Aquaculture 80:147-153.

Hung, S. S. O., F. K. Fynn-Aikins, P. B. Lutes, R. P. Xu. 1989b. Ability of White Sturgeon (*Acipenser transmontanus*) to utilize different carbohydrate sources. The Journal of Nutrition 119:727-733.

Hung, S. S. O, P. B. Lutes, A. A. Shqueir, F. S. Conte. 1993. Effect of feeding rate and water temperature on growth of juvenile White Sturgeon (*Acipenser transmontanus*). Aquaculture 115:297-303.

Hung, S. S. O., W. Liu, H. Li, T. Storbakken, Y. Ciu. 1997. Effect of starvation on some morphological and biochemical parameters in White Sturgeon, *Acipenser transmonatnus*. Aquaculture 151:357-363.

Hung, S. S. O., and D. F. Deng. 2002. Sturgeon *Acipenser* spp. Chapter 24. Pages 334-355 *in* Nutrient Requirements and Feeding of Finfish for Aquaculture. CABI Publishing, Wallingford, UK.

Imanpoor, M. R., T. Bagheri, A. Azimi. 2010a. Serum biochemical change induced by soybean meal in diet on Persian Sturgeon, *Acipenser persicus*. Global Veterinaria 5(1):61-64.

Imanpoor, M. R., T. Bagheri, A. Azimi. 2010b. Effects of replacing fish meal with soybean meal in diets on some morphometric indices of Persian Sturgeon, *Acipenser persicus*. World Journal of Zoology 5(4):320-323.

Kappenman, K. M., W. C. Fraser, M. Toner, J. Dean, M. A. H. Webb. 2009. Effect of temperature on growth, condition, and survival of juvenile Shovelnose Sturgeon. Transactions of the American Fisheries Society 138:927-937.

Kaushik, S. J., P. Luquet, D. Blanc, A. Paba. 1989. Studies on the nutrition of Siberian Sturgeon, *Acipenser baeri* I. Utilization of digestible carbohydrates by sturgeon. Aquaculture 76:97-107.

Kaushik, S. J., J. Breque, D. Blanc. 1991. Apparent amino acid requirements of and protein utilization by Siberian Sturgeon (*Acipenser baeri*), Pages 25-39 *in*: Williot P.(Ed.), *Acipenser*, Actes let coll. Int. by l'Esturgeon, Bordeaux, France, Cemagref-Dicova, Atony, France.

Keenlyne, K. D. 1997. Life history and status of the Shovelnose Sturgeon, *Scaphirhynchus platorynchus*. Environmental Biology of Fishes 48:291-298.

Khajepour, F. and S. A. Hosseini. 2010. Mineral status of juvenile Beluga *Huso huso* fed citric acid supplemented diets. World Applied Sciences Journal 11(6):682-686.

King, K. 2004. Growth, survival, and body composition of juvenile Atlantic Sturgeon fed five commercial diets under hatchery conditions. North American Journal of Aquaculture 66:53-60.

Knudsen, D., P. Uran, A. Arnous, W. Koppe, H. Frokliaer. 2007. Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic Salmon. Journal of Agricultural Food and Chemistry 55:2261-2267.

Lin, J., Y. Cui, S. S. O. Hung, S. Shiau. 1997. Effect of feeding strategy and carbohydrate source on carbohydrate utilization by White Sturgeon (*Acipenser transmontanus*) and hybrid Tilapia (*Oreochromis niloticus* X *O. aueus*). Aquaculture 148:201-211.

Liu, H. X. Wu, W. Zhao, M. Xue, L. Guo, Y. Zheng, Y. Yu. 2009. Nutrients apparent digestibility coefficients of selected proein sources for juvenile Siberian Sturgeon (*Acipenser baerii* Brandt), compared by two cromic oxide analyses methods. Aquaculture Nutrition 15:650-656.

Mayden, R. L. and B. R. Kuhajda. 1996. Systematics, taxonomy, and conservation status of the endangered Alabama Sturgeon, *Scaphirhynchus suttkusi* Williams and Clemmer (Actinopterygii, Acipenseridae). Copeia 241-73.

Mayfield, R. B., and J. J. Cech Jr. 2004. Temperature effects on Green Sturgeon bioenergetics. Transactions of the American Fisheries Society 133:961-970.

Medale, F., Blanc, D., Kaushik, S. J. 1991. Studies on the nutrition of Siberian Sturgeon, *Acipenser baeri* II. Utilization of dietary non-protein energy by sturgeon. Aquaculture 93:143-154.

Merrifield, D. L., R. E. Olsen, R. Myklebust, E. Ringo. 2011. Dietary effect of soybean (*Glycine max*) products on gut histology and microbiota of fish. Soybean and Nutrition:231-250.

Moghim, M., D. Kor, M. Tavakolieshkalak, M. B Khoshghalb. 2006. Stock status of the Persian Sturgeon (*Acipenser persicus* Borodin, 1897) along the Iranian coast of the Caspian sea. Journal of Applied Ichthyology 22(1):99-107.

Mohler, J. W., K. Fynn-Aikins, R. Barrows. 1996. Feeding trials with juvenile Atlantic Sturgeons propagated from wild broodstock. The Progressive Fish-Culturist 58(3):173-177.

Mohseni, M., M. Pourkazemi, M. Bahamanu, B. Falahatkar, H. R. Pourali, M. Salehpour. 2006. Effects of feeding rate and frequency on growth performance of yearling Great Sturgeon, *Huso* Huso. Journal of Applied Ichthyology 22:278-282.

Mohseni, M., M. Sajjadi, M. Pourkazemi. 2007. Growth performance and body composition of sub-yearling Persian Sturgeon, (*Acipenser persicus*, Borodin, 1897), fed different dietary protein and lipid levels. Journal of Applied Ichthyology 23:204-208.

Monaco, G., R. K. Buddington, S. I. Doroshov. 1981. Growth of White Sturgeon (*Acipenser transmontanus*) under hatchery conditions. Journal of World Mariculture Society 12:113-121.

Moore, B. J., S. S. O. Hung, J. F. Medrano. 1988. Protein requirement of hatchery-produced juvenile White Sturgeon (*Acipenser transmontanus*). Aquaculture 71:235-245.

Murat, J. C. and A. Serfaty. Simple enzymatic determination of polysaccharide (glycogen) content of animal tissues. Clinical Chemistry 20(12):1576-1577.

Naylor, R. L., R. W. Hardy, D. P. Bureau, A. Chiu, M. Elliott, A. P. Farrell, I. Forster, D. M Gatlin, R. J. Goldburg, K. Hua, P. D. Nichols. 2009. Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences of the United States of America. 106:15103-15110.

Nematipour, G. R., M. L. Brown, D. M. Gatlin. 1992. Effects of dietary energy:protein ratio on growth characteristics and body composition of hybrid Striped Bass, (*Morone chrysops* ♀ × *M. saxatilis* ♂). Aquaculture 107:359-368.

Ng, W. K. and S. S. O. Hung. 1995. Estimating the ideal indispensable amino acid pattern for growth of White Sturgeon, (*Acipenser transmontanus* Richardson). Aquaculture Nutrition 1:85-94.

Ng, W. K. and S. S. O. Hung. 1994. Amino acid composition of whole body, egg, and selected tissues of White Sturgeon *(Acipenser transmontanus*). Aquaculture 126:329-339.

NRC. 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Washington D.C.

Raskovic, B. S., M. B. Stankovic, Z. Z. Markovic, V. D. Poleksic. 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. Journal of Agricultural Sciences 56:87-100.

Seibert, J. R., Q. E. Phelps, S. J. Tripp, J. E. Garvey. 2011. Seasonal diet composition of adult Shovelnose Sturgeon in the middle Mississippi river. The American Midland Naturalist 165(2):355-363.

Singer, T. D., and J.S. Ballantyne. 2004. Sturgeon and paddlefish metabolism. Pages 167-194 *in* Sturgeons and Paddlefish of North America. Kluwer Academics Publishers, Netherlands.

Small, B. C. and J. H. Soares Jr. 1999. Effect of dietary carbohydrate on growth, glucose tolerance, and liver composition of juvenile Striped Bass. North American Journal of Aquaculture 61:286-292.

Stuart, J. S. and S. S. O. Hung. 1989. Growth of juvenile White Sturgeon (*Acipenser transmontanus*) fed different proteins. Aquaculture 76:303-316.

Tripp, S. J., R. E. Colombo, J. E. Garvey. 2009a. Declining recruitment and growth of Shovelnose Sturgeon in the middle Mississippi river: Implications for conservation. Transactions of the American Fisheries Society 138:416-422.

Tripp, S. J., Q. E. Phelps, R. E. Colombo, J. E. Garvey, M. Brooks, D. P. Herzog, R. A. Hrabik. 2009b. Maturation and reproduction of Shovelnose Sturgeon in the middle Mississippi river. North American Journal of Fisheries Management 29:730-738.

Trushenki, J. T., C. S. Kasper, C. C. Kohler. 2006. Challenges and opportunities in finfish nutrition. North American Journal of Aquaculture 68:122-140.

Uran, P. A., A. A. Goncalves, J. J. Taverne-Thiele, J. W. Schrama, J. A. J. Verreth, J. H. W. M. Rombout. 2008a. Soybean meal induces intestinal inflammation in Common Carp (*Cyprinus carpio* L.). Fish and Shellfish Immunology 25:751-760.

Uran, P. A., J. W. Schrama, J.H.W.M. Rombout, A. Obach, L. Jensen, W. Koppe, J. A. J. Verreth. 2008b. Soybean meal-induced enteritis in Atlantic Salmon (*Salmo salar* L.) at different temperatures. Aquaculture Nutrition 14:324-330.

USFWS (U.S. Fish and Wildlife Service). 1990. Endangered and threatened wildlife and plants: determinations of endangered status for the Pallid Sturgeon. Federal Register 55:173(6 September 1990):36641–36647.

USFWS (U.S. Fish and Wildlife Service). 2010. Endangered and threatened wildlife and plants; threatened status for Shovelnose Sturgeon under the similarity of appearance provisions of the Endangered Species Act. Federal Register 75:169(September 2010):53598-53606.

USGS (U. S. Geological Survey). 2011. Water quality requirements, tolerances, and preferences of Pallid Sturgeon *Scaphirhynchus albus* in the lower Missouri river. Scientific Investigations Report 2011-5185. USGS, Reston, VA: 2011.

Venou, B., M. N. Alexis, E. Fountoulaki, J. Haralabous. 2008. Performance factors, body composition and digestion characteristics of Gilthead Seabream (*Spartus aurata*) fed pelleted or extruded diets. Aquaculture Nutrition 15:390-401.

Voila, S. S. Mokady, U. Rappaport, Y. Arieli. 1982. Partial and complete replacement of fishmeal by soybean meal in feeds for intensive culture of Carp. Aquaculture 26:223-236.

Watanabe T. 2002. Strategies for further development of aquaculture feeds. Fisheries Science 68:242-252.

Webb, K. A. JR., L. T. Rawlinson, G. J. Holt. 2010. Effects of dietary starches and the protein to energy ratio on and feed efficiency of juvenile Cobia, *Rachycentron canadum*. Aquaculture Nutrition 16:447-456.

Wei. Q., J. He, D. Yang, W. Zheng, and L. Li. 2004. Status of aquaculture and sturgeon trade in China: a review based on two recent nationwide surveys. Journal of Applied Ichthyology 20:321-332.

Xiao, H., Y. Ciu, S. S. O. Hung, X. Zhu, Z. Zou, S. Xie. 1999. Growth of Chinese Sturgeon *Acipenser sinensis* fed live and formulated diets. North American Journal of Aquaculture 61:184-188.

Zhang, Y., S. Doroshov, T. Famula, F. Conte, D. Kueltz, J. Linares-Casenave, J. Van Eenennaam, P. Struffenegger, K. Beer, K. Murata. 2011. Egg quality and plasma testosterone (T) and estradiol-17β (E_2) in White Sturgeon (*Acipenser Transmontanus*) farmed for caviar. Journal of Applied Ichthyology 27:558-564.

VITA

Graduate School

Southern Illinois University

Elliott C. Kittel

Elliott_kittel@hotmail.com

University of Arkansas Pine Bluff, Pine Bluff Arkansas
Bachelor of Science, Fisheries Biology, May 2010

Special Honors and Awards:

Hutton Junior Fisheries Biologist Scholar 2005
Hutton Junior Fisheries Biologist Scholar 2006
Arkansas Game and Fish Commission Scholarship 2007 - 2010
USGS BRD National Cooperative Fisheries Scholarship 2007 – 2010
SIU College of Agricultural Sciences Outstanding Masters Researcher 2013

Thesis Title:

> Estimation of optimal protein to energy ratio and percent soybean meal replacement of fish meal in juvenile *Scaphirhynchus* sturgeon diets.

Major Professor: Brian C. Small

Publications:

2012 Kittel, E. C. and B. C. Small, "Research toward the development of *Scaphirhynchus* sturgeon feeds for aquaculture", Aquaculture America 2012, Las Vegas, NV, February 29 2012, p 18.

2010 Kittel, E. C. and A. E. Goodwin, "Survival of bacteria in synthetic digestive fluids: estimating the potential of fish pathogens to successfully pass through reptiles, mammals, and birds", Student/Faculty UAPB Research Symposium, Pine Bluff, AR, March 2010.