SELENIUM NUTRITION OF Morone HYBRIDS INCLUDING DIETARY REQUIREMENTS, BIOAVAILABILITY, TOXICITY AND EFFECTS ON IMMUNE RESPONSES AND DISEASE RESISTANCE

A Dissertation

by

FRANCISCO JARAMILLO, JR.

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2006

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

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ABSTRACT

Selenium Nutrition of *Morone* Hybrids Including Dietary Requirements, Bioavailability, Toxicity and Effects on Immune Responses and Disease Resistance. (May 2006)

Francisco Jaramillo, Jr., B.S., Texas A&M University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. Delbert M. Gatlin, III

Aquacultural production of hybrid striped bass (HSB) *Morone chrysops* × *M. saxatilis* is highly vulnerable to losses from bacterial pathogens such as *Streptococcus iniae*. Therefore, research was conducted to evaluate various dietary factors that may enhance immunocompetence and disease resistance of HSB. In the first experiment, purified and practical diets were supplemented with β-glucan and selenium in a factorial arrangement and fed to juvenile HSB for 6 wk followed by a *S. iniae* challenge. Weight gain (WG) and feed efficiency (FE) were higher for fish fed either practical diets or purified diets supplemented with selenium, but not those supplemented with β-glucan. Survival after disease challenge for fish fed the selenium-supplemented practical and purified diets was 75% and 35%, respectively.

Because selenium supplementation also improved WG and FE, and because selenium and vitamin E have complementary biochemical functions, a second experiment evaluated potential interactions by feeding purified diets with or without vitamin E or sodium selenite (Na_2SeO_3), singularly or in combination, for 12 wk. Dietary selenium significantly affected whole-body selenium concentration but there

was no effect of dietary selenium, vitamin E or their interaction on WG, FE, survival or blood neutrophil oxidative radical production.

Three additional 12-wk experiments were conducted to establish selenium essentiality, toxicity, tissue deposition, dietary requirements, bioavailability and non-specific immune responses using purified diets with a basal selenium level of 0.11 mg/kg. In one experiment, diets had selenium concentrations of 1.19, 2.00, 5.17 and 21.23 mg/kg from Na_2SeO_3. Another experiment had selenium concentrations of 0.90, 1.26 and 2.55 mg/kg from seleno-DL-methionine. The third trial utilized selenium from Na_2SeO_3, seleno-DL-methionine and selenium yeast at approximately 0.15, 0.30 and 0.60 mg/kg diet.

No overt selenium deficiency signs were observed in any of the three latter experiments, but based on selenium retention values, a minimum dietary requirement of approximately 0.1 mg/kg was estimated. Selenium toxicity was observed in fish fed the diet containing more than 20 mg/kg. Bioavailability of selenium sources was ranked as seleno-DL-methionine > selenium yeast > Na_2SeO_3.

DEDICATION

In memory of my great-uncle Guadalupe Molina.

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First, I cannot overstate the contribution that Dr. Delbert M. Gatlin, III, chair of my advisory committee, has made to my academic pursuits. He has provided a real and personal example of how through hard work and commitment one's goals can be realized. Thank you for everything you have done for me, Dr. Gatlin.

Next, I thank the other members of my committee, Drs. William H. Neill, Christopher A. Bailey and Donald H. Lewis, for their constructive criticism, guidance and encouragement throughout the years.

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also like to recognize Alltech, Inc. (Nicholasville, Kentucky, USA), Omega Protein, Inc., (Houston, Texas USA), and US Biochemical Corporation (Cleveland, Ohio, USA) for generously providing the ingredients and Keo Fish Farms (Keo, Arkansas, USA) for providing the fish used in this research.

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CHAPTER I

INTRODUCTION

Hybrid striped bass *Morone chrysops* × *M. saxatilis* has become an important aquacultured fish in the United States. Its desirability relates to its taste appeal, provision of lean, high quality protein for consumers (Hodson et al. 1987), its recreational appeal for angling opportunities and its use in fisheries management (Jahn et al. 1987). Successful aquaculture of any fish species requires an understanding of its nutritional and environmental requirements as well as effective disease management procedures. Over the past 10+ years, research has focused on delineating the nutritional requirements of hybrid striped bass (e.g., Brown et al. 1992, 1993; Nematipour and Gatlin 1993; Gatlin 1997; Kocabas and Gatlin 1999; Sealy and Gatlin 1999a), which has led to the development of nutritious, cost-effective diet formulations for this fish.

In addition to having nutritionally balanced diets, the ability to prevent and manage disease outbreaks in aquaculture systems is necessary for successful and profitable operations. Losses of hybrid striped bass in aquacultural production systems have been attributed to various protozoan and bacterial pathogens including *Streptococcus iniae* (Stoffregen et al. 1996). Current methods of disease management in hybrid striped bass culture have involved the extra-label use of a limited number of antibiotics or other chemotherapeutics in production systems as well as vaccines under laboratory conditions (Plumb 1999). However, to date these methods have not proven to

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This dissertation follows the style of the Journal of the World Aquaculture Society.

be completely effective. Currently, there are only two codified products approved for use in food fish by the United States Food and Drug Administration (USFDA). These are oxytetracycline and Romet 30, a combination of sulfadimethoxine and ormetoprim 5:1, neither of which is specifically approved for use in hybrid striped bass (Stoffregen et al. 1996; Muirhead 2002). In addition to these, the United States Center for Veterinary Medicine recently approved, for the first time in over 20 yr, an antimicrobial for use in a finfish species (FDA Veterinarian 2005). This product, Aquaflor® (florfenicol), is approved as a "Veterinary Feed Directive" for the control of *Edwardsiella ictaluri* in channel catfish *Ictalurus punctatus*. Pharmaceutical companies have limited financial incentive to invest in the research and development of new products or medications for aquaculture due to the complexity of obtaining USFDA approval for new animal drugs (Plumb 1999). Thus, it is not anticipated that additional chemotherapeutics will be available to combat diseases of hybrid striped bass in the near future. Justification of research into methods of controlling or preventing disease in this fish through nutritional modulation deserves consideration. Potential immunomodulating compounds include antioxidant vitamins and minerals as well as various biological components such as killed mycobacteria, muramyl dipeptide and β-1,3-glucans which stimulate non-specific immune responses (Raa et al. 1992; Gatlin 2002). The use of nutritional modulation, with approved feed ingredients, can easily be incorporated into the feeding regime without any additional labor, application cost or governmental restrictions. Therefore, nutritional modulation has several advantages over the use of chemotherapeutics, antibiotics and vaccines.

Nutritional Aspects of Hybrid Striped Bass Culture

Macronutrients such as protein and amino acids, lipid and carbohydrates are often studied first when establishing dietary requirements of an organism. Mineral requirements, both macro and trace, often are given a lower priority (NRC 1993). Such has been the case with regard to investigations of dietary requirements of hybrid striped bass.

The most commonly studied trace minerals for fish are iron, copper, manganese, zinc and selenium. These minerals are important because they serve as components of body fluids, cofactors in enzymatic reactions and as structural units of non-enzymatic macromolecules (Watanabe et al. 1997; Lall 2002). Iron is the oxygen-transporting element in hemoglobin (Lim et al. 2001a). Copper also plays a role in hematopoiesis as well as being a component of a number of enzyme systems, such as cytochrome c oxidase of the electron transport system, superoxide dismutase, and tyrosinase (Lovell 1998). Another cofactor in the superoxide dismutase enzyme system is manganese. This mineral is also associated with enzymes involved in amino acid, fatty acid, and glucose oxidation (Lovell 1998). Along with copper-zinc superoxide dismutase, glutathione peroxidase is another antioxidant enzyme which contains selenium (Lim et al. 2001b). Like manganese, selenium also has been identified as a cofactor in glucose metabolism (Lovell 1998).

Importance of Selenium

The biological significance of selenium did not become apparent until the toxic nature of this element was reported by Franke and Painter (1936). It was not until 1957 that the nutritional essentiality of selenium was recognized by Schwarz and Foltz (1957). Selenium's major function is as a component of the enzyme glutathione peroxidase that protects membranes at both the cellular and subcellular level from oxidative damage by reducing strong pro-oxidants such as hydroperoxides (Arteel and Sies 2001). However, selenium also has been found to reduce the bioaccumulation of mercury in largemouth bass (Southworth et al. 2000). Toxicity due to excess dietary selenium has been reported in salmonids (Hilton et al. 1980; Hilton and Hodson 1983; Hicks et al. 1984), channel catfish (Gatlin and Wilson 1984) and razorback sucker *Xyrauchen texanus* (Hamilton et al. 2002).

Watanabe et al. (1997) reported the selenium requirement of fish to range between 0.05 – 1.0 mg selenium/kg dry diet. Gatlin and Wilson (1984) reported the selenium requirement of channel catfish to be 0.25 mg selenium/kg based upon growth and liver glutathione peroxidase activity. Bell et al. (1985) reported selenium deficiency signs to be absent at an inclusion rate of 0.06 mg selenium/kg in rainbow trout. A diet fortified with 0.1 mg selenium/kg dry diet was found to prevent mortality of Atlantic salmon *Salmo salar* fry caused by a selenium-deficient diet (Poston et al. 1976). A level of 0.15 mg selenium/kg dry diet provided the best growth rate of Atlantic salmon (Poston and Combs 1979). Dietary selenium ranging from 0.15-0.38 mg /kg was found to provide maximum glutathione peroxidase activity in the plasma of rainbow trout

Oncorhynchus mykiss (Hilton et al. 1980). Recently, a dietary selenium concentration of 0.7 mg/kg was recommended for grouper *Epinephelus malabaricus* (Lin and Shiau 2005). However, toxicity due to high levels of selenium occurred at inclusion rates of 13 and 15 mg selenium/kg for rainbow trout (Hilton et al. 1980) and channel catfish (Gatlin and Wilson 1984), respectively. In razorback sucker larvae, dietary selenium concentrations of ≥ 4.6 mg /kg were found to have adverse effects on their survival (Hamilton et al. 2002).

Despite the potential toxicity of dietary selenium, disease resistance of some fish species has been improved by selenium supplementation, as well as inclusion of other antioxidant nutrients such as vitamin C and vitamin E at levels above the established minimum requirement (Blazer and Wolke 1984; van Vleet and Watson 1984; Wahli et al. 1986; Wang et al. 1997).

Selenium Sources

Currently, the most commonly used source of selenium to supplement diets is sodium selenite, an inorganic form. Organic forms of selenium, such as selenocystine, selenocysteine, and selenomethionine, have been identified in plant-derived feedstuffs (Shrift 1969). Recently, selenium yeast, a selenomethionine fortified yeast product was approved by the USFDA for use in poultry, swine, beef cattle and dairy cattle feeds (CFR 2004). Organic forms of selenium, like many other minerals, are thought to be more readily available to animals. The digestibility and bioavailability of dietary selenium from fishmeal, sodium selenite, selenomethionine and selenocystine in Atlantic

salmon was investigated by Bell and Cowey (1989). They found that selenomethionine was the most bioavailable source of selenium (92%) while fishmeal was the least (47%). Wang and Lovell (1997) reported relative bioavailability values for selenomethionine to channel catfish at 336 and 147% based on growth and liver glutathione peroxidase activity, respectively, in comparison to sodium selenite. When channel catfish were challenged with *Edwardsiella ictaluri*, the use of selenomethionine and selenoyeast provided a more potent source of selenium and reduced mortality compared to sodium selenite (Wang et al. 1997).

Research Objectives

In summary, the essentiality of selenium in fish nutrition has been established in addition to its toxicity at excess levels of supplementation (Watanabe et al. 1997). Dietary selenium requirements, as well as detrimental levels, have been reported in salmonids (Bell et al.1985; Hilton et al. 1980; Poston et al. 1976) and channel catfish (Gatlin and Wilson 1984). The use of nutrients with antioxidant properties such as selenium above minimum requirement levels also has been investigated for improvement of disease resistance (Blazer and Wolke 1984; van Vleet and Watson 1984; Wahli et al. 1986; Wang et al. 1997). Therefore, my dissertation research has three fundamental objectives. First, the minimum dietary requirement and toxic levels of selenium for hybrid striped bass will be quantified. Secondly, the suitability of incorporating supplemental selenium from organic sources, with respect to bioavailability, will be evaluated in comparison to inorganic selenium. And lastly, the

dietary level above the minimum requirement needed for enhancement of the immune response and disease resistance of hybrid striped bass, especially against *S. iniae*, will be sought. This research will contribute to the refinement of hybrid striped bass diet formulations because there currently is no established dietary requirement for selenium or quantified toxicity level. In addition, it is also hoped that this research will establish an optimum selenium supplementation level to improve the immune response and disease resistance of hybrid striped bass.

A total of five experiments were undertaken for this dissertation. The first experiment, presented in Chapter II, compared purified and practical diets supplemented with or without β-glucan and selenium on resistance to *S. iniae*. Chapter III presents the second experiment which addressed selenium and vitamin E interactions. Chapter IV is comprised of three experiments, labeled as Experiments 3, 4 and 5, which addressed the essentiality of selenium, selenium toxicity, tissue deposition and estimated dietary requirements. Experiment 3 had four supplemented levels of dietary selenium from sodium selenite. The analytical selenium concentrations for these diets were 1.19, 2.00, 5.17 and 21.23 mg/kg. For Experiment 4, seleno-DL-methionine was used to supplement three diets with analyzed dietary selenium levels at 0.90, 1.26 and 2.55 mg/kg. Experiment 5 utilized selenium from either sodium selenite, seleno-DL-methionine or selenium yeast at approximately one, two and four times the analyzed basal level of 0.11 mg/kg. Chapter V is a comparison of bioavailability and non-specific immune responses of sodium selenite, seleno-DL-methionine and selenium yeast from Experiment 5.

CHAPTER II

COMPARISON OF PURIFIED AND PRACTICAL DIETS SUPPLEMENTED WITH OR WITHOUT β-GLUCAN AND SELENIUM ON RESISTANCE OF HYBRID STRIPED BASS *Morone chrysops* ♀ × *M. saxatilis* ♂ TO *Streptococcus iniae* INFECTION*

Hybrid striped bass *Morone chrysops* × *M. saxatilis* has become an important aquaculture product in the United States. Its desirability stems from its provision of lean, high-quality protein for consumers and its recreational appeal for angling opportunities (Hodson et al. 1987) as well as its use in fisheries management (Jahn et al. 1987). The successful culture of aquatic species requires an understanding of nutritional requirements and disease management. Research has focused on delineating the nutritional requirements of hybrid striped bass (Gatlin 1997; Webster 2002) as well as methods of controlling or preventing disease (Plumb 1997).

Losses of hybrid striped bass in aquacultural production systems have been attributed to various protozoan and bacterial pathogens including *Streptococcus iniae* (Stoffregen et al. 1996; Plumb 1997; Shelby et al. 2003). *S. iniae* was reported to have a prevalence of 7.3% in hybrid striped bass cultured in the United States (Shoemaker et al.

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*Reprinted with permission from "Comparison of purified and practical diets supplemented with or without β-glucan and selenium on resistance of hybrid striped bass *Morone chrysops* × *M. saxatilis* to *Streptococcus iniae* infection" by Jaramillo, F., Jr. and Gatlin, D.M., III, 2004. Journal of the World Aquaculture Society, 35, 245-252.

2001), and it is known to spread rapidly through infected fish (Evans et al. 2000, 2001). Current methods of disease management in hybrid striped bass culture have involved the extra-label use of a limited number of antibiotics or other chemotherapeutics in production systems as well as vaccines under laboratory conditions (Plumb 1999). However, to date these methods have not proven to be completely effective, and the two antibiotics currently approved for use in food fish by the United States Food and Drug Administration (USFDA) are not specifically approved for use in hybrid striped bass (Stoffregen et al. 1996; Muirhead 2002).

Due to the limited number of approved chemotherapeutics available in aquaculture, research into methods of controlling or preventing disease in hybrid striped bass through nutritional modulation deserves consideration. Potential immunomodulating compounds include antioxidant vitamins and minerals as well as various biological components such as nucleotides, killed mycobacteria, muramyl dipeptides, and β-1,3-glucans, which may be found in natural feedstuffs or in purified form and have been shown to stimulate non-specific immune responses (Raa et al. 1992; Sealey and Gatlin 1999; Gatlin 2002). The use of nutritional modulation, with approved feed ingredients, can easily be incorporated into the feeding regime without any additional labor, application cost or governmental restrictions. Therefore, nutritional modulation has several advantages over the use of chemotherapeutics, antibiotics and vaccines. It should be noted that some purified immunostimulants are not currently approved by the USFDA for use in animal feeds. This study evaluated various dietary

factors including basal diet composition, selenium and β-glucans on hybrid striped bass exposed to *S. iniae* via bath immersion.

Materials and Methods

Experimental Diets

Two different types of diet formulations were based on either menhaden fish meal or purified crystalline amino acids and casein/gelatin (Table 1). The crude protein and lipid levels were formulated at 35% and 6%, respectively, for all diets. All other known nutritional requirements of hybrid striped bass were met by the experimental diets. Both the purified and practical fish meal-based diet formulations without selenium supplementation were analyzed by hydride generation and a flame atomic absorption spectrophotometer (Agricultural Analytical Services 2001) to contain 0.03 and 1.03 mg selenium/kg, respectively. Sodium selenite was supplemented at either 0 and 0.2 mg/kg and β-glucan from barley (Sigma Chemical Co., St. Louis, Missouri, USA) was supplemented at either 0 or 0.1% in both purified and practical diets in a 2x2x2 factorial arrangement for a total of eight diets. The diets supplemented with sodium selenite at 0.2 mg/kg contained an additional 0.07 mg selenium/kg on average. The dietary inclusion of 0.1% glucan was based on the mortality levels reported by Jeney et al. (1997) for rainbow trout *Oncorhynchus mykiss* fed various glucan levels and challenged by a spontaneous infection with *Flexibacter columnaris.*

TABLE 1. *Basal formulations (g/kg on a dry-matter basis) of purified and practical type diets.*

Ingredient	Purified	Practical
Amino acid premix [a]	249.0	
Menhaden fish meal [b]		515.2
Casein [c]	90.0	
Gelatin [c]	18.0	
Dextrin [c]	250.0	250.0
Selenium-free mineral premix [d]	40.0	40.0
Vitamin premix [e]	30.0	30.0
Carboxymethyl cellulose [c]	20.0	20.0
Cellulose [c]	243.0	144.1
Menhaden oil [b]	60.0	0.7
Selenium premix [f]	0	0
ß-glucan [g]	0	0

[a] Contained the following L-amino acids (g/kg): Arginine HCl 54.2, Histidine 28.5, Isoleucine 56.2, Leucine 94.4, Lysine 35.1, Methionine 20.0, Cystine 9.2, Phenylalanine 65.9, Tyrosine 62.3, Threonine 57.8, Tryptophan 15.7, Valine 68.7, Aspartate 88.0, Proline 88.0, Glutamate 88.0, Serine 80.0 and Glycine 88.0.

[b] Omega Protein, Inc., Houston, Texas USA.

[c] US Biochemical Corporation, Cleveland, Ohio, USA.

[d] Contained (g/kg): Calcium phosphate monobasic 136.00, Calcium lactate 348.49, Ferrous sulfate 5.00, Magnesium sulfate heptahydrate 132.00, Potassium phosphate dibasic 240.00, Sodium phosphate monobasic 88.00, Sodium chloride 45.00, Aluminum chloride 0.15, Potassium iodide 0.15, Cupric sulfate 0.50, Manganous sulfate 0.70, Cobalt chloride1.00, Zinc sulfate heptahydrate 3.00.

[e] Contained (g/kg): Ascorbic acid 50.0, dl-Calcium pantothenate 5.0, Choline chloride 36.2, Inositol 5.0, Menadione sodium bisulfite 2.0, Niacin 5.0, Pyridoxine hydrochloride 1.0, Riboflavin 3.0, Thiamine mononitrate 0.5, dl-Alpha-tocopherol acetate (250 IU/g) 8.0, Vitamin A palmitate (500,000 IU/g) 0.2, Biotin 0.05, Folic Acid 0.18, Vitamin B_{12} 0.002, Cholecalciferol (40 IU/µg) 0.002, Cellulose 883.866.

[f] Selenium premix: 0.1 g sodium selenite and 499.9 g cellulose.

[g] From barley (Sigma Chemical Co., St. Louis, Missouri, USA)

Fish and Feeding Trial

Juvenile hybrid striped bass were provided by a commercial producer (Keo Fish Farms, Keo, Arkansas) and sorted for uniform size. A recirculating culture system consisting of individual 110-L glass aquaria connected to a settling chamber and biofilter was employed. Prior to initiating the feeding trial, fish were fed either the purified or practical basal diets without selenium or β-glucan supplementation for 7 d. After the conditioning period, aquaria were stocked with 15 fish initially averaging 2.44 ± 0.17 g/fish and assigned either purified or practical diets consistent with the conditioning diet. Each of the eight experimental diets were fed to fish in triplicate aquaria for 2 wk at 6% of body weight daily. During the next 2 wk period, fish previously fed glucan-supplemented diets were fed the same basal diet without glucan supplementation. During the last 2 wk of the trial, daily feeding rate was adjusted to 4% of body weight, and fish in the glucan treatments were once again fed glucan-supplemented diets. Sakai (1999) reported that long-term (more than 28 d) use of immunostimulants resulted in a loss of efficacy. Jeney et al. (1997) also reported that continuous feeding of glucan may result in hypersensitivity. For this reason, glucan supplementation was intermittently administered. Fish were fed every morning and evening throughout the course of the conditioning period and feeding trial and they were weighed as a group weekly.

Bacterial Challenge

At the end of the 6-wk period, 10 fish from each replicate aquarium were pooled by dietary treatment and immersed in a 198-L bath containing 6.2×10^6 colony forming units of *S. iniae* /mL for 2 h. The bacterial bath was prepared from a pure

culture of *S. iniae* obtained from the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) and used to inoculate 400-mL of brain-heart infusion broth and incubated for 18 h at 28 C. After incubation, seven bottles containing 400 mL of brain-heart infusion broth were inoculated with 0.2 mL of the bacterial stock solution and incubated for 24 h at 28 C. A total of 2.8 L of bacterial solution was added to 197 L of water to prepare the bath. Air stones were placed in the bath to provide oxygen and water circulation. Fish did not show any visible signs of stress during immersion. After bacterial exposure, fish were redistributed in 38-L flow-through aquaria receiving well water as triplicate groups of 10 fish per dietary treatment, and fed their respective diets once daily to satiation for the first 9 d, and survival rates were monitored through day 21.

Blood Collection and Lysozyme Assay

Blood was collected from three randomly selected fish from each aquarium prior to the bacterial challenge (wk 6) and from the survivors in each tank after 21 d post challenge. Approximately 0.5 ml of blood was collected from the caudal vasculature using a heparin-treated syringe and 27-gauge needle. The blood was then centrifuged and plasma separated and stored at -80 C until lysozyme determination using the turbidimetric assay described by Parry et al. (1965). The amount of enzyme necessary to produce a decrease in absorbance of 0.001/min/mL of plasma was defined as one lysozyme activity unit.

Statistical Analysis

A 2 x 2 x 2 factorial ANOVA was used to evaluate the main effects and any interactions with regard to weight gain, feed efficiency, serum lysozyme, and survival

after challenge. Survival data were arcsine transformed before analysis. The statistical significance was set at $P < 0.05$. SPSS® 11.0 for Windows (Chicago, Illinois, USA) was used for the statistical computation.

Results

At the end of the 6-wk feeding trial, diet type ($P = 0.005$) and glucan supplementation ($P = 0.041$) had significant effects upon final weight gain with the fish meal-based diets supporting more weight gain than the purified diets, and glucan supplementation slightly but significantly suppressed weight gain (Table 2). Diet type ($P = 0.001$) and glucan supplementation ($P = 0.016$) also had significant effects on feed efficiency similar to those observed for weight gain. An interaction between diet type and selenium supplementation was detected for weight gain ($P = 0.045$) and feed efficiency ($P = 0.011$) responses due to positive effects of selenium supplementation in the purified diets but slightly negative effects in the practical diets.

After the controlled exposure to *S. iniae*, fish in most tanks experienced mortality attributed to *S. iniae*. The cause of death was confirmed by the manifestation of clinical signs such as bulging eyes and hemorrhaging around the head and operculum (Stoffregen et al. 1996). Histopathologic diagnosis of tissue by the TVMDL confirmed infection of gram positive cocci. *S. iniae* was also identified in isolates from brain tissue of dead fish. This study was able to induce an infection with a longer exposure time and lower bacterial concentration compared to the challenge protocol of Shoemaker et al. (2000) with tilapia *Oreochromis niloticus*. Diet type ($P < 0.001$) as well as the

TABLE 2. *Cumulative weight gain, feed efficiency and survival of hybrid striped bass fed purified and practical diets with or without selenium or β-glucan supplementation for 6 wk. Values are means of three replicate groups.*

Diet type	Selenite (mg/kg)	Glucan (%)	Weight gain (% of initial weight)	Feed efficiency	Survival (%)
Purified	0.0	0.0	388	0.62	100
Purified	0.2	0.0	412	0.64	100
Purified	0.0	0.1	382	0.62	100
Purified	0.2	0.1	399	0.63	100
Practical	0.0	0.0	447	0.66	100
Practical	0.2	0.0	424	0.65	100
Practical	0.0	0.1	412	0.65	100
Practical	0.2	0.1	405	0.63	100
ANOVA, $P > F$					
Diet type			0.005	0.001	
Selenium			NS	NS	
Glucan			0.041	0.016	
Selenium x Glucan			NS	NS	
Diet type x Selenium			0.045	0.011	
Diet type x Glucan			NS	NS	
Diet type x Selenium x Glucan			NS	NS	
Pooled SE[a]			11.54	0.62	0.0

[a] Pooled standard error = $\sqrt{mean\ square\ error / number\ of\ replicates}$ (Baker 1986).

interaction between diet type and selenite supplementation ($P = 0.001$) had significant effects on survival after exposure to *S. iniae* (Table 3).

A significant interaction ($P = 0.041$) between selenite and glucan supplementation on plasma lysozyme was detected after the 6 wk feeding trial (Table 3). However, after *S. iniae* exposure, no significant effects on plasma lysozyme activity were found for any dietary factors or their interaction.

Discussion

Diet type had a significant effect on weight gain and feed efficiency of hybrid striped bass in the current study. The slight but significantly better weight gain and feed efficiency of hybrids fed the fish meal-based diets compared to the purified diets was not unexpected given that most fish species utilize diets containing intact protein much more efficiently than those containing crystalline amino acids (NRC 1993). However, in contrast to many other fish species, hybrid striped bass have been observed to use diets with high levels of crystalline amino acids almost as well as diets containing only intact protein (Keembiyehetty and Gatlin 1993). It also is not uncommon for practical diets to be more palatable compared to purified diets, and this also may have contributed to the better performance of fish fed the practical diets.

The naturally occurring selenium for the fish meal based-diet was 1.032 mg selenium/kg compared to 0.033 mg selenium/kg in the purified diet. Fish fed the purified selenium-supplemented diets tended to have higher weight gain and feed efficiency values compared to those fed diets without selenium supplementation.

TABLE 3. *Survival and plasma lysozyme activity of hybrid striped bass fed purified and practical diets with or without selenium or β-glucan supplementation for 6 wk then exposed to* S. iniae *and monitored for 21 d. Values are means of three replicate groups.*

Diet type	Selenite (mg/kg)	Glucan (%)	Pre-exposure plasma lysozyme[a]	Post-exposure plasma lysozyme[a]	Survival (%)
Purified	0.0	0.0	162	108	26.7
Purified	0.2	0.0	107	274	50.0
Purified	0.0	0.1	86	116	20.0
Purified	0.2	0.1	145	255	43.3
Practical	0.0	0.0	259	419	83.3
Practical	0.2	0.0	104	430	66.7
Practical	0.0	0.1	169	320	83.3
Practical	0.2	0.1	207	154	66.7
ANOVA, $P > F$					
Diet type			NS	NS	<0.001
Selenium			NS	NS	NS
Glucan			NS	NS	NS
Selenium x Glucan			0.041	NS	NS
Diet type x Selenium			NS	NS	0.001
Diet type x Glucan			NS	NS	NS
Diet type x Selenium x Glucan			NS	NS	NS
Pooled SE[b]			49.03	103.40	6.77

[a] One lysozyme unit is defined as the amount of enzyme necessary to produce a decrease in absorbance of 0.001/min/mL of plasma.
[b] Same as Table 2.

Apparently the purified diet without selenium supplementation was deficient such that the addition of 0.2 mg sodium selenite/kg diet enhanced weight gain. However, supplementation of sodium selenite to the fish meal-based diets did not provide a similar enhancement due to their higher endogenous level of selenium. Selenium has been established as an essential nutrient in other fish species such as rainbow trout (Hilton et al. 1980) and channel catfish *Ictalurus punctatus* (Gatlin and Wilson 1984).

In the present study, the incorporation of glucans into the diet slightly reduced weight gain and feed efficiency but did not have an effect on disease resistance or plasma lysozyme activity. However, dietary supplementation with β-glucan and selenium did show an interaction ($P = 0.0411$) with basal composition on plasma lysozyme levels prior to exposure to *S. iniae*. None of the dietary factors had a significant effect upon lysozyme activity 21 d after the bacterial challenge.

Efthimiou (1996) reported that weight gain, feed efficiency, and serum lysozyme of dentex *Dentex dentex* were not affected by feeding commercial glucan products derived from yeast or fungi; whereas, mortality from a protozoan parasite was significantly reduced in fish fed either glucan product. These results contrast somewhat with those of Jeney et al. (1997) in which rainbow trout fed 0.1, 0.5 or 1.0% glucan (origin not disclosed) had enhanced phagocytosis and oxidative radical production. A spontaneous infection of *F. columnaris* in that study resulted in 10% mortality in fish fed 0 and 0.5% glucan, 60% mortality in fish fed 1.0% glucan, and no mortality in fish fed 0.1% glucan. Other studies with various fish species have reported increased serum lysozyme and other immune responses due to dietary glucans (e.g., Yano et al. 1989;

Chen and Ainsworth 1992; Engstad et al. 1992; Matsuyama et al. 1992; Dalmo 1996; Santarem et al. 1997). Additionally, increased oxidative capacity of phagocytic cells has been observed as a means by which β-glucans may enhance non-specific immune responses (Anderson and Siwicki 1994; Dalmo et al. 1996). Channel catfish fed diets with either 0.2% β-glucan or 2.7% of the yeast *Saccharomyces cerevisiae* had enhanced macrophage and neutrophil migration and phagocytosis but not increased resistance to infection by *Edwardsiella ictaluri* (Duncan and Klesius 1996). In consideration of these previous studies, substantial variation has been observed in the ability of glucans to enhance disease resistance of various fish species (Wang and Wang 1996, 1997).

In the present study, the most significant factor associated with improved fish survival after exposure to *S. iniae* was the type of basal diet with the practical diets affording the highest survival rate compared to the purified diets. However, selenium supplementation did not improve survival after bacterial exposure. This is in contrast to the results of Wang et al. (1997) who reported that channel catfish survival against *E. ictaluri* was improved with sodium selenite supplementation to provide 0.4 mg selenium/kg diet. The level of selenium supplementation in the present study, especially to the purified diet, may not have been sufficient to exert a beneficial effect.

In summary, dietary supplementation of β-glucan alone did not enhance disease resistance or plasma lysozyme activity of hybrid striped bass. However, a menhaden fish meal-based diet with or without supplemental selenium provided significantly higher survival after *S. iniae* exposure in comparison to purified diets. Thus, influences of basal

diet type and selenium supplementation on disease resistance of hybrid striped bass were most evident in this study and warrant further investigation.

CHAPTER III

SELENIUM AND VITAMIN E INTERACTIONS

Selenium is an essential trace mineral for many animal species (Lall 2002). Despite being in different nutritional categories, selenium and vitamin E share complementary biochemical functions. Selenium is an integral component of glutathione peroxidase which catalyzes the removal of metabolic peroxides to protect the cells and membranes from oxidative damage (Watanabe et al. 1997; Lovell 1998). Vitamin E is referred to as a metabolic free radical or peroxide scavenger which stabilizes highly unsaturated fatty acids such as arachidonic acid in membrane lipids, inhibits phospholipase A2, and prevents excessive activity of superoxide anions and hydrogen peroxide in phagocytes (Sohn et al. 2000).

To date, researchers have investigated the nutritional responses to dietary selenium in a few fish species. When dietary selenium was deficient, growth depression was observed in rainbow trout *Oncorhynchus mykiss* (Hilton et al. 1980) and channel catfish *Ictalurus punctatus* (Gatlin and Wilson 1984). Lall (2002) reported that adequate dietary selenium and vitamin E was required to prevent muscular dystrophy in Atlantic salmon *Salmo salar* (Poston et al. 1976) and exudative diathesis in rainbow trout (Bell et al. 1985) and channel catfish (Gatlin et al. 1986). Walsh et al. (1990) reported the effects of deficient vitamin E and selenium intake on glutathione peroxidase activity of various tissues in cattle.

The interaction of selenium and vitamin E with regard to immune responses has been reported for various terrestrial animals such as rat (Levander et al. 1995; Noaman et

al. 2002; Yilmaz et al. 1997), swine (Lessard et al. 1991), and cattle (Walsh et al. 1990). In fish, this interaction has been studied to various extents in rainbow trout (Poston et al. 1976; Blazer and Wolke 1984), channel catfish (Gatlin et al. 1986), Atlantic salmon (Salte et al. 1988), and Nile tilapia *Oreochromis niloticus* (Kim et al. 2003). Salte et al. (1988) and Kim et al. (2003) were unable to show any increase in immune resistance to disease challenges with introduction of these two nutrients.

Kocabas (1996) investigated the effect of dietary selenium and vitamin E interaction in hybrid striped bass *Morone chrysops* × *M. saxatilis* but did not produce overt signs of selenium deficiency in the presence or absence of vitamin E. This study was conducted to further evaluate dietary vitamin E and selenium interactions in hybrid striped bass based on production indices of percent weight gain, feed efficiency, and survival as well as blood neutrophil oxidative radical production as a measure of non-specific immunity, and final whole-body selenium concentration.

Materials and Methods

Experimental Diets

Four diets, in a 2x2 factorial arrangement, composed of purified crystalline amino acids and casein/gelatin as protein sources were formulated (Table 4). The crude protein and lipid levels were formulated at 35% and 6%, respectively, for all diets. The lipid sources included menhaden oil and tocopherol-free corn oil. With the exception of selenium and vitamin E, all other known nutritional requirements of hybrid striped bass were met by the experimental diets. The basal diet was formulated without any

TABLE 4. *Formulations (g/kg on a dry-matter basis) for the basal diet and those supplemented with vitamin E (Vit. E), selenium (Se) or both.*

	Diet Designation			
Ingredient	Basal	+ Vit. E	+ Se	+ Se/+ Vit. E
Amino acid premix[a]	249	249	249	249
Casein[b]	90	90	90	90
Gelatin[b]	18	18	18	18
Dextrin[b]	250	250	250	250
Selenium-free mineral premix[c]	40	40	40	40
Vitamin E-free Vitamin premix[d]	30	30	30	30
Carboxymethyl cellulose[b]	20	20	20	20
Cellulose[b]	243	243	243	243
Menhaden oil[e]	30	30	30	30
Alpha-tocopherol-free corn oil	30	30	30	30
Selenium premix[f]	0	0	10	10
dl-alpha-tocopherol acetate (250 IU/g)	0	0.24	0	0.24

[a] Contained the following L-amino acids (g/kg): Arginine HCl 54.2, Histidine 31.7, Isoleucine 56.2, Leucine 94.4, Lysine 88.7, Methionine 31.3, Cystine 9.2, Phenylalanine 65.9, Tyrosine 62.3, Threonine 57.8, Tryptophan 15.7, Valine 60.6, Aspartate 88.0, Proline 88.0, Glutamate 88.0, Serine 20.0 and Glycine 88.0.

[b] US Biochemical Corporation, Cleveland, Ohio, USA.

[c] Same as in Table 1.

[d] Contained (g/kg): Ascorbate polyphosphate 50.0, dl-Calcium pantothenate 5.0, Choline chloride 36.2, Inositol 5.0, Menadione sodium bisulfite 2.0, Niacin 5.0, Pyridoxine hydrochloride 1.0, Riboflavin 3.0, Thiamine mononitrate 0.5, Vitamin A palmitate (500,000 IU/g) 0.2, Biotin 0.05, Folic Acid 0.18, Vitamin B_{12} 0.002, Cholecalciferol (40 IU/μg) 0.002, Cellulose 891.866.

[e] Omega Protein, Inc., Houston, Texas USA.

[f] Selenium premix: 0.1 g sodium selenite and 499.9 g cellulose.

supplemental selenium. Two diets were singularly supplemented with either vitamin E from dl-alpha-tocopherol acetate (60 mg/kg diet) or selenium from sodium selenite (1 mg selenium/kg). The supplemental vitamin E level satisfied the minimum requirement of 28 mg/kg diet previously established for hybrid striped bass by Kocabas and Gatlin (1999). The supplemental selenium level was based on established requirements of other fish species (Nutritional Research Council 1993). A fourth diet was supplemented with both vitamin E and selenium. The supplemental selenium and vitamin E premixes were substituted for cellulose. Diets not supplemented with vitamin E or selenium were found to contain 8 IU of total tocopherols/kg and 0.09 mg of selenium/kg, respectively. The analyzed total tocopherols content for the vitamin E-supplemented diets was 75 IU/kg diet. The selenium supplemented diets were analyzed to contain an average of 1.3 mg selenium/kg diet. Selenium analysis was by hydride generation and a flame atomic absorption spectrophotometer (Agricultural Analytical Services 2001).

Fish and Feeding Trial

Juvenile hybrid striped bass were provided by a commercial producer (Keo Fish Farms, Keo, Arkansas) and sorted to uniform size. A recirculating culture system consisting of individual 110-L glass aquaria connected to a settling chamber and biofilter was employed. Prior to initiating the feeding trial, fish were conditioned on the basal diet for 13 d. After the conditioning period, aquaria were stocked with 15 fish initially averaging 2.92 ± 0.12 g/fish. Each experimental diet was fed to fish in triplicate aquaria for 2 wk at 7% of body weight daily. During the next 5-wk period, fish were fed at 6% of body weight daily. During the following 2-wk period, fish were fed at 5% of body

weight daily after which the daily feeding rate was adjusted to 4% of body weight for 1-wk and then to 3% of body weight for the remaining 2 wk. Fish were fed every morning and evening throughout the course of the conditioning period and feeding trial, and they were weighed as a group weekly. At the end of the 12-wk period, weight gain, feed efficiency and survival were computed.

Blood Collection, Neutrophil Oxidative Radical Production Assay and Whole-body Selenium Analysis

At the end of the feeding trial, three randomly selected fish from each aquarium were anesthetized with tricane methane sulfonate for blood collection. Approximately 0.5 ml of blood was obtained from the caudal vasculature using a heparin-treated syringe and 27-gauge needle. Neutrophil oxidative radical production was determined as a measure of the non-specific immune response according to the procedures described by Siwicki et al. (1994) and Li and Gatlin (2003) using nitroblue tetrazolium (NBT).

Three additional anesthetized fish also were randomly selected from each aquarium and homogenized for whole-body selenium analysis. Tissue selenium analysis was by hydride generation using a flame atomic absorption spectrophotometer (Agricultural Analytical Services 2001).

Statistical Analysis

A 2 x 2 factorial ANOVA was used to evaluate the main effects and any interactions with regard to weight gain, feed efficiency, survival, the NBT test and whole-body selenium concentration. The statistical significance was set at $P < 0.05$.

SPSS® 11.0 for Windows (Chicago, Illinois, USA) was used for the statistical computation.

Results

Table 5 provides a summary of the performance data, as well as the NBT test and whole-body selenium concentration of hybrid striped bass fed the various diets. It should be noted that at the end of the seventh week, all fish in one replicate of the fully supplemented diet died due to an undetermined cause. The lowest weight gain, as a percent of the initial weight, was 519% for fish fed the diet supplemented with only vitamin E. The diet supplemented with both selenium and vitamin E had the highest weight gain of 680%. No significant main effects of selenium or vitamin E or interaction between selenium and vitamin E were detected by the factorial ANOVA. Selenium supplementation did show a marginal effect on weight gain at $P = 0.09$. Likewise, feed efficiency ranged from 0.42 to 0.48, for fish fed the vitamin E supplemented diet and the fully supplemented diet, respectively. However, no significant effect of selenium or vitamin E supplementation, or interaction between these two factors was detected by the factorial ANOVA.

The lowest survival rate of 73.3% was observed for fish fed the diet supplemented only with vitamin E. The highest survival rate was 86.7% obtained by fish fed the diet supplemented only with selenium. Even though the ANOVA did not show any significant effects of the factors or interaction, selenium supplementation had a *P*-value of 0.08.

TABLE 5. *Cumulative weight gain, feed efficiency, survival, blood neutrophil oxidative production (NBT test) and whole-body selenium concentration of hybrid striped bass fed purified diets with or without sodium selenite or vitamin E supplementation for 12 wk*[a].

Supplemental		Weight gain (% of initial	Feed efficiency	Survival	NBT test	Whole-body selenium
Selenium	Vitamin E	weight)	(g gain/g feed)	(%)	(mg/ml)[b]	(mg/kg)
–	–	599	0.44	75.6	0.956	0.087
–	+	519	0.42	73.3	0.956	0.070
+	–	646	0.47	86.7	0.886	0.153
+	+	680	0.48	83.3	0.871	0.135
ANOVA, $P > F$						
Selenium		0.09	0.12	0.08	NS	0.001
Vitamin E		NS	NS	NS	NS	NS
Selenium x Vitamin E		NS	NS	NS	NS	NS
Pooled SE[c]		49.8	0.026	4.86	0.224	0.026

[a] Values are means of three replicate groups except for the fully supplemented diet which only had two replicates.
[b] mg NBT/ml.
[c] Pooled standard error = $\sqrt{mean\ square\ error / number\ of\ replicates}$ (Baker 1986).

Fish fed both diets without any selenium supplementation had the highest NBT test values of 0.956 although no significant effects from selenium or vitamin E supplementation or their interaction was found. The fully supplemented diet had the lowest NBT test value at 0.871.

Fish fed diets without any selenium supplementation had lower whole-body selenium concentration values averaging 0.079 mg selenium/kg body weight. Those fed the selenium-supplemented diets had an average of 0.144 mg selenium/kg body weight. The ANOVA detected a significant ($P = 0.001$) effect of selenium supplementation on whole-body selenium concentration. However, no significant main effect of vitamin E or an interaction between vitamin E and selenium was detected.

Discussion

Kocabas (1996) conducted a 12-wk feeding trial evaluating the possible interaction between vitamin E and selenium in hybrid striped bass. Vitamin E supplementation, at 60 mg/kg diet, had a significant effect on both weight gain ($P = 0.0014$) and feed efficiency ($P = 0.0004$). For survival, there was no effect of the factors or interaction. Selenium supplementation of 0.2 mg/kg diet or interaction between vitamin E and selenium did not have a significant effect on either of these parameters. In contrast, the present study did not detect any significant effects of the main factors or interactions between any of the above growth indices. However the dietary selenium was considerably higher at 1 mg/kg in the present study. The whole-body selenium was significantly affected by the selenium supplementation; whole-body

selenium was not measured by Kocabas (1996). For hybrid striped bass, these two investigations did not demonstrate any significant interaction between dietary selenium and vitamin E supplementation.

In channel catfish, Gatlin et al. (1986) revealed selenium and vitamin E interactions in a 26-wk experiment. Selenium supplementation level was identical to that of Kocabas (1996) while vitamin E supplementation was similar at 50 mg/kg diet. A selenium and vitamin E deficiency was exhibited in the basal diet with a survival rate of 69.1 % compared to 100% for the supplemented diets. In addition, weight gain and hematocrit were lower and significantly different ($P < 0.01$) for the basal diet.

Diets without any added selenium, vitamin E or either of these nutrients caused dietary deficiencies within 4-wk of being fed to Atlantic salmon (Poston et al. 1976). A reduction in mortality was observed within 2-wk of supplementing the diets with their respective deficient nutrients of selenium (0.1 mg/kg diet), vitamin E (500 IU /kg diet), or combination of both.

Kim et al. (2003) investigated the effects of dietary supplementation of selenium, alpha-tocopherol acetate, and ascorbic acid in Nile tilapia. This 10-wk feeding trial found that weight gain and feed efficiency were significantly ($P < 0.05$) higher for diets with 150 mg ascorbic acid/kg, 240 mg alpha-tocopherol acetate/kg and 0.2 mg selenium/kg and 2,000 mg ascorbic acid/kg, 240 mg alpha-tocopherol acetate/kg and 0.5 mg selenium/kg compared to the control diet with 150 mg ascorbic acid/kg, 100 mg alpha-tocopherol acetate/kg diet and 0.2 mg selenium/kg diet and 150 mg ascorbic acid/kg, 100 mg alpha-tocopherol acetate/kg diet and 0.5 mg selenium/kg diet. At the

end of the feeding trial, fish were challenged with *Edwardsiella tarda* and no significant difference in mortality was found between any of the diets.

In an effort to evaluate the use of selenium and vitamin E by means other than dietary supplementation, intraperitoneal injections of these two nutrients were administered to Atlantic salmon with "Hitra disease" by Salte et al. (1988). As with Kim et al. (2003), the use of selenium and vitamin E to mitigate the negative effects of exposure to disease were not shown.

Despite the significant effect of dietary selenium on whole-body selenium concentration, the present study did not show any significant responses in weight gain, feed efficiency, survival or non-specific immune response based on the NBT test associated with dietary supplementation of selenium, vitamin E or an interaction between these two factors. These findings are similar to those of Kocabas (1996) in hybrid striped bass and Kim et al. (2003) in Nile tilapia. In addition, dietary supplementation (Kim et al. 2003) or intraperitoneal administration (Salte et al. 1988) of these two nutrients did not provide any additional protection against disease.

CHAPTER IV

SELENIUM ESSENTIALITY, TOXICITY, TISSUE DEPOSITION AND ESTIMATED DIETARY REQUIREMENTS

The dietary essentiality of selenium was demonstrated in rats by Schwarz and Foltz (1957). These researchers reported necrotic liver degeneration due to a selenium deficiency. Because selenium is a component of glutathione peroxidase (GSH-Px), inadequate selenium levels reduce the amount of GSH-Px in an organism. The reduction of available GSH-Px results in the onset of selenium deficiency signs. In fish, selenium deficiency signs including reduced GSH-Px activity, poor growth, anemia, cataracts, muscular dystrophy, and exudative diathesis were summarized by Lall (2002) for various species.

For most animals, the dietary selenium requirement is between 0.05 to 0.30 mg selenium/kg. Schwarz and Foltz (1957) estimated the dietary selenium requirement for the rat to be less than 0.03 mg/kg diet. In fish, the dietary selenium requirement has been quantified to range between 0.15 to 0.38 mg/kg based on optimum growth and maximum plasma GSH-Px activity (NRC 1993). Supplementing diets with either sodium selenite or selenomethionine at 1 or 2 mg selenium/kg for Atlantic salmon *Salmo salar* promoted growth and maintained hepatic GSH-Px activity (Lim et al. 2001b). Lovell (1998) recommended formulation inclusion rates of 0.03, 0.10 and 0.30 mg selenium/kg diet in salmonid, catfish and hybrid striped bass diets, respectively.

Despite selenium being an essential nutrient it is toxic in excessive quantities. Franke and Painter (1936) reported selenium toxicity in animals due to high selenium

content naturally occurring in some plant feedstuffs. They found some grains grown in seleniferous soils to contain approximately 30 mg selenium/kg. Schwarz and Foltz (1957) stated that the chronic toxic level of dietary selenium was 3 to 4 mg selenium/kg for the rat. The feeding of razorback sucker *Xyrauchen texanus* larvae on zooplankton with naturally occurring selenium concentrations of 4.6 mg/g or greater caused a reduction in their survival (Hamilton et al. 2002). Hilton et al. (1980) reported that a dietary selenium concentration of 13 mg/kg produced toxic effects in rainbow trout *Oncorhynchus mykiss*. Toxicity was expressed as reduced growth rate, poor feed efficiency and increased mortalities. Hilton and Hodson (1983) observed an increase in renal calcinosis as well as a decrease in growth and feed efficiency for rainbow trout fed diets containing 10 mg selenium/kg and high available carbohydrates. Hicks et al. (1984) supplemented diets with 11.4 mg selenium/kg and observed induced renal calcinosis, poor growth and feed efficiency, and increased mortality. Gatlin and Wilson (1984) also observed a reduction in growth and feed efficiency of channel catfish *Ictalurus punctatus* attributed to the toxic effects of selenium at 15 mg selenium/kg diet.

The NRC (1983) provided an overview of selenium absorption and retention. It reported that in monogastric animals the largest amount of selenium is absorbed in the last part of the small intestine, the cecum and colon with essentially no absorption in the stomach. Of the selenium that is absorbed, the largest portion is concentrated in the kidneys, followed by the liver and other glandular tissues. Gatlin and Wilson (1984) found a linear correlation between muscle selenium concentrations and dietary selenium concentration in channel catfish. Rainbow trout kidney, liver and carcass selenium

levels also were strongly correlated with dietary selenium levels (Hilton and Hodson 1983); Hilton et al. (1980) reported similar results with rainbow trout kidney and liver tissues.

In the present study, three experiments were undertaken to establish selenium as an essential trace mineral, as well as characterize its tissue deposition, toxicity and estimate the dietary selenium requirement of hybrid striped bass *Morone chrysops* × *M. saxatilis* based on weight gain, feed efficiency, survival, liver GSH-Px activity and whole-body selenium balance.

Materials and Methods

Experimental Diets

For the following three experiments (labeled 3-5), all diets were composed of purified crystalline amino acids and casein/gelatin as protein sources with the crude protein and lipid levels formulated at 35% and 6%, respectively. In each feeding trial, a basal diet was formulated to meet the known nutritional requirements of hybrid striped bass, except for selenium. This basal diet was analyzed to contain 0.1 mg selenium/kg on a dry matter basis. Selenium premixes, with cellulose as the diluent, replaced the cellulose portion of the basal formulation to provide the targeted selenium levels.

The basal diet formulation shown in Table 4, of Chapter III, is the same basal diet utilized in Experiments 3 and 4. For Experiment 3, the basal diet was fortified with four levels of a sodium selenite premix and analyzed to contain 1.19, 2.00, 5.17 and 21.23 mg selenium/kg on a dry-matter basis. For Experiment 4, the basal diet was

supplemented with three levels of selenium from seleno-DL-methionine. The analyzed selenium concentrations of these diets were 0.90, 1.26 and 2.55 mg selenium/kg.

For Experiment 5, the basal diet (Table 6) was slightly modified in its amino acid composition based on refined understanding of the hybrid striped bass requirements and fortified with either sodium selenite, seleno-DL-methionine or selenium yeast to provide approximately one, two and four times the analyzed basal level of 0.11 mg selenium/kg.

Fish and Feeding Trial

For experiments 3 and 5, juvenile hybrid striped bass were provided by a commercial producer (Keo Fish Farms, Keo, Arkansas) and sorted for uniform size. A recirculating culture system consisting of individual 110-L glass aquaria connected to a settling chamber and biofilter was employed. Prior to initiating the feeding trial, fish were conditioned on the basal diet for 13-d.

For Experiments 3 and 4, after a 13-d conditioning period, aquaria were stocked with 15 fish initially averaging 2.94 ± 0.13 g/fish and 2.92 ± 0.12 g/fish, respectively. Each experimental diet was fed to fish in triplicate aquaria for 2 wk at 7% of body weight daily followed by 6% of body weight during the next 5-wk period. During the next 2-wk period, fish were fed 5% of body weight daily and then 4% of body weight for one week followed by 3% of body weight for the remaining 2-wk period. Fish were fed every morning and evening throughout the course of the conditioning period and feeding trial and weighed by tank as a group weekly.

For Experiment 5, fish were conditioned for 7-wk on the basal diet to deplete tissue stores of selenium. After the conditioning period, aquaria were stocked with fish

TABLE 6. *Basal formulation, on a dry-matter basis, for Experiment 5.*

Ingredient	g/kg
Amino acid premix[a]	249.0
Casein[b]	90.0
Gelatin[b]	18.0
Dextrin[b]	250.0
Selenium-free mineral premix[c]	40.0
Vitamin premix[d]	30.0
Carboxymethyl cellulose[b]	20.0
Cellulose[b]	243.0
Menhaden oil[e]	60.0

[a] Contained the following L-amino acids (g/kg): Arginine HCl 54.2, Histidine 31.7, Isoleucine 56.2, Leucine 94.4, Lysine 88.7, Methionine 31.3, Cystine 9.2, Phenylalanine 65.9, Tyrosine 62.3, Threonine 57.8, Tryptophan 15.7, Valine 60.6, Aspartate 88.0, Proline 88.0, Glutamate 88.0, Serine 20.0 and Glycine 88.0.

[b] US Biochemical Corporation, Cleveland, Ohio, USA.

[c] Contained (g/kg): Calcium phosphate monobasic 136.00, Calcium lactate 348.49, Ferrous sulfate 5.00, Magnesium sulfate heptahydrate 132.00, Potassium phosphate dibasic 240.00, Sodium phosphate monobasic 88.00, Sodium chloride 45.00, Aluminum chloride 0.15, Potassium iodide 0.15, Cupric sulfate 0.50, Manganous sulfate 0.70, Cobalt chloride 1.00, Zinc sulfate heptahydrate 3.00.

[d] Contained (g/kg): Ascorbic acid 50.0, dl-Calcium pantothenate 5.0, Choline chloride 36.2, Inositol 5.0, Menadione sodium bisulfite 2.0, Niacin 5.0, Pyridoxine hydrochloride 1.0, Riboflavin 3.0, Thiamine mononitrate 0.5, dl-Alpha-tocopherol acetate (250 IU/g) 8.0, Vitamin A palmitate (500,000 IU/g) 0.2, Biotin 0.05, Folic Acid 0.18, Vitamin B_{12} 0.002, Cholecalciferol (40 IU/μg) 0.002, Cellulose 883.866.

[e] Omega Protein, Inc., Houston, Texas USA.

initially averaging 3.62 ± 0.28 g. Due to the number of fish available and to achieve a uniform size within each experimental unit, either 14 or 15 fish were stocked per aquarium. Each experimental diet was fed to fish in triplicate aquaria for 3 wk at 6% of body weight daily and then 5% of body weight daily for the next 4-wk period. Feeding rate was further adjusted to 4% of body weight for one week and then to 3% of body weight for the remaining 4-wk period. Fish were fed every morning and evening throughout the course of the conditioning period and feeding trial, and fish in each tank were weighed as a group weekly.

Tissue Collection

Prior to tissue and blood collection, fish were anesthetized with tricane methane sulfonate (100 mg/L). For all experiments, three fish per aquarium (9 fish per dietary treatment) were randomly selected and homogenized for whole-body selenium analysis. All tissues from fish in each aquarium were combined, and homogenized prior to analysis.

For Experiment 5, selenium content of liver and muscle tissues also was determined for three additional fish per aquarium that were randomly selected. The liver and muscle tissues were frozen at -80 C and then finely ground prior to selenium analysis. Tissue for the liver GSH-Px activity was stored at -80 C until it could be assayed as described by Gatlin and Wilson (1984).

Statistical Analysis

ANOVA was used to evaluate the main effects and any interactions with regard to weight gain, feed efficiency, survival, and non-specific immune responses. Mean

separation procedures included Duncan's multiple range test and a 2-sided Dunnett's t test. Linear regressions were based on whole-body retention as described by Shiau and Ning (2003), and broken-line analysis for liver GSH-Px activity (Robbins et al. 1979; Robbins 1986) was used to estimate requirements. The statistical significance was set at $P < 0.05$. SPSS® 11.0 for Windows (Chicago, Illinois, USA) was used for all statistical computations.

Results

Experiment 3

Cumulative weight gain, feed efficiency and survival of hybrid striped bass fed purified diets containing total selenium levels of 1.19, 2.00, 5.17 or 21.23 mg selenium/kg from sodium selenite for 12-wk are shown in Table 7. In this experiment, the level of dietary selenium supplementation had a significant ($P < 0.001$) effect on cumulative weight gain, feed efficiency, and survival. Fish fed the lowest supplemental selenium level (1.19 mg selenium/kg) had the highest cumulative weight gain at 680%; whereas, those fed the diet supplemented over 20 mg selenium/kg produced the lowest weight gain at 68%, with significant differences detected among treatments. One tank of fish fed the 1.19 mg selenium/kg diet was lost due to a failure in the aeration system. A similar pattern as weight gain was seen in the feed efficiency ratios with 0.12 and 0.48 being the lowest and highest, respectively. The diet with 5.17 mg selenium/kg yielded the highest survival rate of 86.7%; whereas, fish fed the diet with the highest selenium concentration had the poorest survival at 17.8% by the end of the 12-wk period.

TABLE 7. *Cumulative weight gain, feed efficiency and survival of hybrid striped bass fed purified diets with various levels of selenium from sodium selenite for 12 wk in Experiment 3*[1].

Analyzed selenium (mg/kg)	Weight gain (% of initial weight)	Feed efficiency	Survival (%)
0.10	519 [b, 2]	0.42	73.3
1.19	680 [c, 2]	0.48	83.3
2.00	560 [b, c, 2]	0.46	75.6
5.17	607 [b, c, 2]	0.45	86.7
21.23	68 [a, *, 2]	0.12 [a, *, 2]	17.8 [a, *, 2]
ANOVA, $P > F$			
	<0.001	<0.001	<0.001
Pooled SE[3]	36.7	0.026	6.77

[1]Values are means of three replicate groups except for the 1.19 mg selenium/kg diet which only had two replicates.

[2]The asterisk indicates a significant ($P < 0.05$) difference from the basal diet without any selenium supplementation, determined by the Dunnett's t test. Similar alphabetic superscripts indicate no significant ($P < 0.05$) difference between dietary selenium levels based on Duncan's multiple range test.

[3]Pooled standard error = $\sqrt{mean\ square\ error / number\ of\ replicates}$ (Baker 1986).

Table 8 presents selenium intake and whole-body selenium balance for fish in Experiment 3. The relationship between dietary selenium concentration and whole-body selenium retention is shown for all dietary treatments except the one with the highest selenium concentration (Figure 1). The regression line and equation shown in Figure 1 predicts a dietary selenium requirement of 0.195 mg selenium/kg when sourced from sodium selenite.

Experiment 4

The cumulative weight gain, feed efficiency and survival of hybrid striped bass fed purified diets containing dietary selenium levels of 0.10, 0.90, 1.26 and 2.55 mg selenium/kg from seleno-DL-methionine for 12-wk are shown in Table 9. Fish fed the basal diet had the lowest cumulative weight gain at 519% while those fed the diet with 0.90 mg selenium/kg had the highest gain of 690%. The means for these two diets were significantly different based on Duncan's multiple range test. Dietary selenium level tended to have a significant effect on weight gain with a *P*-value of 0.076. The feed efficiency ranged from 0.42 to 0.49 for the basal diet and the lowest selenium supplemented diet with a *P*-value of 0.1 detected. Survival ranged from 73.3%, for fish fed the basal diet, to 84.4% for those fed the 0.90 and 2.55 mg selenium/kg and the means were not significantly different.

Table 10 presents selenium intake and whole-body selenium balance for fish fed the various diets in Experiment 4. Regression analysis of dietary selenium concentration, from seleno-DL- methionine, against whole-body selenium retention predicted a dietary selenium requirement of 0.059 mg selenium/kg (Figure 2).

TABLE 8. W*hole-body selenium (Se) balance of hybrid striped bass fed diets containing various levels of Se from sodium selenite for 12 wk in Experiment 3. Values are means of three replicate groups except the 1.19 mg Se/kg group which had two replicates.*

Analyzed dietary (mg Se/kg)	Initial whole-body Se content (µg)	Total Se gained (µg)	Total dietary Se fed (µg)	Whole-body Se retention (µg)
0.10	30.61	58.83	54.93	3.90
1.19	31.11	122.29	592.61	-470.32
2.00	30.93	226.67	1,096.20	-869.53
5.17	27.72	429.04	2,939.04	-2,510.00
21.23	34.76	400.83	4,894.11	-4,493.28

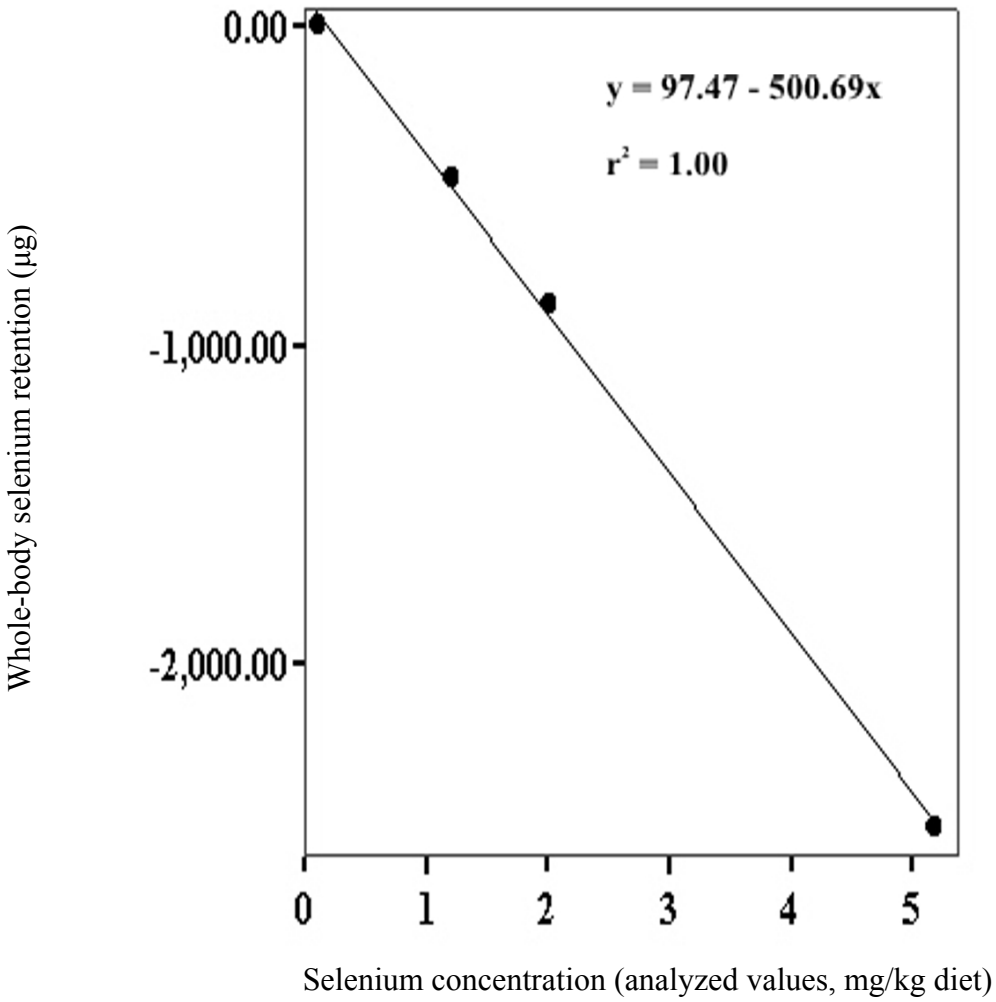

FIGURE 1. *Relationship between dietary selenium concentration and whole-body selenium retention from sodium selenite in Experiment 3. Each point represents the mean of three replicate groups of fish except the 1.19 mg Se/kg group which had two replicates.*

TABLE 9. *Cumulative weight gain, feed efficiency and survival of hybrid striped bass fed purified diets with various levels of selenium from seleno-DL-methionine for 12 wk in Experiment 4*[1].

Analyzed selenium (mg/kg)	Weight gain (% of initial weight)	Feed efficiency	Survival (%)
0.10	519 [a]	0.42 [a]	73.3
0.90	690 [b]	0.49 [b]	84.4
1.26	550 [a, b]	0.44 [a, b]	75.6
2.55	653 [a, b]	0.47 [a, b]	84.4
ANOVA, $P > F$			
	0.076	0.101	NS
Pooled SE[2]	44.6	0.018	4.30

[1]Values are means of three replicate groups. Means with similar superscripts are not significantly ($P < 0.05$) different selenium levels based on Duncan's multiple range test.

[2]Pooled standard error = $\sqrt{mean\ square\ error / number\ of\ replicates}$ (Baker 1986).

TABLE 10. W*hole-body selenium (Se) balance of hybrid striped bass fed diets containing various levels of Se from seleno-DL-methionine for 12 wk in Experiment 4. Values are means of three replicate groups.*

Analyzed dietary (mg Se/kg)	Initial whole-body Se content (µg)	Total Se gained (µg)	Total dietary Se fed (µg)	Whole-body Se retention (µg)
0.10	30.61	58.83	54.93	3.90
0.90	29.98	264.97	544.90	-279.93
1.26	31.98	313.27	692.05	-378.78
2.55	29.49	719.65	1,482.34	-762.69

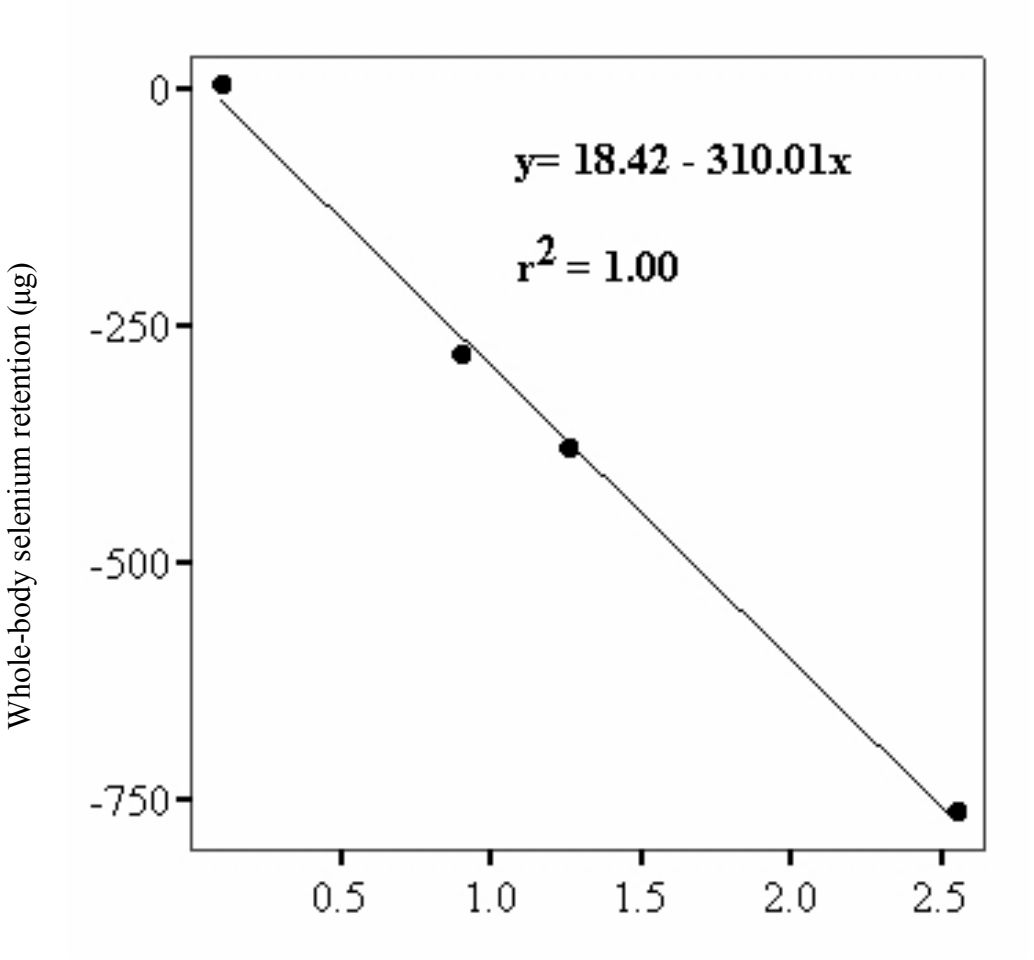

FIGURE 2. *Relationship between dietary selenium concentration from seleno-DL-methionine and whole-body selenium retention in Experiment 4. Each point represents the mean of composite samples of three fish from each three replicate tanks.*

Experiment 5

The basal diet contained 0.11 mg selenium/kg while the lowest selenium supplemented diet (1X) was analyzed to contain approximately 0.15 mg selenium/kg while the 2X and 4X levels averaged 0.252 and 0.558 mg selenium/kg, respectively, based on all three sources. Cumulative weight, feed efficiency, survival and liver GSH-Px activity of hybrid striped bass fed different levels of selenium from the different sources are presented for Experiment 5 (Table 11). By the end of the seventh week, all fish died in one replicate of the 4X selenium yeast treatment due to a mechanical failure of the aeration system in that aquarium. Thus this treatment only had two replicate groups rather than three as in the other treatments. The weight gain values ranged from 475% for fish fed the 2X selenium yeast level to 598% for those fed 1X selenium level from seleno-DL-methionine while the basal diet yielded a weight gain of 587%. Fish fed the 2X supplemented diets with seleno-DL-methionine and selenium yeast, as well as the 4X sodium selenite diet had the lowest feed efficiency values of 0.49 while fish fed the 1X diet from seleno-DL-methionine exhibited the highest feed efficiency at 0.55. Fish fed the basal diet had the highest survival rate of 100% while those fed the 4X supplemented seleno-DL-methionine diet had the lowest at 85.7%. None of these performance indices showed significant effects of selenium level or source based on factorial ANOVA.

Liver GSH-Px activity was significantly affected ($P < 0.001$) by the selenium level. No main effect from selenium source or interaction between level and source was

TABLE 11. Cumulative weight gain, feed efficiency, survival and liver glutathione peroxidase (GSH-Px) activity of hybrid striped bass fed purified diets with various formulated levels of selenium from sodium selenite, seleno-DL-methionine or selenium yeast for 12 wk in Experiment 5[1].

Selenium level	Selenium source	Weight gain (% of initial weight)	Feed efficiency	Survival (%)	Liver GSH-Px activity (nmol of NADPH oxidized/minute/g)
Basal	None added	587	0.53	100.0	0.404
1X	Sodium selenite	581	0.52	95.2	0.478
1 X	Seleno-DL-methionine	598	0.55	95.2	0.450
1 X	Selenium yeast	555	0.53	95.2	0.477
2 X	Sodium selenite	582	0.53	97.6	0.540
2 X	Seleno-DL-methionine	487	0.49	95.2	0.556
2 X	Selenium yeast	475	0.49	90.6	0.538
4 X	Sodium selenite	504	0.49	97.8	0.608
4 X	Seleno-DL-methionine	501	0.52	85.7	0.595
4 X	Selenium yeast	526	0.52	93.3	0.559
ANOVA, P > F					
Level		NS	NS	NS	<0.001
Source		NS	NS	NS	NS
Level x Source		NS	NS	NS	NS
Pooled SE[2]		57.7	0.032	4.67	0.0408

[1]Values are means of three replicate groups except for the 4X selenium yeast diet which had only two replicates.

[2]Pooled standard error = $\sqrt{mean\ square\ error\ /\ number\ of\ replicates}$ (Baker 1986).

detected. The values for liver GSH-Px activity represent the nmol of nicotinamide adenine dinucleiotide phosphate reduced form (NADPH) oxidized per minute per g of liver tissue. The basal diet had the lowest activity level at 0.404. The mean liver GSH-Px activity for selenium supplementation levels 1X, 2X and 4X were 0.4683, 0.5447, and 0.5873, respectively. In Figure 3, liver GSH-Px activity and broken-line analysis was used to calculate dietary selenium supplementation rates of 0.294 mg/kg, 0.207 mg/kg and 0.145 mg/kg for sodium selenite, seleno-DL-methionine and selenium yeast, respectively.

Selenium intake and whole-body selenium balance data for Experiment 5 are presented in Table 12. Figure 4 shows the relationships between dietary selenium concentration and whole-body selenium retention for the three different selenium sources. Regression analysis of dietary selenium against whole-body retention predicted dietary requirements of 0.140 mg selenium/kg, 0.181 mg selenium/kg, and 0.189 mg selenium/kg for sodium selenite, seleno-DL-methionine and selenium yeast, respectively.

Discussion

With the exception of the sodium selenite diet supplemented with over 20 mg selenium/kg in Experiment 3, fish fed the basal diets had the lowest weight gain, feed efficiency and survival for Experiments 3 and 4. There was no significant difference ($P < 0.05$) between fish fed the basal diet and the second and third levels of selenium supplementation for means of weight gain, feed efficiency, and survival. In Experiment

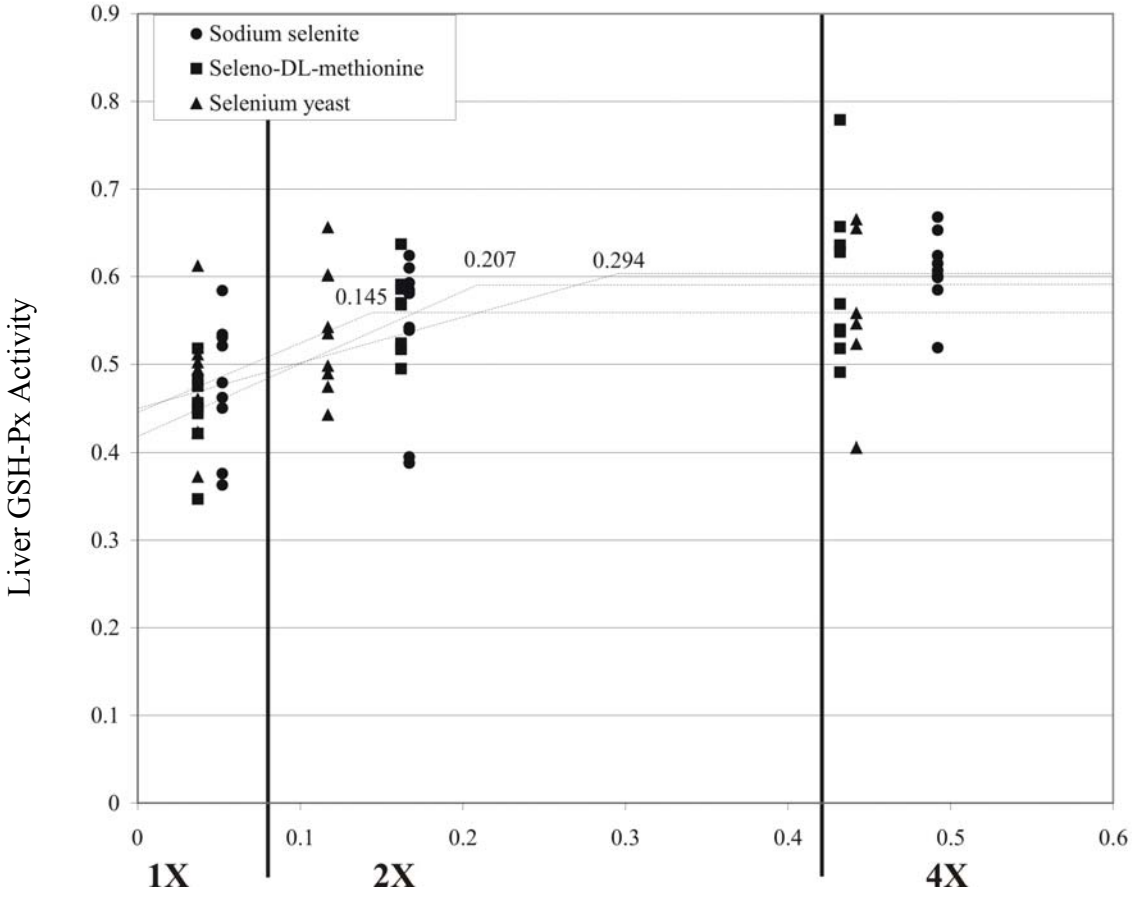

FIGURE 3. *Regression of liver glutathione peroxidase activity (GSH-Px) and dietary selenium concentration from sodium selenite (•), seleno-DL-methionine (■), and selenium yeast (▲). Activity is expressed as the nmol of NADPH oxidized/minute/g liver.*

TABLE 12. Whole-body selenium (Se) balance of hybrid striped bass fed diets containing various levels of Se for 12 wk in Experiment 5. Values are means of three replicate groups except for the 4X of selenium yeast diet which had two replicate groups.

Supplemental Se source	Analyzed dietary (mg Se/kg)	Initial whole-body Se content (µg)	Total Se gained (µg)	Total dietary Se fed (µg)	Whole-body Se retention (µg)
Basal level	0.11	18.83	70.34	62.31	8.03
Sodium selenite/1X	0.17	18.41	82.41	93.86	-11.45
Sodium selenite/2X	0.29	19.77	105.72	170.25	-64.53
Sodium selenite/4X	0.65	19.39	114.77	353.22	-238.45
Seleno-DL-methionine/1X	0.15	18.72	99.17	85.89	13.28
Seleno-DL-methionine/2X	0.29	18.46	121.15	144.02	-22.87
Seleno-DL-methionine/4X	0.59	19.03	221.39	299.65	-78.26
Selenium yeast/1X	0.15	18.88	91.77	83.41	8.36
Selenium yeast/2X	0.24	18.69	123.87	116.60	7.27
Selenium yeast/4X	0.60	20.74	212.79	330.46	-117.67

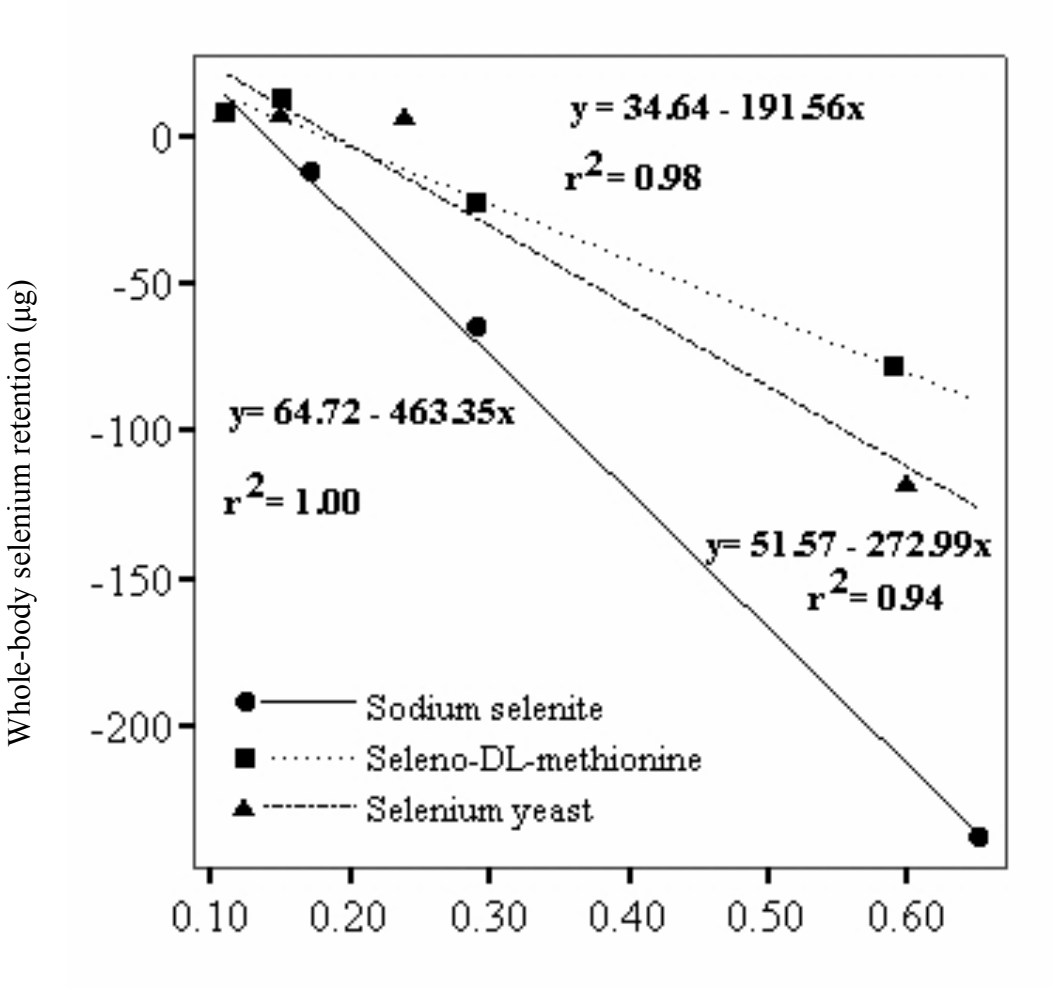

FIGURE 4. *Relationship between dietary selenium concentration and whole-body selenium retention in Experiment 5. Each point represents the mean of composite samples of three fish from each of three replicate tanks except for the highest concentration of selenium yeast which had two replicate groups.*

5, there was no significant difference in measured performance parameters for fish fed any of diets.

In Experiments 3 and 4, the diet with the lowest supplemented level of selenium had the highest mean and significantly different ($P < 0.05$) weight gain and feed efficiency for both sources. Even though the sodium selenite diet had over 30% more selenium than the seleno-DL-methionine diet, the weight gain and feed efficiency were similar. This may be explained in that selenium from organic sources has been found to have higher bioavailability than selenium from inorganic sources (Bell and Cowey 1989; Wang and Lovell 1997; Wang et al. 1997; Wantanabe et al. 1997).

Dietary selenium levels of 2.55 mg/kg and 5.17 mg/kg for seleno-DL-methionine and sodium selenite, respectively, did not have any adverse effects on weight gain, feed efficiency or survival of hybrid striped bass in the present study. Obvious selenium toxicity occurred in the diet containing 21.23 mg selenium/kg from sodium selenite. Weight gain, feed efficiency and survival were significantly ($P < 0.001$) reduced in fish fed this diet compared to the other diets. These finding are consistent with other fish species for which selenium toxicity was observed when dietary selenium content exceeded 10 mg/kg (Gatlin and Wilson 1984; Hicks et al. 1984; Hilton et al. 1980; Hilton and Hodson 1983).

Regression analysis of dietary selenium against whole-body retention predicted an average dietary requirement of 0.17 mg selenium/kg from sodium selenite for Experiments 3 and 5. The mean predicted selenium dietary requirement from seleno-DL-methionine was 0.12 mg selenium/kg for Experiments 4 and 5. The estimated

requirement values for selenium from sodium selenite and seleno-DL-methionine were the same as those for channel catfish based on liver GSH-Px activity (Wang and Lovell 1997). For grouper *Epinephelus malabaricus*, the dietary selenium requirement from selenomethionine was estimated at 0.7 mg/kg based on weight gain and whole-body selenium retention (Lin and Shiau 2005). For selenium yeast, the predicted dietary selenium requirement for hybrid striped bass was 0.189 mg/kg in the present study. Even though selenium yeast had the lowest relative bioavailability, the broken-line analysis indicated that it needed the least supplementation compared to seleno-DL-methionine and sodium selenite. A cause for this inconsistency may be attributed to the wider range of results obtained for liver GSH-Px activity due to the inherent variability of enzyme analysis compared to the measurement of metals. The higher incorporation rate for selenium yeast into whole-body, muscle and liver tissue may have resulted in less selenium being available for GSH-Px. Conversely, because seleno-DL-methionine was utilized more efficiently for GSH-Px activity, less was available for incorporation into tissue mass. Because GSH-Px activity is what affords protection to cells and membranes from oxidative damage by catalyzing the removal of metabolic peroxides (Watanabe et al. 1997; Lovell 1998), this measurement may be more important when evaluating bioavailability and maintenance of a healthy immune system.

In summary, a dietary selenium concentration above 20 mg/kg is sufficient to induce selenium toxicity in hybrid striped bass. The dietary selenium requirement of hybrid striped bass based on regression analysis of dietary selenium against whole-body

selenium retention predicted minimum requirements between 0.12 and 0.20 mg/kg, which are similar to the range reported by the NRC (1993).

CHAPTER V

COMPARATIVE BIOAVAILABLITY OF SODIUM SELENITE, SELENO-DL-METHIONINE AND SELENIUM YEAST AND EFFECTS ON NON-SPECIFIC IMMUNE RESPONSES OF HYBRID STRIPED BASS

The bioavailability of nutrients contained within a feedstuff is just as important as the quantity of the nutrient being provided (Paripatananont and Lovell 1997). The importance of this issue is not limited to the animal production standpoint but also from an environmental one. If a nutrient is not readily available for digestion and metabolism by the targeted organism, then its feeding efficiency will be decreased. Likewise, if a nutrient is not digested and absorbed it is simply excreted as waste into the environment. There has been considerable interest and different approaches to improving the bioavailability of nutrients in prepared diets and feedstuffs including various processing technologies, the use of enzymes, direct-fed microorganisms and the complexing of trace minerals (Hardy and Barrows 2002). In recent years some of the interest has been directed toward selenium nutrition.

The two primary inorganic sources of selenium are sodium selenite and sodium selenate. Mahan and Moxon (1978) evaluated selenium bioavailability from sodium selenite, fish meal, brewers grains, and distillers grains and solubles; fish meal had the poorest selenium retention of all the products evaluated in young swine. Bell and Cowey (1989) evaluated the bioavailability of dietary selenium from sodium selenite,

fishmeal, selenomethionine, and selenocystine in Atlantic salmon *Salmo salar*. Wang and Lovell (1997) evaluated the bioavailability of sodium selenite, selenomethionine and selenium yeast in channel catfish *Ictalurus punctatus*. Paripatananont and Lovell (1997) compared net absorption between sodium selenite and selenium proteinate in channel catfish. In these various studies, organic sources of selenium were reported to have higher bioavailability than inorganic sources (Bell and Cowey 1989; Wang and Lovell 1997; Wang et al. 1997; Wantanabe et al. 1997).

Presently in the United States of America, the only approved sources of selenium to supplement diets are sodium selenite and sodium selenate as well as a single organic source of selenium yeast (CFR 2004; Senesac 2006). This selenium-enriched yeast is a selenomethionine-fortified product that is currently only approved for use in poultry, swine, beef cattle and dairy cattle feeds (CFR 2004). The present experiment was conducted to provide additional information on the comparative bioavailability of selenium from an inorganic source, sodium selenite, and two organic sources, seleno-DL-methionine and selenium yeast. Whole-body, muscle, and liver selenium concentrations, liver glutathione peroxidase (GSH-Px) activity, plasma lysozyme activity, blood neutrophil oxidative radical production, hematocrit and intracellular and extracellular superoxide anion production of head kidney macrophages were measured to compare these various sources of selenium. This information may assist with the potential approval of these ingredients for use in aquafeeds.

Materials and Methods

The diets, fish and feeding regime are the same as those in Experiment 5 discussed in Chapter IV. The basal diet (Chapter IV, Table 6) was analyzed and found to contain 0.11 mg selenium/kg. The basal diet was fortified with either sodium selenite, seleno-DL-methionine or selenium yeast to provide approximately one, two and four times the basal selenium concentration.

Sample Collection and Analysis

At the end of the 12-wk feeding period but prior to tissue and blood collection, fish were anesthetized with tricane methane sulfonate (100 mg/L). One fish was randomly selected from each aquarium to obtain blood, heart, liver and muscle tissues. The cardiac and muscle tissues were fixed in formalin for histopathological evaluation by the Texas Veterinary Medical Diagnostic Laboratory (TVMDL). The remaining muscle tissue was stored at -80 C until the selenium analyses could be conducted. Three additional fish per aquarium were randomly selected for samples of blood, liver and muscle tissues. Approximately 0.5 ml of blood was collected from the caudal vasculature using a heparin-treated syringe and 27-gauge needle. The blood was then centrifuged and plasma separated and stored at -80 C until lysozyme determination using the turbidimetric assay described by Parry et al. (1965). The amount of enzyme necessary to produce a decrease in absorbance of 0.001/min/mL of plasma was defined as one lysozyme activity unit. Blood neutrophil oxidative radical production was determined using the procedures described by Siwicki et al. (1994) and Li and Gatlin (2003) using nitroblue tetrazolium (NBT). Tissue for the liver GSH-Px activity was

stored at -80 C until it could be assayed as described by Gatlin and Wilson (1984). The remaining fish in each dietary treatment were combined into individual aquaria and fed their respective diet for 30-d to allow for more growth. After the additional grow-out period, the three largest fish per aquarium were selected and head kidney tissue removed for macrophage isolation and determination of extracellular and intracellular superoxide anion as described by Li and Gatlin (2003).

Statistical Analysis

Factorial ANOVA was used to evaluate the main effects and any interactions associated with serum lysozyme activity, macrophage assays, and neutrophil oxidative radical production. Slope-ratio analysis as described by Wang and Lovell (1997), and broken-line regression analysis (Robbins et al. 1979; Robbins 1986) were used for the statistical computations with SPSS® 11.0 for Windows (Chicago, Illinois, USA). The statistical significance was set at $P < 0.05$.

Results

Histopathological evaluation by TVMDL detected no abnormalities in either the cardiac or muscle tissue submitted from fish fed any of the dietary treatments. Discussion of weight gain, feed efficiency, survival and liver GSH-Px activity is presented in Chapter IV and summarized on Table 11 of the same chapter. Briefly, weight gain, feed efficiency and survival ranges were 475% to 598%, 0.49 to 0.55, and 85.7% to 100%, respectively. By the end of the seventh week, all fish died in one replicate of the 4X selenium yeast treatment due to a mechanical failure of the aeration

system in that aquarium. None of these performance indices showed any significant effects of selenium level or source based on factorial ANOVA. However, liver GSH-Px activity significantly ($P < 0.001$) increased with each selenium level.

Plasma lysozyme activity, blood neutrophil oxidative radical production, and intracellular and extracellular superoxide anion production of head kidney macrophages were the measured non-specific immune response assays. Plasma lysozyme activity (Table 13) ranged from 163 to 381 units. Significant effects of selenium level ($P = 0.009$) and selenium source ($P = 0.028$) on plasma lysozyme activity were detected with factorial ANOVA; no significant interaction between the main effects was detected. Dunnett's t test determined the mean plasma lysozyme activity for the 2X selenium level and sodium selenite source to be different from the basal diet. The mean plasma lysozyme activities for the 2X selenium level and sodium selenite source were 298.0 and 291.7, respectively.

Blood neutrophil oxidative radical production results are presented on Table 14. These values ranged from 1.28 mg NBT/ml for fish fed the diet supplemented with seleno-DL-methionine at a 2X supplementation level to 1.75 mg NBT/ml for fish fed the diet supplemented with sodium selenite at a 4X level. The factorial ANOVA detected that the selenium level had a significant ($P = 0.037$) effect on the neutrophil oxidative radical production and no significant effect from the selenium source or interaction. Duncan's multiple range test did not separate any differences among the means for the selenium source. A 2-sided Dunnett's t test also failed to detect a difference in the treatment means from the basal diet.

TABLE 13. *Plasma lysozyme activity of hybrid striped bass fed purified diets with various formulated levels of selenium from sodium selenite, seleno-DL-methionine or selenium yeast for 12 wk in Experiment 5*[1].

Selenium Level	Selenium source	Plasma lysozyme[2]
Basal	None added	163
1X	Sodium selenite	275[4]
1 X	Seleno-DL-methionine	231
1 X	Selenium yeast	224
2 X	Sodium selenite	381[3,4]
2 X	Seleno-DL-methionine	285[3]
2 X	Selenium yeast	228[3]
4 X	Sodium selenite	219[4]
4 X	Seleno-DL-methionine	217
4 X	Selenium yeast	217
ANOVA, $P > F$		
Level		0.009
Source		0.028
Level x Source		0.205
Pooled SE[5]		53.07

[1]Values are means of three replicate groups except for the 4X selenium yeast diet which had only two replicates.

[2]One lysozyme unit is defined as the amount of enzyme necessary to produce a decrease in absorbance of 0.001/min/mL of plasma.

[3]Selenium level significantly ($P < 0.05$) different from the basal, determined by the Dunnett's t test.

[4]Selenium source significantly ($P < 0.05$) different from the basal, determined by the Dunnett's t test.

[5]Pooled standard error = $\sqrt{mean\ square\ error / number\ of\ replicates}$ (Baker 1986).

TABLE 14. *Blood neutrophil oxidative radical production (NBT test) of hybrid striped bass fed purified diets with various formulated levels of selenium from sodium selenite, seleno-DL-methionine or selenium yeast for 12 wk in Experiment 5*[1].

Selenium Level	Selenium source	NBT test (mg NBT/ml)[2]
Basal	None added	1.43
1X	Sodium selenite	1.57
1 X	Seleno-DL-methionine	1.61
1 X	Selenium yeast	1.41
2 X	Sodium selenite	1.44
2 X	Seleno-DL-methionine	1.28
2 X	Selenium yeast	1.49
4 X	Sodium selenite	1.75
4 X	Seleno-DL-methionine	1.67
4 X	Selenium yeast	1.44
ANOVA, $P > F$		
Level		0.037
Source		0.242
Level x Source		0.147
Pooled SE[3]		0.1673

[1]Values are means of three replicate groups except for the 4X selenium yeast diet which had only two replicates.

[2]Values are means of two replicates for O.D. at 620 nm.

[3]Pooled standard error = $\sqrt{mean\ square\ error / number\ of\ replicates}$ (Baker 1986).

Table 15 also contains the assay results for hematocrit and intracellular and extracellular superoxide anion production of head kidney macrophage. The factorial ANOVA showed no significant effects on the hematocrit due to selenium level, source or interaction. For both the intracellular and extracellular superoxide anion production, the selenium source had a significant effect ($P = 0.002$) and ($P < 0.001$), respectively. The ANOVA failed to detect a significant effect of these two measures for either selenium level or interaction.

Intracellular superoxide anion production was the lowest (0.04) for fish fed the 1X selenium yeast diet and highest (1.00) for those fed the 4X sodium selenite diet. The mean extracellular superoxide anion production for the 1X selenium yeast was non-detectable as a result of negative values being generated for the assay. The 1X sodium selenite diet yielded the highest extracellular superoxide anion production of 4.24. The mean extracellular superoxide anion production for fish fed the sodium selenite supplemented diet was significantly different from those fed the basal diet based on a 2-sided Dunnett's t test; no other means for the remaining selenium sources were deemed different from the non-supplemented diet. Duncan's multiple range test found the extracellular superoxide anion production for fish fed the sodium selenite diets to be significantly different than all other selenium sources.

Slope-ratio analysis was based on the incorporation rate of selenium into tissue as well as GSH-Px activity at various dietary selenium concentrations for the various selenium sources. This ratio multiplied by 100 will yield a relative bioavailability value. Sodium selenite was used as the reference standard because it has been most commonly

TABLE 15. *Hematocrit, intracellular (Intra-) and extracellular (Extra-) superoxide anion production of head kidney macrophages of hybrid striped bass fed purified diets with various formulated levels of selenium from sodium selenite, seleno-DL-methionine or selenium yeast for 12 wk in Experiment 5*[1].

Selenium Level	Selenium Source	Hematocrit (%)	Intra-	Extra-
Basal	None added	37.8	0.439	1.010
1X	Sodium selenite	41.7	0.574	4.240 *, 2
1 X	Seleno-DL-methionine	46.1	0.539	0.835
1 X	Selenium yeast	36.6	0.036	0
2 X	Sodium selenite	46.1	0.440	4.070 *, 2
2 X	Seleno-DL-methionine	42.9	0.577	2.285
2 X	Selenium yeast	42.4	0.250	0.995
4 X	Sodium selenite	46.1	1.001	4.190 *, 2
4 X	Seleno-DL-methionine	49.1	0.319	1.095
4 X	Selenium yeast	43.8	0.216	0.275
ANOVA, $P > F$				
Level		0.329	0.450	0.375
Source		0.273	0.002	<0.001
Level x Source		0.785	0.053	0.776
Pooled SE[3]		3.88	0.1245	0.6648

[1]Values are means of three individual fish from each treatment.

[2]For a significance level of $P < 0.05$, the asterisk indicates difference from the basal diet determined by the Dunnett's t test.

[3]Pooled standard error = $\sqrt{mean\ square\ error / number\ of\ replicates}$ (Baker 1986).

used in feeds. For the whole-body selenium data, slope-ratio analysis found seleno-DL-methionine and selenium yeast to have relative bioavailability values of 414% and 275%, respectively (Figure 5). The muscle selenium concentration against dietary selenium level is plotted in Figure 6 for each selenium source. Due to lack of a slope for the sodium selenite treatments, it could not be used as the reference for slope-ratio analysis. Liver tissue had relative bioavailability values of 121% and 111% for seleno-DL-methionine and selenium yeast, respectively (Figure 7). The plot for GSH-Px activity at various dietary selenium concentrations for the various selenium sources is shown in Figure 4 of Chapter IV. Slope-ratio analysis computed for liver GSH-Px activity showed selenium yeast to have a relative bioavailability value of 61% with respect to sodium selenite; whereas, for seleno-DL-methionine, the relative bioavailability value was 120%. The slope-ratio analysis of seleno-DL-methionine to selenium yeast as the reference, yielded relative bioavailability values of 150%, 222%, 109% and 196% for tissue selenium concentrations in whole-body, muscle, liver and liver GSH-Px activity, respectively.

Discussion

As reported for other fish species (Bell and Cowey 1989; Wang and Lovell 1997; Wang et al. 1997; Wantanabe et al. 1997), this study demonstrated the ability of hybrid striped bass to incorporate selenium into tissue and increase liver glutathione peroxidase activity with selenium more readily from organic sources than an inorganic source. Of the two organic sources evaluated, seleno-DL-methionine generally had a higher relative

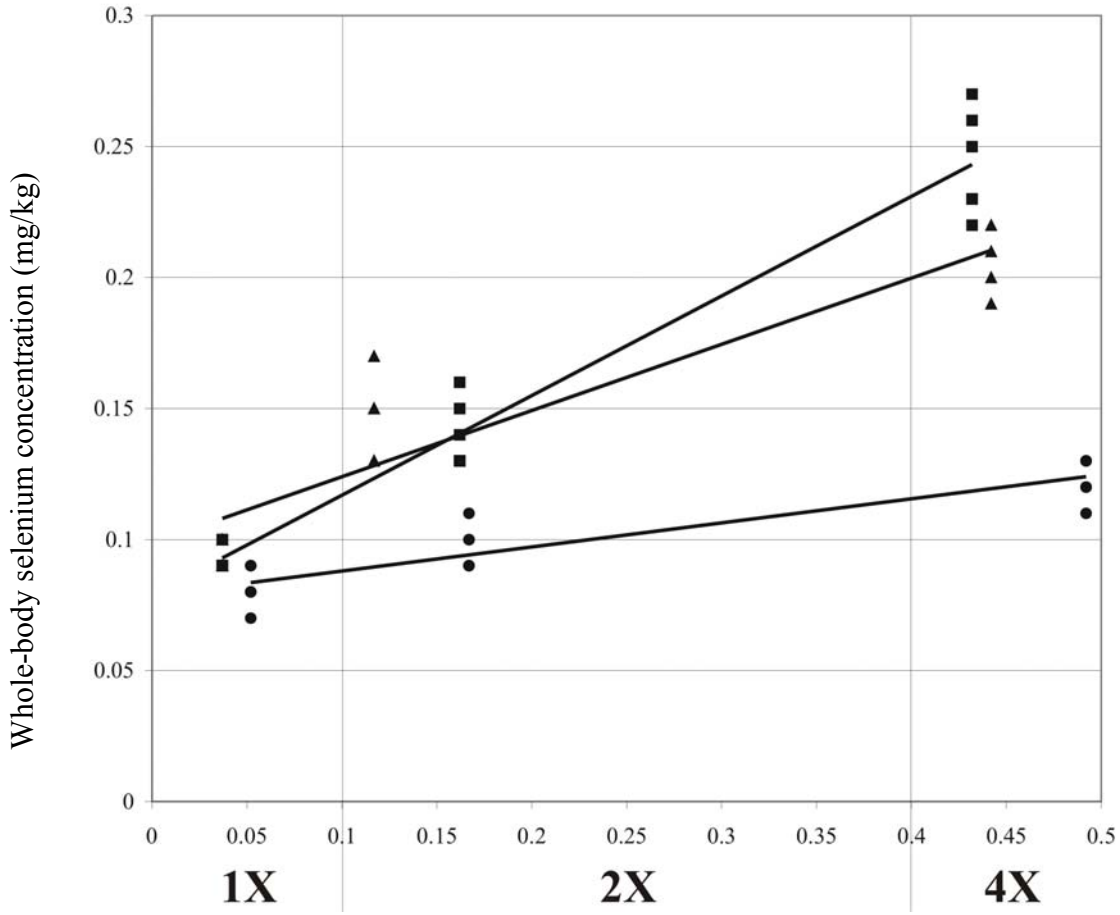

FIGURE 5. *Regression of whole-body selenium concentration and supplemental dietary selenium from sodium selenite (•), seleno-DL-methionine (■), and selenium yeast (▲).*

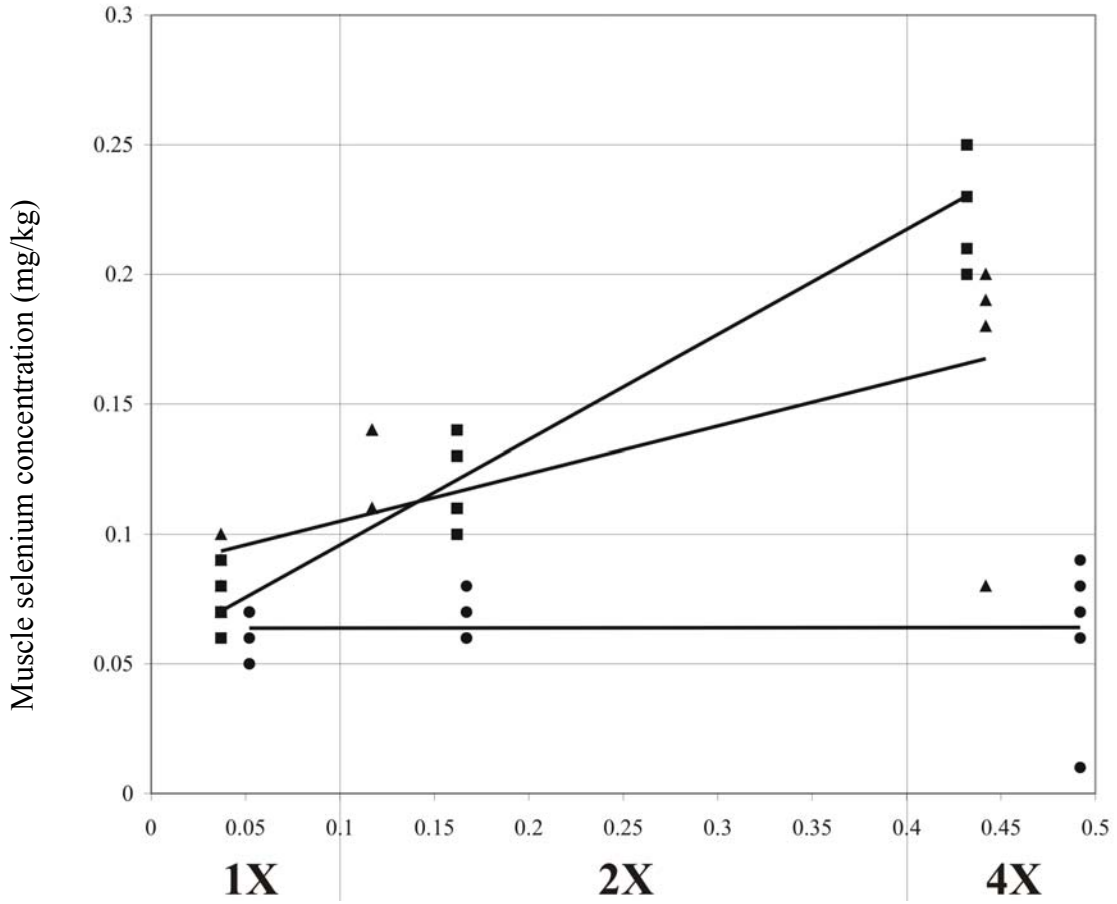

FIGURE 6. *Regression of muscle selenium concentration and supplemental dietary selenium from sodium selenite (•), seleno-DL-methionine (■), and selenium yeast (▲).*

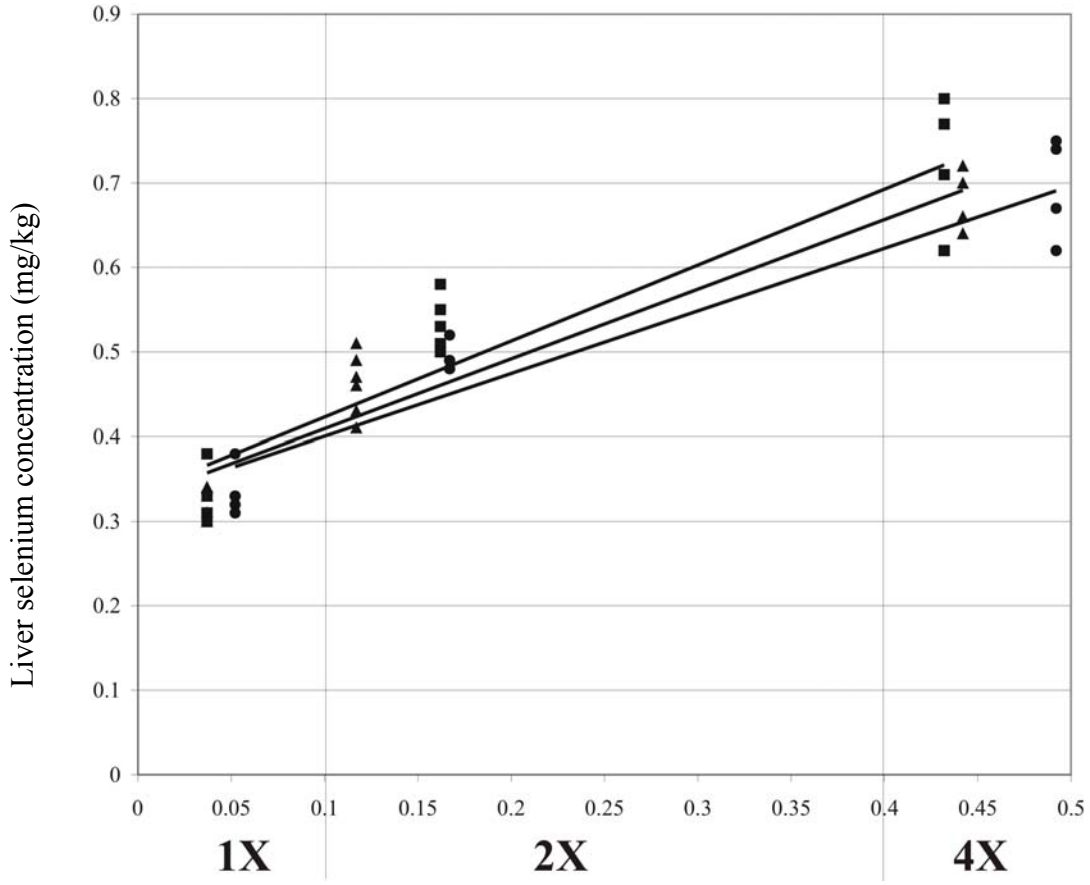

FIGURE 7. *Regression of liver selenium concentration and supplemental dietary selenium from sodium selenite (•), seleno-DL-methionine (■), and selenium yeast (▲).*

bioavailability.

In channel catfish, Wang and Lovell (1997) reported relative bioavailability values, for selenomethionine to selenium yeast, of 106%, 107%, and 99% based on selenium concentration in muscle and liver tissue and liver glutathione peroxidase activity, respectively. In comparison to the present study, the relative bioavailability value based on liver selenium concentration was similar (109%). However for muscle selenium concentration and liver glutathione peroxidase activity the relative bioavailability values to hybrid striped bass were more than double, at 222% and 196%, respectively.

Even though the bioavailability of seleno-DL-methionine was greater than that of selenium yeast based on liver GSH-Px activity, the selenium source did not have a significant effect (Chapter IV, Table 11) on this assay. Bell and Cowey (1989) reported the same observation with plasma and liver glutathione activity in Atlantic salmon smolts.

The main factors, selenium source and selenium level, had significant ($P < 0.05$) effects on the various non-specific immune responses measured in the present study. In contrast to the results obtained in Chapter II, the selenium level significantly ($P = 0.009$) affected plasma lysozyme levels. This is most likely due to the greater selenium supplementation concentrations used in the present study. In addition, the significantly higher plasma lysozyme levels for the 2X sodium selenite treatment would suggest that this is the optimum combination to maximize plasma lysozyme. Even though selenium supplementation level had a significant effect on the neutrophil oxidative radical

production, none of the treatment means were found to be different. The mean intracellular superoxide production of head kidney macrophages for selenium yeast was significantly lower than the other supplemental selenium sources. In channel catfish, Wise et al. (1993) found increased intracellular superoxide anion production of head kidney macrophages from fish fed sodium selenite supplemented diets containing 0.8 mg selenium/kg compared to those fed 0.06 mg selenium/kg.

The present study found the mean extracellular superoxide anion production of head kidney macrophages from fish fed sodium selenite was approximately four times higher than the basal diet and different from all other treatments based on source. The European Commission Scientific Committee on Food has expressed concern with the use of organic sources of selenium related to tissue retention and toxicity (Rayman 2004). As a result of the higher bioavailability of organic selenium sources, the reduced extracellular superoxide anion production may be a manifestation of acute selenium toxicity affecting macrophage viability. A long-term investigation with periodic assessment of intracellular and extracellular superoxide anion production of head kidney macrophages is warranted to further evaluate these selenium sources.

This study found seleno-DL-methionine to have a higher degree of bioavailability than selenium yeast and sodium selenite, which was the lowest, based on the incorporation of selenium into the whole-body, muscle and liver tissues. However, when GSH-Px activity was the response being considered, seleno-DL-methionine had the highest degree of bioavailability with selenium yeast having less than even sodium selenite. The measured non-specific immune responses including plasma lysozyme,

NBT test and intracellular and extracellular superoxide production of head kidney macrophages were significantly affected by either selenium supplementation level or selenium source with plasma lysozyme being significantly affected by both factors. In conclusion, further consideration should be given to the approval of organic selenium sources suitable for supplementing fish diets and various biochemical assays, as well as selenium retention, should be used in this evaluation.

CHAPTER VI

CONCLUSIONS

Characterization of the optimal quantity of specific nutrients, selection of the most efficient source and incorporating the most suitable technology to process the ingredients to maximize animal production are all important considerations of nutritionists. The use of nutritional modulation to enhance the immune system of fish has been investigated (e.g., Raa et al. 1992; Sealey and Gatlin 1999b; Gatlin 2002). Some of the benefits of nutritional modulation over chemotherapeutics, antibiotics, and vaccines include ease of implementation and lack of governmental regulations. Antioxidant nutrients such as vitamin E and selenium as well as biological components like glucans have been evaluated as immunomodulators in various fish species (Yano et al. 1989; Chen and Ainsworth 1992; Anderson and Siwicki 1994; Efthimiou 1996, Jeney et al. 1997; Santarem et al. 1997; Wang et al. 1997; Kim et al. 2003). In addition to identifying compounds that may enhance the immune system, it is also important to quantify various nutritional requirements, determine potential toxicity levels, evaluate potential nutrient interactions and establish the bioavailability of various nutrient sources for cultured species of economic importance. This research evaluated with hybrid striped bass the potential use of selenium and β-glucan as dietary immunomodulators, estimated dietary selenium requirement and toxicity levels, evaluated selenium and vitamin E interactions, determined bioavailability of organic selenium sources to sodium selenite, and measured selenium tissue deposition from different selenium sources.

The first experiment compared practical menhaden fish meal-based and purified crystalline amino acids/casein/gelatin diets supplemented with or without β-glucan and selenium on resistance of hybrid striped bass to *Streptococcus iniae* infection. As expected, the hybrids fed the practical diets had significantly better weight gain and feed efficiency. Higher weight gain and feed efficiency also was observed in fish fed the selenium-supplemented purified diets; selenium supplementation in the practical diets did not have a similar effect. Apparently the non-selenium supplemented purified diet's endogenous 0.033 mg selenium/kg concentration was deficient so that 0.2 mg sodium selenite/kg diet enhanced weight gain. Diets supplemented with β-glucan slightly reduced weight gain and feed efficiency without having an effect on disease resistance or plasma lysozyme activity. Substantial variation has been observed in the ability of glucans to enhance disease resistance of various fish species (e.g, Wang and Wang 1996, 1997; Gatlin 2002). However, dietary supplementation with selenium and β-glucan interacted significantly ($P = 0.041$) with basal composition on plasma lysozyme levels prior to *S. iniae* exposure. In summary, dietary supplementation of β-glucan alone did not enhance disease resistance or plasma lysozyme activity of hybrid striped bass. However, a menhaden fish meal-based diet with or without supplemental selenium provided significantly higher survival after *S. iniae* exposure in comparison to purified diets. Thus, influences of basal diet type and selenium supplementation on disease resistance of hybrid striped bass were most evident in this study and warranted further investigation.

The second study evaluated the interaction between selenium and vitamin E on growth performance and non-specific immune response. Despite the significant effect of dietary selenium on whole-body selenium concentration, this study did not show any significant responses in weight gain, feed efficiency, survival or non-specific immune response (neutrophil oxidative radical production) associated with dietary supplementation of selenium, vitamin E or an interaction between these two factors. These findings are similar to those reported by Kocabas (1996) for hybrid striped bass and Kim et al. (2003) for Nile tilapia.

The results of Experiments 3, 4 and 5 in Chapter IV indicated a dietary selenium concentration above 20 mg/kg is sufficient to induce selenium toxicity in hybrid striped bass. The dietary selenium requirement based on regression analysis of dietary selenium against whole-body selenium retention predicted minimum requirements between 0.12 and 0.20 mg/kg which are similar to values reported by the NRC (1993) for various fish species. However, supplementing diets with 1 mg selenium/kg generally yielded optimal performance based on weight gain, feed efficiency and survival of hybrid striped bass in the present experiments.

Organic mineral sources generally have been considered to be more available than inorganic sources. Organic sources of selenium have been reported to have higher bioavailability than inorganic sources for several fish species (Bell and Cowey 1989; Wang and Lovell 1997; Wang et al. 1997; Wantanabe et al. 1997). Therefore, experiment 5 as discussed in Chapter V evaluated different sources of selenium with hybrid striped bass. Seleno-DL-methionine was shown to have a higher degree of

bioavailability than selenium yeast and sodium selenite, which was the lowest, based on the incorporation of selenium into the whole-body, muscle and liver tissues. However, when GSH-Px activity was the response being considered, seleno-DL-methionine had the highest degree of bioavailability with selenium yeast having even less than sodium selenite. The measured non-specific immune responses such as plasma lysozyme, blood neutrophil oxidative radical production and intracellular and extracellular superoxide production of head kidney macrophages were significantly affected by either selenium supplementation level or selenium source with plasma lysozyme being significantly affected by both factors.

In conclusion, the minimum dietary selenium requirement of hybrid striped bass appears to be approximately 0.1 mg selenium/kg as evidenced by lack of selenium deficiency signs being induced in fish fed this level during any of the experiments. However, dietary selenium supplementation of 1 mg/kg generally produced the greatest weight gain, feed efficiency and survival of hybrid striped bass but this level well exceeds current regulatory limits of the United States (CFR 2004). Selenium toxicity was observed in hybrid striped bass fed diets containing more than 20 mg selenium. Seleno-DL-methionine had the highest bioavailability of the three sources evaluated with hybrid striped bass but is currently not approved for use in animal feeds; whereas, selenium yeast, which is approved for some terrestrial species (CFR 2004), provided a more readily available source of selenium compared to sodium selenite. Further consideration should be given to the approval of additional organic selenium sources based on tissue retention and various biochemical assays such as GSH-Px activity.

In summary, these various experiments have provided a greater understanding of selenium nutrition for hybrid striped bass. This information will be used to further refine diet formulations to enhance the efficiency of hybrid striped bass production in aquaculture.

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VITA

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Along with research experience, Mr. Jaramillo has experience in feed production. In 1993, he was employed as the Quality Assurance Manager at Rangen, Inc. located in Angleton, Texas. In 1995, he accepted a position as an Investigator I for the South Plains area with the Texas Feed and Fertilizer Control Service within the Office of the Texas State Chemist. In 1997, he was promoted to his current position of Registration Associate for this agency. He is a member in the Association of American Feed Control Officials, the Association of American Plant Food Control Officials, and the Association of Southern Feed, Fertilizer and Pesticide Control Officials in which he served as President in 2001-2002 and chairs the Feed Control Program.

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