DIETARY VITAMIN E REQUIREMENT OF SUNSHINE BASS (*MORONE CHRYSOPS* ♀ x *M. SAXATILIS* ♂) AND APPARENT LACK OF INTERACTIONS BETWEEN VITAMIN E AND SELENIUM

A Thesis

by

ARIF MURAT KOCABAS

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

MASTER OF SCIENCE

December 1996

Major Subject: Wildlife and Fisheries Sciences

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ABSTRACT

Dietary Vitamin E Requirement of Sunshine Bass (*Morone chrysops* ♀ x *M. saxatilis*♂) and Apparent Lack of Interactions between Vitamin E and Selenium.

(December 1996)

Arif Murat Kocabas, B.S., Ankara University

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

A 12-week feeding trial was conducted to determine the dietary vitamin E requirement of juvenile sunshine bass (*Morone chrysops* ♀ x *M. saxatilis* ♂), and to investigate the possible interactions between dietary vitamin E and selenium. Sunshine bass initially averaging 1.8 ± 0.08 g (mean ± s.d.) were fed semi-purified diets supplemented with 0.2 mg Se/kg from Na_2SeO_3 and either 0 (basal), 10, 20, 40, 60, or 80 mg vitamin E/kg as dl-α-tocopheryl acetate. Fish fed the basal diet, which contained 5.8 mg α-tocopherol/kg dry weight, were darker in color and had reduced hematocrit values but did not exhibit reduced survival. Diets containing the lowest levels of vitamin E caused significantly ($P<0.05$) reduced weight gain and feed efficiency of fish compared to those fed diets supplemented with vitamin E at 20 to 80 mg/kg. Dietary supplementation of vitamin E caused incremental increases in the concentration of α-tocopherol in both plasma and liver tissues. Regression analysis of weight gain data using the broken-line model indicated a dietary vitamin E requirement of 28 mg/kg dry diet. Two additional semi-purified diets containing either 0 or 60 mg vitamin E/kg without supplemental

selenium also were included in the feeding trial and provided a 2 x 2 factorial arrangement to evaluate potential interactions between selenium and vitamin E. At the end of the 12-week feeding trial, sunshine bass fed diets without supplemental vitamin E had significantly reduced weight gain and feed efficiency but selenium supplementation did not influence these responses. Dietary vitamin E and selenium also did not have significant effects on hematocrit of sunshine bass. Dietary vitamin E had a significant effect on plasma glutathione peroxidase (GSH-Px) activity which was generally reduced at higher levels of vitamin E intake; however, dietary selenium had no significant effect on liver and plasma GSH-Px activity. Based on the results of this study, a dietary selenium deficiency was not induced and there were no interactions between dietary vitamin E and selenium in the nutrition of juvenile sunshine bass.

DEDICATION

To my parents and family for their love and support in my endeavors as a student.

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION AND LITERATURE REVIEW

Hybrid striped bass are crosses between striped bass (*Morone saxatilis*) and white bass (*M. chrysops*). The "original cross" hybrid striped bass was first produced in South Carolina in the mid-1960s using eggs from striped bass and sperm from white bass. The accepted common name of this cross is the palmetto bass (Robins et al., 1991). More recently, the "reciprocal cross" between white bass females and striped bass males also has been produced. The accepted common name of this cross is the sunshine bass. Sunshine bass and palmetto bass both have gained widespread acceptance as sport fish for recreational purposes and as food fish in aquacultural production.

Hybrid striped bass have become highly desirable substitutes for the declining striped bass seafood industry. As a food fish, the hybrid exhibits a mild taste and firm texture. Aquaculturists have found these hybrids to be well-suited to pond culture, and current research is helping to improve culture techniques (Lareau, 1987). The increasing popularity of hybrid crosses between striped bass and white bass for production in aquaculture is largely a result of their capacity for rapid growth and ability to live in water ranging widely in salinity (Setzler et al., 1980; Kerby, 1986). Specifically, Tuncer et al. (1990) have shown that the palmetto bass demonstrated exceptional growth and survival, and lower metabolic energy needs in comparison to striped bass. In addition, Hodson (1989) stated that the sunshine bass also has shown desirable qualities such as rapid growth and disease resistance.

The journal Aquaculture was used as a model of style.

Nutritional Requirements of Hybrid Striped Bass

Several studies have been carried out during the last few years to investigate various nutritional requirements of sunshine bass to improve and refine practical diet formulations for use in aquacultural production. The dietary protein requirement of juvenile sunshine bass for maximum growth and feed efficiency has been determined in both fresh water and brackish water to be approximately 40 % of dry diet (Brown et al., 1992). In a study by Nematipour et al. (1992a), it was determined that the optimum energy:protein ratio (E:P) was 8 kcal/g protein, based on high weight gain and protein efficiency ratio values, as well as lower lipid deposition in the abdominal cavity. Furthermore, it was concluded that sunshine bass were able to efficiently utilize soluble carbohydrate (dextrin) for energy, and dietary lipid could be partially replaced with carbohydrate to improve fish quality and productivity (Nematipour et al., 1992b).

Keembiyetty and Gatlin (1992, 1993) determined both the dietary lysine and the total sulfur amino acid requirements of sunshine bass for maximum growth and feed efficiency. The lysine requirement was 1.41% of dry diet (4.0% of dietary protein), and the total sulfur amino acid requirement was 1.0 % of dry diet (2.9% of dietary protein). An arginine requirement also was determined to be 1.55% of diet or 4.4% of dietary protein (Griffin et al., 1994a).

Nematipour and Gatlin (1993) found that highly unsaturated fatty acids (HUFA) of the n-3 series were essential for sunshine bass. Approximately 1% of n-3 HUFA in the diet (20 % of dietary lipid) was required by sunshine bass.

In addition, Brown et al. (1993) found the available phosphorus requirement of sunshine bass to be approximately 0.5% of diet. The dietary choline requirement also has been determined to be 500 mg choline/kg diet for maximum weight gain and prevention of increased liver lipid concentration in juvenile sunshine bass (Griffin et al., 1994b). However, no other mineral or vitamin requirements of hybrid striped bass have been determined to date. Two nutrients that have been shown to be important in fish nutrition are selenium and vitamin E; therefore, these nutrients were investigated in the present study.

Selenium and Vitamin E Nutrition in Aquaculture

Vitamin E is the generic name for a group of compounds that are lipid-soluble intracellular antioxidants, essential for the regulation of many metabolic functions. A primary function of vitamin E is protecting polyunsaturated fatty acids (PUFA) in biomembranes from oxidation. Vitamin E is required in the diet of all animals including fish (Poston et al., 1976; Bell and Cowey, 1985; Halver, 1985; National Research Council, 1993).

Established vitamin E requirements are 30 mg/kg diet for salmonids (National Research Council, 1993); 30-50 mg/kg diet for channel catfish (Murai and Andrews, 1974; Wilson et al., 1984); 200-300 mg/kg diet for common carp (Watanabe et al., 1977); and 25-50 mg/kg diet for Nile tilapia (Lam, 1985; Satoh et al., 1987). In addition, it has been suggested that the dietary vitamin E requirement of blue tilapia be expressed as a function of dietary lipid level with 3-4 mg of vitamin E for each percent of corn oil in the diet

(Roem et al., 1990). Furthermore, He and Lawerence (1993) determined that the vitamin E requirement of *Penaeus vannami* was approximately 100 mg/kg diet, because that level provided optimal growth and minimal ascorbic acid-stimulated membrane lipid peroxidation of shrimp.

Selenium is an essential trace element that has a detoxifying role in the reduction of cytosolic hydroperoxides as a component of glutathione peroxidase (GSH-Px) (Rotruck et al., 1973; Koller and Exon, 1986). According to Sheffy and Schultz (1979), selenium also functions in cell-mediated and humoral immune responses, and a dietary surplus of vitamin E and selenium has been shown to stimulate immunological responses in several animal species. Vitamin E synergistically works with selenium to form an important antioxidant defense system in animal tissues (Lucy, 1972). Furthermore, Gatlin et al. (1986) observed that vitamin E and selenium interacted physiologically in the nutrition of channel catfish. The interrelationships between vitamin E and selenium have been studied with several other species of fish including Atlantic salmon (Poston et al., 1976) and rainbow trout (Bell et al., 1985).

The requirement for selenium by rainbow trout was found to be in the range of 0.15-0.38 mg Se/kg of dry feed (Hilton et al., 1980), while channel catfish required 0.25 mg/kg diet (Gatlin and Wilson, 1984), based on maximum plasma and liver GSH-Px activity. The dietary requirements of hybrid striped bass for vitamin E and selenium previously have not been investigated.

Research Objectives

This study was undertaken to evaluate dietary vitamin E and selenium effects on sunshine bass. The specific objectives of this research were to determine the dietary vitamin E requirement of sunshine bass, and to investigate potential interactions between vitamin E and selenium in the nutrition of this fish.

MATERIALS AND METHODS

Experimental Design and Diets

A feeding trial was conducted using a completely randomized design to determine the dietary vitamin E requirement of sunshine bass, and a 2 x 2 factorial design also was included to evaluate potential interactions between selenium and vitamin E. Eight semi-purified diets (Table 1) were formulated to contain 35 % crude protein from lipid-extracted red drum muscle and crystalline amino acids to minimally satisfy the protein requirement of sunshine bass (Brown et al., 1992). This formulation contained an optimal energy:protein ratio of 8 kcal available energy/g of protein (Nematipour et al., 1992a). Diets were supplemented with menhaden fish oil (Zapata Haynie Corporation, Reedville, VA), and tocopherol-stripped corn oil (ICN Biomedicals Inc., Aurora, OH) to provide a total of 5% lipid. The supplemented oil did not contain synthetic antioxidants. Minerals and vitamins used in the premixes and diets were of high purity and obtained from usual sources. The vitamin premix did not contain vitamin E. Six experimental diets were prepared by adding incremental levels of a vitamin E premix containing 10 mg dl-α-tocopheryl acetate/g in cellulose to the basal diet while removing corresponding amounts of cellulose. Supplemental vitamin E concentrations ranged from 0 to 80 mg/kg. The basal diet was analyzed (Erickson, 1992) to contain 5.8 mg α-tocopherol/kg, and supplemental levels in experimental diets were confirmed by analysis. Each of the diets used to quantify the vitamin E requirement was supplemented with 0.20 mg Se/kg diet (from a selenium premix containing 200 μg Se/g from Na_2SeO_3 in cellulose). This level of supplemental selenium previously had been determined to satisfy the

Table 1

Composition of the basal diet.

Ingredient	g/100 g dry weight
Red drum muscle[1] (lipid extracted)	11.23
Amino acid premix[2]	23.00
Dextrin[3]	23.80
Menhaden oil[4]	3.00
Corn oil[5]	2.00
Mineral premix (Se-Free)[6]	4.00
Vitamin premix (Vitamin E-free)[7]	3.00
Carboxymethyl cellulose[3]	2.00
$CaHPO_4 \cdot 2H_2O$	1.00
Aspartate/Glutamate premix[8]	3.00
Cellulose[3]	23.87
Selenium premix[9]	0.10
Vitamin E premix[10]	------

[1]Prepared in this laboratory by lyophilizing muscle of adult fish from wild stocks and contained (as g/100 g): protein 93.5; lipid 1.8.
[2]Same as Keembiyehetty and Gatlin (1993).
[3]United States Biochemical Corp., Cleveland, Ohio.
[4]Winterized (cold-pressed) product, donated by Zapata Haynie Corp., Reedville, Virginia.
[5]Tocopherol-stripped corn oil, ICN Biomedicals Inc., Aurora, Ohio.
[6]Contained (as g/kg): $Ca(H_2PO_4)H_20$, 136.00; $Ca(C_6H_{10}O_6)5H_2O$, 348.49; $FeSO_4 \cdot 7H_2O$, 5.00; $MgSO_4 \cdot 7H_2O$, 132.00; K_2HPO_4, 240.00; $NaH_2PO_4H_2O$, 88.00; NaCl, 45.00; $AlCl_3 \cdot 6H_2O$, 0.15;

Table 1 (continued)

KI, 0.15; $CuSO_4 5H_2O$, 0.5; $MnSO_4 H_2O$, 0.7; $CoCl_2 6H_2O$, 1.00; $ZnSO_4 7H_2O$, 3.00; Cellulose, 0.01.

[7]Contained (as g/kg): Ascorbic acid, 50.00; dl-calcium pantothenate, 5.00; Choline chloride, 36.20; Inositol, 5.00; Menadione sodium bisulfite, 2.00; Niacin, 5.00; Pyridoxine HCl, 1.00; Riboflavin, 3.00; Thiamin 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 (40IU/µg), 0.002; cellulose, 891.87.

[8]Contained (as g/kg): Aspartate, 500 g; Glutamate, 500 g.

[9]Contained 200 µg Se/g from Na_2SeO_3 in cellulose.

[10]Contained 10 mg dl-α-tocopheryl acetate/g in cellulose.

requirement of channel catfish (Gatlin and Wilson, 1984). Two additional diets were prepared by supplementing either 0 or 60 mg vitamin E/kg without supplemental selenium to investigate potential interactions between these nutrients. All diets were adjusted to pH 7 with 6 N NaOH, formed into 3-mm pellets as previously described (Moon and Gatlin, 1991), and kept in a freezer (-18°C) before feeding.

Fish and Feeding Trial

The feeding trial was conducted at the Texas A&M University Aquacultural Research and Teaching Facility (ARTF), College Station, TX, using juvenile sunshine bass (*Morone chrysops* ♀ x *M. saxatilis* ♂) obtained from a private hatchery (Keo Fish Farm, Keo, AR). The experimental fish initially averaged 1.8 ± 0.08 g (mean ± s.d.). All fish were conditioned for a 2-week period by feeding the basal diet to satiation two times per day.

The culture system for the feeding trial consisted of 38-l rectangular glass aquaria connected as a closed, recirculating system with a delivary rate of approximately 1 l/min. Water quality was maintained within standards suggested for striped bass culture (Bonn et al., 1976; Lewis and Heidinger, 1981; Rogers et al., 1982) by mechanical and biological filtration. Water hardness was maintained at 290 mg/l as $CaCO_3$. Ammonia, nitrite and pH were monitored weekly and averaged 0.080 ± 0.04 mg/l (mean ± s.d.), 0.027 ± 0.01 mg/l and 7.88 ± 0.34, respectively. Water temperature was maintained at 25 ± 2 °C with an in-line water chiller, and dissolved oxygen was maintained near saturation by continuous aeration; levels were monitored several times weekly with a YSI 51B

temperature and oxygen meter (YSI, Yellow Springs, OH). A light:dark cycle of 12:12 h was maintained using fluorescent lighting controlled by an automatic electric timer.

After the conditioning period, randomly selected groups of 11 fish weighing 19.3 ± 0.8 g/group were then stocked in individual aquaria for the feeding trial. Each diet was fed on a dry-matter basis to fish in three randomly selected aquaria initially at 8% of body weight per day equally divided into morning and evening feedings. Feeding rate was progressively reduced to 4% of body weight during the experiment to minimize overfeeding while maintaining a level approaching satiation. Waste material was siphoned from each aquarium every other day. Group weight measurements were made at weekly intervals and feed allotments adjusted for the next week. The feeding trial continued for 12 weeks.

Sample Collection and Analyses

At the end of 12 weeks, all fish were weighed and counted in order to calculate weight gain and survival. Tissue samples also were collected from representative fish in each group. After anesthetizing with tricaine methanesulfonate (MS-222), blood samples were taken from three fish in each group by heparinized syringe from the caudal vasculature. Hematocrit was determined by the micro method (Brown, 1980). Plasma then was separated by centrifugation (Centricone, Precision Scientific, Chicago, IL), removed and stored at -80°C until analyzed for tocopherol (Bai and Gatlin, 1993). Then whole livers were excised from three fish per group and stored at -80°C before being assayed for tocopherol (Erickson, 1992). In addition, whole liver and blood samples from

three fish in each group fed diets with 0 or 60 mg vitamin E/kg diet, with and without supplemental selenium were assayed for GSH-Px (E.C. 1.11.1.9) activity according to previously described procedures (Gatlin and Wilson, 1984). Appendix I describes the methodology for determination of vitamin E and GSH-Px activity.

All statistical analyses were performed with SAS (1988) routines (i.e., ANOVA, GLM, and NLIN procedures); statistical significance was set at $P<0.05$. Pooled standard error values were reported for estimates of variance (Baker, 1986). All data from the requirement experiment were subjected to non-linear regression analysis to obtain objective estimates of response breakpoints (Freund and Littell, 1986).

RESULTS

Dietary Vitamin E Requirement

Survival of sunshine bass ranged from 67 to 85% after the 12-week feeding trial and was not affected by dietary vitamin E or selenium levels (Table 2). However, fish fed the basal diet had a much darker appearance than fish fed diets supplemented with vitamin E. Fish consuming diets supplemented with less than 20 mg vitamin E/kg had depressed weight gain and feed efficiency compared to fish fed diets containing higher levels of vitamin E (Table 2). Broken-line regression of sunshine bass weight gain against graded levels of dietary vitamin E resulted in a requirement estimate (± s.e.) of 22 (± 3) mg/kg diet. Broken-line regression of feed efficiency data against graded levels of dietary vitamin E provided a requirement estimate of 21 (± 3) mg vitamin E /kg diet. Therefore, based on these data and in consideration of the endogenous α-tocopherol provided by the basal diet, a minimum of approximately 28 mg vitamin E/kg dry diet was required for acceptable growth and feed efficiency of juvenile sunshine bass.

Hematocrit of fish fed the basal diet was numerically lower but not significantly different from that of fish fed the diets with supplemental vitamin E (Table 3). Dietary vitamin E significantly affected both plasma and liver α-tocopherol concentrations (Table 3). Levels of α-tocopherol in plasma and liver generally increased in response to increasing dietary vitamin E level.

Table 2

Response of juvenile sunshine bass fed various levels of vitamin E as dl-α-tocopheryl acetate for 12 weeks[1].

Supplemental vitamin E (mg/kg)	Weight gain[2] (%)	Feed efficiency[3] (g/g)	Survival (%)
0	676	0.28	70
10	679	0.32	67
20	1105	0.36	85
40	978	0.36	73
60	1035	0.35	79
80	1222	0.38	82
Analysis of variance			
Pr > F	0.0165	0.0002	
Pooled s.e.	109.7	0.0095	
Requirement			
estimate ± s.e.[4]	21.8 ± 3.1	20.6 ± 2.8	

[1]Means of three replicate groups.
[2]Expressed as percent increase in initial body weight at the end of week 12; initial weight of fish was 1.8 ± 0.08 g (mean ± s.d.).
[3]Wet weight gain/dry weight feed.
[4]Based on least-squares regression using the broken-line model.

Table 3

Hematocrit, liver α-tocopherol and plasma α-tocopherol concentrations of juvenile sunshine bass fed various levels of vitamin E as dl-α-tocopheryl acetate for 12 weeks[1].

Supplemental vitamin E (mg/kg)	Hematocrit (%)	Plasma α-tocopherol (μg/ml)	Liver α-tocopherol (μg/g)
0	26	0.22	0.17
10	32	2.33	1.52
20	32	1.96	2.86
40	32	3.69	4.61
60	30	5.74	11.24
80	33	6.34	11.47
Analysis of variance			
Pr > F	0.2653	0.0012	0.011
Pooled s.e.	2.44	0.83	2.21

[1]Means of three fish in each of three replicate groups, except two fish in each replicate group were analyzed for α-tocopherol in plasma and liver.

Evaluation of Interactions between Dietary Vitamin E and Selenium

Neither dietary vitamin E nor selenium significantly affected survival of juvenile sunshine bass. Sunshine bass fed diets without supplemental vitamin E had significantly reduced weight gain and feed efficiency compared to those fed diets containing 60 mg dl-α-tocopheryl acetate/kg; whereas, selenium supplementation did not influence weight gain or feed efficiency (Table 4).

Hematocrit values of sunshine bass were not statistically influenced by dietary supplementation of vitamin E or selenium, although fish fed the diets without supplemental vitamin E tended to have lower hematocrit values regardless of selenium supplementation (Table 5). Dietary vitamin E did significantly affect both plasma and liver α-tocopherol concentrations but there was no effect of dietary selenium or interaction between the dietary factors on these responses (Table 5).

As seen in Table 6, GSH-Px activity in blood plasma was significantly influenced by dietary vitamin E with reduced activity observed in fish fed diets supplemented with vitamin E. In contrast, sunshine bass fed the various levels of vitamin E and selenium exhibited no significant differences in liver GSH-Px activity.

Table 4

Responses of juvenile sunshine bass fed different levels of selenium and vitamin E for 12 weeks[1].

Nutrient supplement		Weight gain[2]	Feed efficiency[3]	Survival
Selenium (mg/kg)	Vitamin E	(%)	(g/g)	(%)
0	0	798	0.30	76
0.2	0	657	0.28	70
0	60	1181	0.37	79
0.2	60	1034	0.35	79
Analysis of variance				
Vitamin E		0.0014[4]	0.0004	
Selenium		0.1079	0.1361	
Vitamin E x Selenium		0.9731	0.9672	
pooled s.e.		79.4	0.012	

[1]Means of three replicate groups.
[2]Expressed as the percentage increase in initial body weight at the end of week 12; average initial weight of fish was 1.8 ± 0.08 g (mean ± s.d.).
[3]Wet weight gain:dry weight feed.
[4]Significance (Pr > F).

Table 5

Hematocrit, liver α-tocopherol and plasma α-tocopherol concentrations of juvenile sunshine bass fed diets containing different levels of selenium and vitamin E for 12 weeks[1].

Nutrient supplement		Hematocrit	Plasma α-tocopherol	Liver α-tocopherol
Selenium (mg/kg)	Vitamin E	(%)	(μg/ml)	(μg/g)
0	0	26.3	0.21	0.32
0.2	0	26.4	0.22	0.17
0	60	29.6	6.3	11.24
0.2	60	30.0	5.7	6.87
Analysis of variance				
Vitamin E		0.1157[2]	0.0009	0.0021
Selenium		0.8951	0.8228	0.3156
Vitamin E x Selenium		0.9588	0.8147	0.2841
Pooled s.e.		1.97	1.13	1.97

[1]Means of three fish from each of three replicate groups, except two fish in each replicate group were analyzed for α-tocopherol in plasma and liver.
[2]Significance (Pr > F).

Table 6

Glutathione peroxidase (GSH-Px) activity in liver and plasma from juvenile sunshine bass fed diets containing different levels of selenium and vitamin E for 12 weeks[1].

Nutrient supplement		Liver GSH-Px	Plasma GSH-Px
Selenium (mg/kg)	Vitamin E	(µmole units)[2]	(µmole units)[3]
0	0	1.99	13.03
0.2	0	1.95	13.13
0	60	1.68	8.30
0.2	60	1.94	7.36
Analysis of variance			
Vitamin E		0.331[4]	0.0016
Selenium		0.4901	0.7212
Vitamin E x Selenium		0.3510	0.6588
Pooled s.e.		0.15	1.13

[1]Means of two fish from each of three replicate groups.
[2]Micromoles of NADPH oxidized/(minute·g).
[3]Micromoles of NADPH oxidized/(minute·dl).
[4]Significance (Pr >F).

DISCUSSION

Dermal depigmentation has been noted as a gross vitamin E deficiency sign in channel catfish (Murai and Andrews, 1974; Gatlin et al., 1986) and chinook salmon (Thorarinsson et al., 1994). Lovell et al. (1984) also found that channel catfish fed a diet deficient in vitamin E were lighter in color. However, in the present study fish fed the basal diet were distinctly darker in color compared to fish fed diets supplemented with vitamin E.

Sunshine bass consuming diets deficient in vitamin E grew at a slower rate than those consuming diets adequate in vitamin E. Not all fish species have responded to a vitamin E deficiency with reduced growth (Hung et al., 1980, 1981; Cowey et al., 1981, 1983; Satoh, 1987; Wilson et al., 1984). It was determined that 28 mg vitamin E/kg diet produced greater weight gain and feed efficiency of juvenile sunshine bass than lower levels of vitamin E. Thus, the present study indicated the minimum dietary vitamin E requirement of sunshine bass for acceptable growth and feed efficiency was approximately 28 mg vitamin E/kg. The minimum dietary requirements for vitamin E by chinook salmon (Woodall et al., 1964), Atlantic salmon (Lall et al., 1988), channel catfish (Murai and Andrews, 1974; Wilson et al., 1984), common carp (Watanabe et al., 1977), and Nile tilapia (Lam, 1985; Satoh et al., 1987) were reported to be 30 mg/kg, 30 mg/kg, 30-50 mg mg/kg, 200-300 mg/kg, and 25-30 mg/kg, respectively, in the presence of selenium.

Reductions in hematocrit values in fish fed diets deficient in vitamin E were reported by Woodall et al. (1964), Poston et al. (1976) and Gatlin et al. (1986). However, in these previous studies hematocrit values were considerably lower (7-21%) than those

measured in the present study (26-32%). Gatlin et al. (1986) reported that catfish fed a basal diet without supplemental selenium and vitamin E had significantly lower hematocrit value (24%) than those fed diets supplemented with either selenium or vitamin E (31-33%).

The responses of sunshine bass to increasing levels of dietary vitamin E in the present study were similar to those of poultry (Marusich et al., 1975; Bartov and Bornstein, 1977; Rethwill et al., 1981; Lin et al., 1989) and fish (Boggio et al., 1985; Murai and Yamauchi, 1989; Frigg et al., 1990; Gatlin et al., 1992; Bai and Gatlin, 1993) in previous studies where supplementation of vitamin E at elevated levels increased the concentration of tocopherol in various tissues. Although weight gain and feed efficiency responses were used to estimate the dietay vitamin E requirement of sunshine bass, saturation of α-tocopherol in liver tissues also could be used as a conservative measure of their dietary vitamin E requirement. Concentrations of α-tocopherol in liver tissue were consistently increased as the level of vitamin E increased in the diet until reaching a plateau at approximatelly 60 mg vitamin E/kg diet. The dietary requirement based on liver saturation of α-tocopherol is within the range of requirement values reported for various species (National Research Council, 1993).

Cowey et al. (1983) and Wilson et al. (1984) were successful in utilizing the ascorbic acid-stimulated lipid peroxidation assay in liver microsomes as a sensitive index of vitamin E status in rainbow trout and channel catfish. Furthermore, Satoh et al. (1987) demonstrated that lipid peroxidation values (thiobarbituric acid values) in muscle and liver

could be used as an index of vitamin E status in Nile tilapia. However, these specific measurements were not made in the present study.

In previous studies with Atlantic salmon (Poston et. al., 1976), rainbow trout (Hilton et al., 1980), and channel catfish (Gatlin and Wilson, 1984; Gatlin et al., 1986) GSH-Px activity has been shown to decrease in fish fed diets deficient in selenium. However, dietary selenium did not affect plasma or liver GSH-Px activity in the present study. Additionally, dietary selenium did not affect plasma and liver α-tocopherol levels. This observation agrees with the findings of Bell et al. (1987) and Thorarinsson et al. (1994). However, Poston et al. (1976) and Bell et al. (1985, 1986) found that elevated dietary selenium increased liver α-tocopherol levels. The disagreement in these results may be due to different dietary levels of antioxidants other than selenium in the test diets.

Data from the present study indicated a negative correlation between plasma vitamin E levels and GSH-Px activity in plasma of juvenile sunshine bass. The lower GSH-Px activity in plasma of sunshine bass fed diets supplemented with vitamin E at 60 mg/kg may have reflected a reduced need for protection against oxidation in this tissue. Gatlin et al. (1986) found that selenium and not vitamin E was the main factor influencing GSH-Px activity in channel catfish.

CONCLUSIONS

For acceptable growth and feed efficiency, a minimum dietary vitamin E requirement of 28 mg/kg was established for juvenile sunshine bass. This requirement value is somewhat similar to those previously reported as minimum dietary requirements for salmon, channel catfish and Nile tilapia. Sunshine bass were not sensitive to lack of supplemental dietary selenium, and there were no significant interactions between dietary vitamin E and selenium in the nutrition of sunshine bass.

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APPENDIX I

METHODOLOGY FOR ANALYSIS OF VITAMIN E AND GLUTATHIONE PEROXIDASE (GSH-Px) ASSAY

A. Vitamin E Determination via HPLC

Reagents:

Saponification mixture
18% KOH and 3% Ascorbic acid in 25% ethanol (make fresh every 48 hours)

Extraction solution
10% ethyl acetate in hexane (HPLC grade)

7.5% Ascorbic acid in deionized water

Absolute ethanol

Procedure: (e.g. tissue samples)

Weigh 1.0g or less of tissue into a 20 ml screw cap test tube.
Place 10ml of saponification mixture in tube and vortex briefly.
Heat sample at 70 °C for 45 min.
Invert samples once or twice during heating to mix.
Remove sample and allow to cool.
Pipette in 2 ml of extraction solution and vortex.
Let stand ca. 10 min allowing phases to separate and remove upper phase.
Perform extraction 3 times and pool upper phase into 10 test tube.
Dry down upper phase under nitrogen and cap with paraffin.
Store at -20 °C until analyzed.
Reconstitute dried sample in 1 ml hexane, vortex and filter through a 0.25-micron polypropylene encased syringe filter.
Inject 20 µl.

Reference:

Erickson, M. C., 1992. Lipid and tocopherol composition of farm-raised striped and hybrid striped bass. Comp. Biochem. Physiol., 101 A: 171-176.

Procedure: (e.g. plasma samples)

Pipette 100 µl of plasma into a 10 ml screw top tube.
Add 0.5 ml ethanol and vortex.
Add 2 ml hexane and vortex.
Centrifuge at 4000 rpm for 10 min on Marathon 13K centrifuge.
Remove an aliquot of the hexane upper phase, filter through a 0.25-micron polypropelene encased syringe filter.
Inject 20 µl.

Reference:

Bai, S.C. and Gatlin, D.M., III, 1992. Dietary vitamin E concentration and duration of feeding affect tissue α-tocopherol concentrations of channel catfish. Aquaculture, 113: 129-135.

Equipment:

High-pressure liquid chromatography (HPLC):
Waters 501 solvent delivery system using a gradient controller
Waters 401 scanning fluorescence detector.
Column: Nova-Pak silica 39 x 150 mm 0.4 µm
Precolumn: Nova-Pak silica (part no. 20790)
Mobile phase: 0.3% isopropanol in hexane
Flow rate: 0.6 ml per min isocratic

Detector settings:

Ex: 295 Em: 335
Atten: 64 Gain: x100 Filter: 1.5 sec

Run time: 12 min

B. Glutathione Peroxidase Assay

Reagents:

10mM EDTA (disodium ethylenediaminetetraacetate)
10mM NaN_3 (sodium azide)
10mM GSH (reduced glutathione)
2mM NADPH (reduced nicotinamide-adenine dinucleotide phosphate)
500mM Phosphate buffer (0.5M K_2HPO_4, 0.5M KH_2PO_4; pH 7.0)
2.5M H_2O_2 (hydrogen peroxide) as substrate
0.25M Sucrose, crystal

Homogenize livers in 6 volumes of 0.25M sucrose.
Spin at 14,000xg for 15 minutes at 4°C.

Assay cocktatil: (e.g.)

4ml from 500mM phosphate buffer
4ml from 10mM EDTA
4ml from 10mM NaN_3
4ml from 2mM NADPH
0.025ml from 3900 units/ml GSSG reductase (oxidized glutathione)
4ml from 10nM GSH
11.98ml from DI water

Use 0.8ml of cocktail, 0.1ml enzyme source, and 0.1ml H_2O_2

Equipment:

UV/Vis Spectrophotometer
wavelenght: 340nm
chart speed: 30mm/min
sensitivity: 1000mV
thermostated cuvette holder
measure against DI water

Temperature: 25°C.

Run time: 1min.

Reference:

Gatlin, D.M., III and Wilson, R.P., 1984. Dietary selenium requirement of channel catfish. J. Nutr., 114: 627-633.

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