EVALUATION OF AMINO ACID SUPPLEMENTATION OF SOYBEAN-MEAL-BASED DIETS FOR HYBRID STRIPED BASS

A Thesis

by

L. CHRISTINE SAVOLAINEN

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 2008

Major Subject: Wildlife and Fisheries Sciences

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ABSTRACT

Evaluation of Amino Acid Supplementation of Soybean-Meal-Based Diets for Hybrid Striped Bass. (December 2008)

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

Hybrid striped bass (*Morone chrysops* x *M. saxatilis*) aquaculture is a major commercial enterprise in the United States and internationally. Efforts to decrease diet costs and limit dependence on fishmeal, have led to the development of plant-based diets as an alternative aquafeed. Due to limiting concentrations of amino acids such as methionine, cystine, and taurine in plant meals such as soybean meal, supplementation of these plant-based diets with methionine or other sulfur amino acid compounds is typically required. Therefore, the current study was conducted to evaluate different amino acid supplements in soybean-meal-based diets for hybrid striped bass for possible refinement of diets and reduction of production costs.

One feeding trial evaluated methionine hydroxy analog (MHA) and Mintrex® which is MHA containing chelated zinc relative to L-methionine. The second trial evaluated the effects of supplemental taurine or arginine on fish performance and potential health benefits.

In the first feeding trial, a basal soybean-meal-based diet (56% soybean meal and 15% fishmeal) marginally deficient in total sulfur amino acids (TSSA) (1.10%

methionine and cystine) was supplemented with either L-methionine, Mintrex® or MHA calcium salt and fed to triplicate groups of juvenile hybrid striped bass for 10 weeks. The different methionine supplements provided similar weight gain, feed efficiency ratio (FER) and protein efficiency ratio (PER) values that tended to be greater than observed in fish fed the basal diet. Mintrex® supplementation provided much higher plasma zinc concentrations compared to fish fed the other methionine supplements.

In the second feeding trial, soybean-meal based diets which satisfied the requirement for TSAA were supplemented with either taurine or arginine at 1.5% of dry weight. Supplemental taurine or arginine did not provide any improvements in weight gain, FER, PER or survival compared to the basal diet. Thus, taurine or arginine supplementation of soybean-meal-based diets does not appear warranted. However, sulfur amino acid supplementation of plant-based diets is critical, and Mintrex® appears to be an effective supplement to meet the methionine and zinc needs of hybrid striped bass.

DEDICATION

To Äiti, Isä, Misi, Lucy, M'aggie, Roux, Niles;

and to all of the fish I've ever killed.

ACKNOWLEDGEMENTS

I thank my committee chair, Dr. Gatlin, for his patience, guidance, and for taking a chance on an unknown kid in the summer of ‘07. Much gratitude goes to my committee members, Drs. Neill, Stallone, and Varner, for their support, assistance and suggestions.

Many thanks also go to my friends, colleagues, faculty and the staff of the Department of Wildlife and Fisheries Sciences for making my time at Texas A&M University an exceptional experience. Special thanks go to Dr. Alejandro Buentello, Brian Ray, Brandon Booth, Gary Burr, Camilo Pohlenz, Thiago Raggi and last but certainly not least, Kcal the overnight farm supervisor; without their help I’d still be collecting data out at the fish farm...

I also want to extend my appreciation to the Office of the Texas State Chemist, Agricultural Analytical Services for assisting with zinc analysis, Keo Fish Farm for providing the experimental fish, Novus International for supporting the research, and the graduate and undergraduate workers at the Texas A&M University Aquacultural Research and Teaching Facility.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (continued)

INTRODUCTION

The sunshine bass, a reciprocal cross hybrid between white bass (*Morone chrysops*) and striped bass (*M. saxatilis*), is an established seafood delicacy and has become a strong staple in aquaculture. Hybrid striped bass production is a fast growing segment of U.S. aquaculture, with commercial production beginning in the mid 1980s after wild striped bass catches began to decline (O'Neill, 2005). In 1990, U.S. production was approximately 1.6 million pounds (FAO Fisheries Department, 2005), but by 2004 U.S. production had increased to 11.5 million pounds as shown in Figure 1 (Carlberg et al., 2004).

Hybrid striped bass are produced as a cross between the white bass and the striped bass. The original cross is commonly known as the palmetto bass (*M. saxatilis*♀ x *M. chrysops* ♂) while the reciprocal cross is known as the sunshine bass (*M. chrysops*♀ x *M. saxatilis* ♂) (Harrell, 1997; Garber and Sullivan, 2006). The sunshine bass is used more commonly in aquaculture due to far greater egg production and ease of handling of the female white bass compared to the female striped bass (Harrell, 1997). The resulting hybrid offspring from both crosses produce improved qualities such as enhanced disease resistance, increased growth and survival rates, and greater tolerance to varied water quality conditions (Hodson, 1989; Harrell, 1997; Morris et al., 1999; O'Neill, 2005). Not only do the offspring exhibit heterosis in regard to the previously mentioned traits, but they also have a lower routine metabolism compared to that of the

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This thesis follows the style and format of the journal *Aquaculture*.

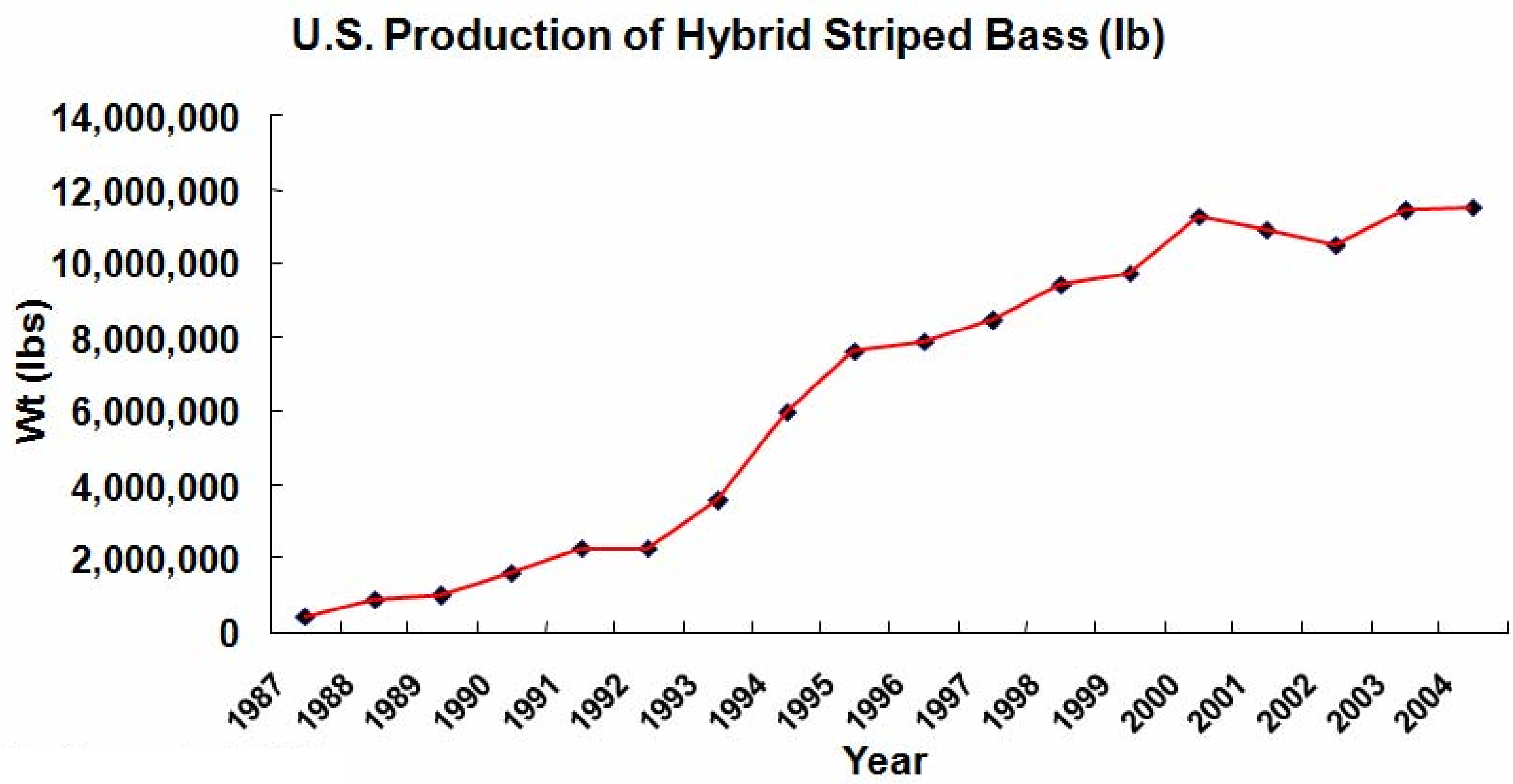

Figure 1 – The U.S. production of hybrid striped bass from 1987 to 2004 (Carlberg et al., 2004).

striped bass and as a result are able to direct more dietary energy for growth (Tuncer et al., 1990). In production settings, hybrid striped bass are typically raised in large ponds or indoor tank systems with water temperatures of 25-30°C (Harrell, 1997).

Traditionally, carnivorous fishes such as the hybrid striped bass have been fed diets high in fishmeal in order to meet their relatively high protein requirements and increase diet palatability (Gatlin, 1997). Hybrid striped bass require approximately 40% crude protein in the diet, as well as the total sulfur amino acids, methionine and cystine, at approximately 2.9% of dietary protein. Fishmeal is the most nutritionally complete protein feedstuff but it also is the most expensive (Nematipour et al., 1992; Gatlin, 1997; USDA, 2008). There are ongoing concerns regarding the use of fishmeal in aquaculture especially considering the limited but stable supply of wild-caught finfish used to produce fishmeal and the increasing demand and consequent cost due to rapid growth of aquaculture (Naylor et al., 2000; Weber, 2003; USDA, 2008). These combined factors have created an interest in reducing the amounts of fishmeal used in commercial feeds by means of ingredient substitution.

The ability to reduce the amount of fishmeal in diets of carnivorous fishes with alternatives such as soybean meal will likely decrease costs. However, with the substitution of fishmeal in the diet, limiting components such as the sulfur amino acid methionine will need to be supplemented. Table 1 shows a clear economic advantage to the use of soybean meal as an alternative to fishmeal in aquafeeds, as well as the need for methionine supplementation. Research is needed to aid in the further refinement of diets for carnivorous fishes, such as the hybrid striped bass, to determine optimal amino acid levels as well as economic benefits.

In addition to the sulfur-containing amino acids, other amino acids and nutrient groups are typically found at lower concentrations in various plant feedstuffs compared to fishmeal. Table 2 shows the percent composition of selected amino acids and other nutrients in fishmeal and soybean meal. Soybean meal is the most readily available and extensively studied plant feedstuff to partially replace fishmeal in the diet of carnivorous species (Tacon et al., 1983; Mohsen and Lovell, 1990; Reigh and Ellis, 1992; Gallagher, 1994; Kaushik et al., 1995; Keembiyehetty and Gatlin, 1997; McGoogan and Gatlin, 1997; Gummadi and Reigh, 2003). Gallagher (1994) reported that soybean meal could provide up to 75% of the protein in the diet of the original-cross hybrid striped bass without a significant decline in growth performance. However, sulfur amino acids are most limiting in this plant feedstuff and may require supplementation (Keembiyehetty and Gatlin, 1997; Goff and Gatlin, 2004).

Low dietary levels of methionine have been shown to suppress growth of sunshine bass and increase susceptibility to fungal disease resulting in high mortality (Keembiyehetty and Gatlin, 1993). Red drum, *Sciaenops ocellatus,* also were found to have higher survival and increased feed efficiency, protein efficiency ratio, and protein conversion efficiency when fed diets supplemented with sulfur amino acids compared to fish fed a basal diet deficient in methionine (Goff and Gatlin, 2004).

Various forms of sulfur amino acids are available and have been evaluated with numerous fish species including sunshine bass. Sunshine bass fed diets supplemented

Table 1 – Comparison of menhaden fishmeal to soybean meal (NRC, 1993; Feedstuffs, 2008; USDA, 2008).

	Menhaden fishmeal	Soybean meal, dehulled and solvent extracted
% Protein	64.5%	50%
U.S.D./kg	$0.96	$0.38
% Methionine	1.75%	0.68%

Table 2 – Percent composition (as fed basis) of amino acids in soybean meal and menhaden fishmeal. Note: soybean meal is solvent extracted, without hulls (NRC, 1993; IFFO, 2006).

Amino Acid	Menhaden Fishmeal	Soybean Meal
Arginine	3.82	3.67
Cystine	0.56	0.75
Histidine	1.45	1.22
Isoleucine	2.66	2.14
Leucine	4.48	3.63
Lysine	4.72	3.08
Methionine	1.75	0.68
Phenylalanine	2.41	2.44
Taurine	0.5	--
Tyrosine	1.94	1.76
Threonine	2.50	1.89
Tryptophan	0.65	0.69
Valine	3.22	2.55
Crude Protein	64.5	50.0

with DL- methionine and acetylmethionine had weight gain and feed efficiency values comparable to those fed a control diet containing L-methionine, while methionine hydroxy analog (MHA) calcium salt was not efficiently utilized and resulted in significantly reduced growth (Keembiyehetty and Gatlin, 1997). Results from another study also indicated MHA calcium salt utilization was only 75% as effective compared to L-methionine (Keembiyehetty and Gatlin, 1995). MHA was shown to be only 26% as effective as L-methionine in promoting weight gain of channel catfish, *Ictalurus punctatus* (Robinson et al., 1978). In contrast, juvenile red drum were found to use crystalline MHA as readily as L-methionine based on growth performance (Goff and Gatlin, 2004). Recently a new product called Mintrex® has been introduced in which the trace mineral zinc is chelated to MHA and thus may provide an organic form of zinc as well as MHA.

Another sulfur-containing amino acid, taurine, is of interest because it is found at relatively high concentrations in animal feedstuff; however, concentrations in plant feedstuffs are extremely low (Lunger et al., 2007). Taurine is typically considered a nonessential amino acid; however, it has been established as an essential amino acid for another carnivore, the domestic cat *Felis silvestris catus* (Hand et al., 2000). In recent feeding trials using plant-based diets for carnivorous fish species, taurine supplementation enhanced feed efficiency and weight gain of rainbow trout, *Oncorhynchus mykiss* (Gaylord et al., 2006), Japanese olive flounder, *Paralichthys olivaceus* (Park et al., 2002; Kim et al., 2005), and cobia, *Rachycentron canadum* (Lunger et al., 2007). These results indicate a need to supplement taurine in plant-based diets for carnivorous fish. However, addition of taurine to a methionine-deficient diet did not increase growth performance of channel catfish (Robinson et al., 1978) or hybrid striped bass (Keembiyehetty and Gatlin, 1995), indicating that methionine was the first limiting amino acid in this diet.

Arginine is another essential amino acid found in relatively high concentrations in fishmeal and some plant feedstuffs. It is important for producing optimal growth and acting as the sole precursor of nitric oxide which may aid in immunity and disease resistance (Reyes et al., 1994; Akarid et al., 1995). Buentello and Gatlin (1999) reported an arginine-dependent metabolic pathway for nitric oxide production in channel catfish. Additionally, an increased level of dietary L-arginine enhanced channel catfish survival and resistance to *Edwardsiella ictaluri* infection following a controlled disease challenge (Buentello and Gatlin, 2001). Arginine supplementation may enhance macrophage function which plays a key role in the immune system by aiding in phagocytosis of infectious agents. When macrophages encounter microbial products, they instigate the production of nitric oxide synthase which uses L-arginine to generate nitric oxide (Hibbs et al., 1987; 1988; Marletta et al., 1988; Hirsh et al., 2004). Nitric oxide acts as a vasodilator to increase blood flow to the site of infection in addition to oxidizing bacteria, viruses, parasites and fungi, thereby killing the infectious microorganisms (Hibbs et al., 1988; De Groote and Fang, 1995).

Neutrophils and macrophages are specialized leucocytes which play an active role in non-specific immune responses. They are able to distinguish invading bacteria from body cells and proceed to phagocytize and kill bacteria with the production of nitric oxide and lysozyme among other chemicals in their biochemical arsenal (Hirsh et al., 2004). Lysozyme exerts its effect by breaking down polysaccharide bacterial cell walls and allowing for ease of phagocytosis by leucocytes. In terrestrial animals, lysozyme is found in the blood and mucosal secretions such as tears and saliva, and in avian species in high concentrations in egg whites (Hirsh et al., 2004). Lysozyme production by white blood cells has been reported in rainbow trout (Jorgensen et al., 1993).

An arginine requirement of 1.0% of diet (4.3% of dietary protein) has been established for channel catfish (Robinson et al., 1981), while 2.4% of diet (6.0% of dietary protein) was reported for Chinook salmon, *Oncorhynchus tshawytscha* (Klein and Halver, 1970), 1.55% of diet (4.4% of dietary protein) established for hybrid striped bass (Griffin et al., 1994) and red drum (Barziza et al., 2000). The arginine requirement of rainbow trout ranged from 0.8-2.5% of the diet (2.0-5.3% dietary protein) (Kaushik et al., 1988). However, supplementation of arginine above the minimum requirement to potentially enhance immune function and disease resistance, as observed in channel catfish (Buentello and Gatlin, 2001), has not been investigated with other species including hybrid striped bass. Arginine supplementation of soybean-meal-based diets may enhance the innate immunity and disease resistance of hybrid striped bass; thereby, reducing disease occurrence and lessening the need for medicated feeds in the production setting.

When formulating aquafeeds with plant feedstuffs, consideration also must be given to anti-nutritional factors such as phytic acid, which is found in appreciable concentrations in plant ingredients such as soybean meal (Lo et al., 1981). Phytic acid is able to bind trace elements such as zinc and render them unavailable to the animal, a fact documented in both terrestrial and aquatic species (Oberleas et al., 1962; O'Dell et al., 1964; Savage et al., 1964; Lo et al., 1981; Gatlin and Wilson, 1984; Gatlin and Phillips, 1989; Lowe et al., 1994). However, when organic chelated forms of minerals—instead of those from inorganic sources—have been provided in diets, livestock and poultry have shown increased mineral uptake (bioavailability) as well as enhanced growth and disease resistance (Sanford, 1976; Brethour, 1984; Wedekind et al., 1992).

Zinc is a principal trace mineral on which specific enzymes depend for regulation and cellular turnover, in addition, zinc has a role in immune response via antibody production (Park et al., 2004). A five-fold increase in dietary zinc supplementation, reported for practical channel catfish soybean-meal-based diets in comparison to purified diets, was presumably attributed to the increased phytic acid content of the practical diets (Gatlin and Wilson, 1984). In a subsequent study with channel catfish, chelated zinc was also demonstrated to reduce the metabolic requirement for zinc by three to five times when compared to inorganic zinc in purified and practical diets (Paripatananont and Lovell, 1997). Thus, there is continued interest in evaluating organic forms of zinc supplementation of plant-based feedstuffs to increase its bioavailability in aquafeeds. In the recently developed commercial product Mintrex®, the zinc atom is chelated between two molecules of MHA; thereby, providing a source of both organic zinc and an essential sulfur amino acid. To date, this product has not been evaluated in feeds for aquatic species.

Objectives

Based on the preceding background information, the purpose of this study is to evaluate the effects of MHA and Mintrex® as possible sulfur amino acid substitutes for L-methionine in soybean-meal-based diets for hybrid striped bass. The bioavailability of zinc in the commercial supplement Mintrex® also will be assessed. Finally, the study will evaluate the effect of taurine and arginine supplementation in soybean-meal-based diets on hybrid striped bass.

MATERIALS AND METHODS

Six diets with 40% crude protein, 10% lipid and 2.96 kcal digestible energy (DE)/g were manufactured as described by Keembiyehetty and Gatlin (1995). All diets met or exceeded the established nutrient requirements of hybrid striped bass with the exception of the basal diet (Table 3) which contained 1.1% total sulfur amino acids (TSAA), 5% below the established TSAA minimal requirement of hybrid striped bass (Keembiyehetty and Gatlin, 1993). The other experimental diets provided 1.2% TSAA on a dry-weight basis from the various supplements which were provided on an equal sulfur basis. These diets were maintained isonitrogenous with aspartic acid. Specific ingredient substitutions of the minor ingredients in the experimental diets are provided in Table 4.

Each diet was fed to triplicate groups of eleven fingerling hybrid striped bass in a system consisting of 38-liter aquaria according to well established procedures (Keembiyehetty and Gatlin, 1997; Li and Gatlin, 2003; Kelly et al., 2006). The aquaria operated in a recirculating system, with the temperature maintained at 25-26°C by managing ambient air temperature and brackish water was maintained at 4 ppt dissolved solids by mixing synthetic sea water and sodium chloride with fresh well water.

Fish were fed twice daily, morning and evening, while the feeding rate was maintained at a fixed level close to apparent satiation for 10 weeks. Once a week, fish were netted and then group-weighed by placing them into a tared container of water on an electronic balance; this procedure minimized handling stress. The experimental

Table 3 - Basal diet formulation.

Ingredient	g/100g dry weight
Menhaden fishmeal	15.00
Soybean meal	56.00
Dextrin	7.50
Menhaden oil	7.20
Mineral premix	4.00
Vitamin premix	3.00
Carboxymethyl cellulose	2.00
Inosine monophosphate	0.50

Table 4 – Ingredient composition of experimental diets.

Ingredient (g/100 g dry weight)	Basal	L-met	Mintrex®	MHA	Taurine	Arginine
Mintrex®	--	--	0.60	--	--	--
MHA	--	--	--	0.57	--	--
Taurine	--	--	--	--	1.50	--
L-arginine HCL	--	--	--	--	--	1.50
L-methionine	--	0.50	--	--	0.50	0.50
L-alanine/L-aspartate	4.04	3.68	4.04	4.04	2.40	--
Celufil	0.28	0.28	--	--	0.28	0.30

aquaria and associated equipment were located at the Texas A&M University System's Aquacultural Research and Teaching Facility in Burleson County, TX.

At the time of trial termination, two fish from each aquarium were collected at random and their weights and lengths recorded to compute body condition indices as previously described (Keembiyehetty and Gatlin, 1997). To gather further information about the health and overall composition of growth in the fish, the exsanguinated carcass was dissected to collect and weigh the liver, intraperitoneal fat (IPF), and total muscle weight for computation of hepatosomatic index (HSI), IPF ratio and muscle ratio, respectively. Dissected body parts were then recombined with the remainder of the carcass, and the whole carcass analyzed for proximate composition (percent moisture, protein, ash, lipid) according to established procedures (Nematipour et al., 1992).

Each of the two representative fish was exsanguinated via veinepuncture of the caudal vasculature with heparinized needles approximately 12h after their final feeding. Plasma was separated from whole blood via centrifugation (2000 x *g*, 10 min) and analyzed for lysozyme activity (Ellis, 1990; Jorgensen et al., 1993; Li and Gatlin, 2003). In addition, plasma samples were stored at -80°C until such time that free amino acid analysis could be performed via high-performance liquid chromatography according to the procedures of Buentello and Gatlin (2001).

Diet and serum samples were analyzed for zinc by the Office of the Texas State Chemist via atomic absorption spectrophotometry using a Varian Spectr-AA 220 FS AA. Diet samples were prepared for analysis using a dry-wet-dry ashing procedure as previously described (Scarpa and Gatlin, 1992). Plasma samples were treated with trichloroacetic acid according to the methods of Parker et al. (1967) prior to analysis.

One-way analysis of variance (ANOVA) and Duncan's multiple-range test was used to analyze all data from the feeding trial (SAS Institue, 1996). Responses were considered significant at $P < 0.05$.

RESULTS

Sulfur amino acid feeding trial

After 10 weeks of feeding the experimental diets, fish fed the MHA-supplemented diet displayed a weight gain of 256% of initial weight, while fish fed the basal diet displayed a weight gain of 190%; however, this difference was not significant (Table 5). Feeding efficiency ratio (FER) values of fish fed the various diets ranged between 0.59 and 0.72 and were not statistically different (P=0.1595). Although the FER value of fish fed the basal diet was numerically lowest (Table 5). Fish fed the diet supplemented with MHA had the highest protein efficiency ratio (PER) value while fish fed the basal diet had the lowest value, with intermediate responses produced by fish fed the diets supplemented with L-methionine and Mintrex®; however, these trends were not significant (P = 0.11; Table 5). Survival during the trial was high and not affected by dietary treatment (Table 5).

The various dietary treatments did significantly affect some body condition indices of hybrid striped bass with the highest HSI value observed in fish fed the L-methionine-supplemented diet (Table 6). Fish fed the basal diet had the lowest HSI value while those fed MHA and Mintrex® had intermediate values. The highest muscle ratio values were observed in fish fed the sulfur amino acid supplemented diets, particularly those fed the diets supplemented with L-methionine and Mintrex®. Fish fed the basal diet had the lowest muscle ratio value although statistical significance was marginal (P<0.0592) (Table 6). The IPF ratio and condition factor indices were not significantly affected by dietary treatment (Table 6).

Whole-body composition of fish fed the various diets was not significantly affected by the various dietary treatments (Table 7). However, whole-body protein and lipid of fish fed the basal diet tended to be lower than that of fish fed all other diets. Moisture and ash were very similar for fish fed all of the dietary treatments. When both composition of fish weight gain and feed consumption were jointly considered, fish fed the diets supplemented with sulfur amino acids had significantly greater net energy utilization in comparison to those fed the basal diet (Table 7). Fish fed the diets supplemented with L-methionine, Mintrex®, and MHA also tended to have higher net protein utilization values compared to those fed the basal diet although differences were not statistically distinct (Table 7). These results indicate fish fed the basal diet were not efficiently using dietary protein for protein synthesis, and consequently energy utilization also was impaired.

Free methionine concentrations in plasma of fish fed the various diets was significantly different with those fed the MHA-supplemented diet having the lowest value (Table 8). In contrast, fish fed the Mintrex®-supplemented diet had the highest methionine concentration.

Table 5 – Performance of juvenile hybrid striped bass fed diets with different amino acid supplements in soybean-meal-based diets for 10 weeks.[1]

Diet	Weight gain (% of initial weight)	Feed efficiency ratio (g gain/g dry feed)	Protein efficiency ratio (g gain/g protein fed)	Survival (%)
Basal	190	0.59	1.28	88
+ L-methionine	227	0.64	1.56	97
+ Mintrex®	225	0.67	1.55	88
+ MHA	256	0.72	1.77	97
pooled SE	44.78	0.10	0.34	16.97
ANOVA $P>F$[2]	0.1010	0.1595	0.1101	0.5523

[1]Means of triplicate groups.

[2]Significance probability associated with the F statistic.

Table 6 – Body condition indices of juvenile hybrid striped bass fed diets with different amino acid supplements in soybean-meal-based diets.[1, 2]

Diet	HSI[3]	Muscle ratio[4]	IPF ratio[5]	Condition Factor[6]
Basal	1.17[b]	33.0	2.80	2.03
+ L-methionine	1.60[a]	37.6	2.51	2.07
+ Mintrex®	1.23[b]	37.2	2.63	2.04
+ MHA	1.33[ab]	36.2	2.88	2.13
pooled SE	0.24	3.03	0.66	0.25
ANOVA $P>F$[7]	0.0331	0.0592	0.6837	0.8482

[1]Means of three fish from each of three replicate groups.

[2]Values within in the same column with different letters are significantly different ($P<0.05$).

[3]Hepatosomatic index (HSI) = Liver weight x 100/body weight

[4]Muscle weight x 100/body weight

[5]Intraperitoneal Fat (IPF) ratio = IPF weight x 100/body weight

[6]Condition Factor = weight of fish x 100/length of fish^3

[7]Significance probability associated with the F statistic.

Table 7 – Whole-body composition of juvenile hybrid striped bass fed diets with different amino acid supplements in soybean-meal-based diets for 10 weeks.[1, 2, 3]

Diet	Moisture	Ash	Protein	Lipid	Net energy utilization[4] (%)	Net protein utilization[5] (%)
	-------------------% of fresh weight-------------------					
Basal	69.9	5.2	14.6	5.8	24.3[b]	19.7
+ L-methionine	70.2	4.8	18.2	7.2	38.3[a]	30.7
+ Mintrex®	70.0	5.1	17.3	6.8	36.7[a]	29.1
+ MHA	69.2	5.1	16.5	7.5	41.3[a]	30.7
pooled SE	1.78	0.64	2.78	1.26	9.39	9.06
ANOVA $P>F$[6]	0.7425	0.5356	0.1441	0.1182	0.0296	0.1139

[1]Means of composite samples of two fish from each of three replicate groups.

[2]Fish initially contained 72.6% moisture, 4.5% ash, 13.3% protein, and 5.9% lipid.

[3]Values within in the same column with different letters are significantly different ($P<0.05$).

[4](kcal gain/kcal fed) x 100

[5](g protein gain/g protein fed) x 100

[6]Significance probability associated with the F statistic.

Zinc contribution of sulfur amino acid diets

A primary difference in the sulfur amino acid supplements evaluated in this trial was that Mintrex® provided chelated zinc in association with the MHA compound. Thus, the diet supplemented with Mintrex® had a much higher zinc concentration than diets containing the other supplements (Table 9). After 10 weeks of feeding, fish fed the Mintrex®-supplemented diet displayed significantly higher serum zinc concentrations compared to fish fed the other diets (Table 9).

Taurine and arginine feeding trial

In this trial, a nutritionally replete soybean-meal-based diet supplemented with L-methionine to satisfy the total sulfur amino acid requirement of sunshine bass (Keembiyehetty and Gatlin, 1993) was used as a control diet, and the experimental diets were further supplemented with either arginine or taurine. No statistical differences were detected in weight gain, FER or PER values between fish fed these three different diets (Table 10). All three diets supported high survival at the end of the 10-week feeding trial (Table 10).

In terms of body condition indices, HSI, muscle ratio and condition factor values were similar for fish fed the three diets (Table 11). In contrast, the IPF ratio value of fish fed the taurine-supplemented diet tended ($P<0.0592$) to be higher than that of fish fed the arginine-supplemented and control diets (Table 11).

Whole-body composition of fish fed these three diets was generally similar except for a tendency for fish fed the control diet to have higher whole-body lipid compared to those fed the two supplemented diets (Table 12). Net energy utilization of

Table 8 – Free methionine plasma profile of juvenile hybrid striped bass fed diets with different amino acid supplements in soybean-meal-based diets for 10 weeks.[1]

Diet	Methionine nmol/ml
Basal	8.6[a]
+ L-methionine	6.6[a, b]
+ Mintrex®	9.4[a]
+ MHA	4.0[b]
pooled SE	5.9
ANOVA $P>F$[2]	0.0399

[1]Means of two fish from each of three replicate groups.

[2]Significance probability associated with the F statistic.

Table 9 – Zinc concentration of soybean-meal-based diets supplemented with different amino acid compounds and serum zinc of juvenile hybrid striped bass fed the diets for 10 weeks.

Diet	Dietary zinc (µg/g dry diet)[1]	Serum zinc (µg/100 ml)[2, 3]
Basal	79.8	532.8[b]
+ L-methionine	79.0	347.2[b]
+ Mintrex®	1059.0	1414.0[a]
+ MHA	77.2	601.2[b]
pooled SE		366.16
ANOVA $P>F$[4]		<0.0001

[1]Means of duplicate samples.

[2]Means of two fish from each of three replicate groups.

[3]Values within the same column with different letters are significantly different ($P<0.05$). [4]Significance probability associated with the F statistic.

fish fed the control diet also tended (*P*=0.0794) to be greater than that of fish fed the arginine- and taurine-supplemented diets (Table 12). Net protein utilization values ranged between 24.6 and 30.7% and were not affected by dietary treatment (Table 12).

Fish fed the control diet exhibited the highest serum lysozyme activity (12 Units/L), while lower activity was noted in fish fed the diets supplemented with taurine (9.3 U/L) and arginine (9.6 U/L). However, these differences were not significant (P=0.5994).

Free amino taurine and arginine levels in plasma of fish fed the taurine- and arginine-supplemented diets were significantly higher than those of fish fed the control diet (Table 13).

Table 10 – Performance of juvenile hybrid striped bass fed nutritionally replete soybean-meal-based diets supplemented with either arginine or taurine for 10 weeks.[1]

Diet	Weight gain (% of initial weight)	Feed efficiency ratio (g gain/g dry feed)	Protein efficiency ratio (g gain/g protein fed)	Survival (%)
Control	227	0.64	1.56	97
+ Taurine	247	0.65	1.55	94
+ Arginine	245	0.67	1.57	94
pooled SE	65.28	0.18	0.41	7.35
ANOVA $P>F$[2]	0.8376	0.9638	0.9973	0.7290

[1]Means of triplicate groups.

[2]Significance probability associated with the F statistic.

Table 11 – Body condition indices of juvenile hybrid striped bass fed nutritionally replete soybean-meal-based diets supplemented with either taurine or arginine.[1]

Diet	HSI[2]	Muscle ratio[3]	IPF ratio[4]	Condition Factor[5]
Control	1.60	37.6	2.51	2.07
+ Taurine	1.23	38.0	3.04	2.10
+ Arginine	1.27	37.8	2.41	2.17
pooled SE	0.28	1.96	0.38	0.15
ANOVA $P>F$[6]	0.1149	0.9554	0.0569	0.5484

[1]Means of three fish from each of three replicate groups.

[2]Hepatosomatic index (HSI) = (Liver weight x 100)/body weight.

[3]Muscle weight x 100/body weight.

[4]Intraperitoneal Fat (IPF) ratio = IPF weight x 100/body weight.

[5]Condition Factor = weight of fish x 100/length of fish^{3}.

[6]Significance probability associated with the F statistic.

Table 12 – Whole-body composition of juvenile hybrid striped bass fed nutritionally replete soybean-meal-based diets supplemented with either taurine or arginine for 10 weeks.[1,2]

Diet	Moisture	Ash	Protein	Lipid	Net energy utilization[3] (%)	Net protein utilization[4] (%)
	-------------------% of fresh weight-------------------					
Control	70.2	4.8	18.2	7.2	38.3	30.7
+ Taurine	69.3	4.9	15.6	6.4	32.0	25.4
+ Arginine	70.2	5.2	15.1	5.3	29.0	24.6
pooled SE	0.68	0.32	11.66	1.08	5.85	6.03
ANOVA $P>F$[5]	0.0858	0.1537	0.0760	0.0557	0.0794	0.2405

[1]Means of composite samples of three fish from each of three replicate groups.

[2]Fish initially contained 72.6% moisture, 4.5% ash, 13.3% protein, and 5.9% lipid.

[3](kcal gain/kcal fed) x 100.

[4](g protein gain/g protein fed) x 100.

[5]Significance probability associated with the F statistic.

Table 13 – Free amino acids in plasma of juvenile hybrid striped bass fed diets with different amino acid supplements in soybean-meal-based diets for 10 weeks.[1]

Diet	Taurine	Arginine	Methionine
	--------nmol/ml--------		
Control	63.1[b]	19.0[b]	6.6[a]
+ Taurine	89.6[a]	21.4[b]	3.5[b]
+ Arginine	62.9[b]	30.4[a]	2.5[b]
pooled SE	24.8	7.9	2.4
ANOVA $P>F$[2]	0.0142	0.0032	0.0007

[1]Means of two fish from each of three replicate groups.

[2]Significance probability associated with the F statistic.

DISCUSSION

Sulfur amino acid feeding trial

TSAA are important components to monitor in plant-based diets, especially when fed to carnivorous fish, which tend to have higher requirements for TSAA than do herbivorous fishes (NRC, 1993). Sulfur amino acids are the most limiting factor in plant-based diets such as those containing high levels of soybean meal. Signs of deficiency associated with TSAA include reduced weight gain, FER and PER values. In order to combat deficiency, diets are typically supplemented with DL-methionine or MHA; however, several studies have shown variable utilization of MHA (Katz and Baker, 1975; Harter and Baker, 1977; Robinson et al., 1978; Dibner et al., 1990; Han et al., 1990; Dibner et al., 1992; McCollum et al., 2000; Goff and Gatlin, 2004; Richards et al., 2005; Kelly et al., 2006; Kim, 2006; Richards et al., 2007; Santos, 2007; Yi et al., 2007; Wilson et al., 2008). Mintrex® is a form of MHA which also provides an organic form of chelated zinc which presumably provides greater bioavailability of zinc to the fish.

Results of the present study in which MHA and Mintrex® were shown to be suitable substitutes for L-methionine in soybean-meal-based diets for hybrid striped bass were generally similar to recent observations in which the liquid form of MHA (Alimet™) and crystalline MHA calcium salt were evaluated in semi-purified diets with hybrid striped bass of similar size (Kelly et al. 2006). Alimet™ is a commercially available L-methionine supplementation source, formulated as an 88% aqueous solution of 2-hydroxy-4-(methylthio) butanoic acid (Dibner et al., 1990; Yi et al., 2007).

Crystalline MHA calcium salt consists of 84% 2-hydroxy-4-(methylthio) butanoic acid. The conversion of Alimet™ and MHA calcium salt into L-methionine is a two-step pathway: first, the alpha carbon to 2-oxo-4-(methylthio) butanoic acid (keto-methionine) is oxidized and then the second carbon undergoes transamination to produce L-methionine (Dibner and Knight, 1984). Varied efficacy of Alimet™ and crystalline MHA relative to L-methionine has been attributed to differential uptake of MHA which is mediated by a sodium independent carrier system associated with L-lactate transport (Brachet and Puigserver, 1987). This uptake mechanism is quite different from that of L-methionine, which is rapidly absorbed via passive diffusion in low pH environments such as the upper gastrointestinal tract (Dibner, 2003; Richards et al., 2005).

In considering weight gain response of hybrid striped bass, Kelly et al. (2006) reported Alimet™ to be 73% as effective as L-methionine at the same concentration, while MHA was 83% as effective. In contrast, Keembiyehetty and Gatlin (1995, 1997) found that hybrid striped bass fed semi-purified and practical diets supplemented with MHA calcium salt on an equal-sulfur basis as the control diet containing L-methionine had significantly reduced weight gain and feed efficiency values compared to fish fed the control diet. They estimated that MHA calcium salt was only 75 % as effective as L-methionine (Keembiyehetty and Gatlin, 1995). In contrast, MHA was found to be only 26% as effective as L-methionine when fed to channel catfish (Robinson et al., 1978).

In the present study, insight was provided by the methionine concentration in plasma samples of hybrid striped bass fed the various methionine sources with fish fed MHA having the lowest circulating methionine concentration. Free methionine levels in plasma indicated fish fed the basal and the Mintrex®-supplemented diets were significantly higher than those of fish fed MHA. The difference in absorption methods may explain the varied findings regarding biological utilization of these sulfur amino acid compounds.

In the current study, fish fed the MHA-supplemented diet gained similar weight compared to fish fed the other supplemented diets and while not significantly different, MHA-fed fish exhibited numerically greater weight gain in comparison to those fed the basal diet. Results from these various studies with hybrid striped bass have shown some differences in utilization of liquid MHA as compared to MHA calcium salt. For example, using a similar diet formulation as in the present study, Keembiyehetty and Gatlin (1997) reported significantly greater weight gain of hybrid striped bass fed diets supplemented with L-methionine compared to MHA calcium salt. Studies with other fish species also have reported variable utilization of MHA calcium salt. In channel catfish, MHA calcium salt was only 26% as effective as L-methionine in promoting weight gain (Robinson et al., 1978). In contrast, juvenile red drum were found to use crystalline MHA as proficiently as L-methionine based on growth performance (Goff and Gatlin, 2004). Chicks also have been reported to use MHA with greatly variable efficiency (Katz and Baker, 1975; Harter and Baker, 1977). The addition of MHA to methionine-deficient diets of juvenile pigs significantly improved weight gain and feed efficiency (Reifsnyder et al., 1984). In another study, pigs fed soybean-meal based diets supplemented with MHA had similar growth rates as pigs fed diets supplemented with L-methionine (Chung and Baker, 1992). The bioavailability of MHA to serve as a sulfur amino acid directly relates to its absorption and the body's ability to convert MHA into L-methionine once it is absorbed (Han et al., 1990). It has been suggested that MHA absorption may be impaired due to the inability of carrier proteins to distinguish its recognition site (Dibner and Knight, 1984; Keembiyehetty and Gatlin, 1997). MHA has been described to interact with a component of the L-lactate transport system in the brush boarder of the jejunum in rats which is a different pathway than used for L-methionine absorption (Brachet and Puigserver, 1987). Mintrex® containing zinc was determined to be a fully available source of methionine to broiler chicks (Richards et al., 2007; Yi et al., 2007) and can be used as a source of L-methionine in sheep as well (McCollum et al., 2000), thus supporting the results of the present study in which Mintrex® supported similar fish performance as the other sulfur amino acid compounds.

A marginal deficiency of TSAA in the basal diet in the current study presumably resulted in the lowest fish performance observed in this experiment. Keembiyehetty and Gatlin (1993) first reported that low dietary levels of methionine or TSAA suppressed growth of hybrid striped bass and increased susceptibility to fungal disease, resulting in high mortality. In the current study, due to the high survivability and slightly reduced performance of fish fed the basal diet, it is evident that TSAA were only marginally deficient in the diet formulation which contained 15% menhaden fishmeal and 56% soybean-meal. The basal diet was calculated to contain 1.1% TSAA based on the concentration of TSAA in these ingredients. Because the level of TSAA in the basal diet used in the present study was only 5% below the established requirement for TSAA, it did not cause severe growth depression but generally demonstrated a need to supplement the soybean-meal based diet with sulfur amino acids as previously indicated (Griffin et al., 1994; Keembiyehetty and Gatlin, 1997).

In the current study, PER values were statistically similar among all dietary treatments although fish fed the basal diet tended to have the lowest values. Fish in other studies were found to have higher PER when fed diets supplemented with various sulfur amino acid compounds compared to fish fed a basal diet deficient in TSAA (Keembiyehetty and Gatlin, 1993; 1995; 1997; Goff and Gatlin, 2004). Higher PER values achieved by fish fed the supplemented diets relate to the role of methionine in protein synthesis. In addition, methionine can be converted to S-adenosylmethionine (SAM), which is essential for numerous chemical reactions within the body (Eloranta, 1977; Finkelstein, 1990), such as acting as a methyl group donor in the methylation of proteins (Lobley, 1992; Chiang et al., 1996; Loest et al., 2002).

Cost comparison among these different supplements should be given consideration by feed producers and aquaculturists as feed cost is typically the greatest expense in aquaculture. Mintrex® is by far the costliest supplement at $2.50 USD/lbs, followed by L-methionine at $1.12 USD/lbs and MHA in liquid form (Alimet™) at $1.00 USD/lbs (Novus International, 2008).

Zinc contribution

Hybrid striped bass fed the Mintrex®-supplemented diet had significantly higher zinc serum concentrations than fish fed the other diets which may be related to the far greater bioavailability of the chelated zinc in Mintrex®. Similar results concerning high zinc bioavailability have been reported for zinc-methionine compounds in chickens (Yi et al., 2007) and humans (Brown, 2002). Due to space limitations in the culture system, a direct comparison between organic and inorganic sources of zinc could not be made in the present study by including another treatment with a similar level of supplemented zinc from zinc sulfate as provided by Mintrex®. However, it appears the supplemented zinc provided by Mintrex® (1058.99 µg/g vs. an average of 78.66 µg/g supplemented by the other diets) was readily absorbed by hybrid striped bass based on the much higher plasma zinc levels of fish fed that diet. Absorption of trace minerals from practical plant-based diets has been reported to be quite low in chickens and livestock due to physiological and diet ingredient antagonism (Leeson and Summers, 2001; Underwood and Suttle, 2001; Dibner, 2005). In the stomach and upper gastrointestinal tract of chickens and other animals, low pH dissociates inorganic trace mineral salts and dietary antagonists such as phytic acid, reduce the bioavailability of zinc for absorption later in the gastrointestinal tract (Leeson and Summers, 2001; Dibner, 2005). By chelating a zinc molecule to two MHA molecules, the zinc complex is able to better survive the upper gastrointestinal tract and is delivered to the absorptive portion of the small intestine, thus greatly increasing its bioavailability (Leeson and Summers, 2001; Dibner, 2005; Chien et al., 2006; Yi et al., 2007). Zinc-methionine (an equal 1:1:1 ratio of zinc, DL- or L- methionine, and a sulfate anion) is an organic source of zinc. Chien et al. (2006) demonstrated increased bioavailability of zinc from zinc-methionine in both human and animal trials. Zinc-methionine also has been shown to have greater bioavailability compared to zinc sulfate in chicks (Wedekind et al., 1992).

Based on the previously mentioned cost comparisons, Mintrex® is by far the costliest supplement (Novus International, 2008). However, some savings may be realized with Mintrex® because it also provides an available source of zinc.

Taurine and arginine feeding trial

In the past, animal-based feedstuffs in fish diets have provided a major source of taurine. However, with the increased use of taurine-deficient soybean meal and other plant feedstuffs as alternative protein sources in fish diets, taurine supplementation has been investigated with great interest, especially for carnivorous fishes. Although taurine is categorized as a non-essential amino acid, it has been found to be at least provisionally essential in some carnivorous fish species (Takeuchi, 2001). Increased weight gain in rainbow trout (Gaylord et al., 2006, 2007) and Japanese flounder (Park et al., 2002; Kim et al., 2003, 2005) has been reported with the addition of taurine to plant-based diets. Rainbow trout fed plant-based diets supplemented with 5 g/kg of taurine were reported to show equivalent growth rates as fish fed fishmeal-based diets with no taurine supplementation. However, growth on the plant-based diets was not improved beyond the taurine supplementation level of 5 g/kg diet (Gaylord et al., 2006). In the present study, taurine was supplemented to the soybean-meal-based diet at 15 g/kg. This level of taurine added to the diet should have been sufficient to elicit a growth response if taurine was inadequate in the control diet. Such an enhanced growth response was not observed.

Rainbow trout also have been found to use cysteine as a precursor to produce taurine (Yokoyama et al., 1997). Methionine can be converted to cysteine, which is oxidized with the enzyme cysteine dioxygenase to form cysteinesulphinate. Cysteinesulphinate then undergoes a decarboxylation reaction by L-cysteinesulphinate decarboxylase (CSD) to form hypotaurine and then taurine (Yokoyama et al., 1997). CSD activity has been reported in Japanese flounder and rainbow trout as well as other carnivorous fish species, although the amount of activity is variable (Yokoyama et al., 1997; Yokoyama, 2001). This enzyme activity appears to be limited as rainbow trout are not capable of producing enough taurine to sustain maximal growth when fed plant-based diets (Gaylord et al., 2006). In the current study, the taurine-supplemented diet did not significantly increase weight gain of hybrid striped bass compared to the control diet. Thus, the hybrid striped bass may have the ability to synthesize an adequate amount of taurine from cysteine, although the activity of CSD was not measured.

Masuda (1984) reported dietary taurine raised neutrophil concentrations in the body as well as boosted host defense mechanisms by increasing neutrophil membrane stability. Oral administration of taurine also has been shown in rats to increase phagocytic and bactericidal capabilities of neutrophils against the bacteria *Escherichia coli* with rising levels of taurine (Masuda, 1984). However, following the release of myeloperoxidase, a peroxidase enzyme produced by neutrophil granulocytes, lysozyme release was inhibited causing a decrease in lysozyme activity (Masuda, 1984). Such a response may explain the lower lysozyme activity of fish fed the taurine-supplemented diet in the current study. That diet did provide an increase in plasma free taurine compared to fish fed the control diet, but no obvious benefits of taurine supplementation were observed.

The supplementation of arginine to diets of fish above the minimum level required for normal growth and protein synthesis has received considerable attention in recent years due to the role of arginine in enhancing disease resistance as the sole precursor for nitric oxide production (Buentello and Gatlin, 1999). In the present study, lysozyme activity was measured as a general index of non-specific immunity. Lysozyme activity ranged from 9.3 to 12.0 Units/L and did not greatly differ among the dietary treatments. Nitric oxide production by channel catfish macrophages has been reported to increase in the presence of arginine and glutamine (Buentello and Gatlin, 1999), and supplementation of 2% arginine (8.3% of dietary protein) significantly increased survival against *Edwardsiella ictaluri* (Buentello and Gatlin, 2001). In the current study, the control diet which was computed to contain 2.63% arginine and was supplemented with arginine at 1.5 % of diet (3.75% of dietary protein) producing a diet with 4.13% arginine. That diet provided increased plasma free arginine relative to fish fed the control diet, however, no significant effect was noted in terms of lysozyme production as an index of non-specific immunity.

Further evaluation of the potential benefits of dietary arginine supplementation is needed not only for nutritional and immunological purposes, but for potentially improving environmental conditions as well. Tulli et al. (2007) reported juvenile European sea bass fed plant-meal-based diets excreted greater amounts of nitrogenous wastes than did their experimental counterparts fed fishmeal-based diets, when expressed as a percentage of nitrogen intake. Once the arginine dietary requirement was satisfied, plasma urea levels dramatically increased, and a strong linear relationship between increased amounts of dietary arginine, arginase activity in the liver, and urea-nitrogen excretion was observed; however, total nitrogenous waste excretion was not affected (Tulli et al., 2007). Similar results were reported in turbot and rainbow trout fed fishmeal-based and plant-meal-based diets with varying levels of arginine (Fournier et al., 2003). A major pathway in ammonia detoxification and urea formation is the ornithine-urea cycle. Ornithine transcarbamylase is a liver enzyme in the ornithine-urea cycle. Low levels of ornithine transcarbamylase were reported in sea bass and it appears to be influenced by arginine levels (Huggins et al., 1969; Wright and Land, 1998).

CONCLUSIONS

The present study confirmed the efficacy of various dietary methionine supplements and established the efficient utilization of Mintrex® by hybrid striped bass. Fish fed diets supplemented with L-methionine and MHA performed similarly; however, fish fed the diet supplemented with Mintrex® had the highest circulating levels of zinc and methionine in comparison to fish fed the other supplements. Thus, the Mintrex® product appears to have considerable potential as a supplement to increase the concentration of TSAA and zinc in practical diets.

Hybrid striped bass fed arginine- and taurine-supplemented diets did not exhibit any significant differences in performance compared to fish fed the control diet. Therefore, supplementation of these amino acids in nutritionally complete plant-based diets does not appear warranted, although detailed assessments of disease resistance and immune responses were not made in this study.

REFERENCES

Akarid, K., Sinet, M., Desforges, B., Gougerot-Pocidalo, M.A., 1995. Inhibitory effect of nitric oxide on the replication of a murine retrovirus in vitro and in vivo. J. Virol. 69-11, 7001-7005.

Barziza, D.E., Buentello, J.A., Gatlin, D.M., 2000. Dietary arginine requirement of juvenile red drum (*Sciaenops ocellatus*) based on weight gain and feed efficiency. J. Nutr. 130, 1796-1799.

Brachet, P., Puigserver, A., 1987. Transport of methionine hydroxy analog across the brush borded membrane of rat jejunum. J. Nutr. 117, 1241-1246.

Brethour, J.R., 1984. Zinc methionine in steer finishing rations. In: University, K.S. (Ed.), Report of Progress, Kansas, USA, pp. 11.

Brown, K.H., 2002. Effect of supplemental zinc on the growth and serum zinc concentrations of pre-pubertal children: a meta-analysis of randomized, controlled trials. Am. J. Clin. Nutr. 75, 1062-1071.

Buentello, J.A., Gatlin, D.M., 1999. Nitric oxide production in activated macrophages from channel catfish (*Ictalurus punctatus*): influence of dietary arginine and culture media. Aquaculture 179, 513-521.

Buentello, J.A., Gatlin, D.M., 2001. Effects of elevated dietary arginine on resistance of channel catfish to exposure to *Edwardsiella ictaluri*. J. Aquatic Animal Health 13, 194-201.

Carlberg, J.M., Massingill, M.J., Chamberlain, R.J., Van Olst, J.C., 2004. U.S. production and sales of hybrid striped bass 1987-2004. http://aquanic.org/sbga/H

SBversion5.ppt.

Chiang, P.K., Gordon, R.K., Tal, J., Zeng, G.C., Doctor, B.P., Pardhasaradhi, K., McCann, P.P., 1996. S-adenosylmethionine and methylation. FASEB J. 10, 471-480.

Chien, X.X., Zafra-Stone, S., Bagchi, M., Bagchi, D., 2006. Bioavailability, antioxidant and immune-enhancing properties of zinc methionine. BioFactors 27, 231-244.

Chung, T.K., Baker, D.H., 1992. Utilization of methionine isomers and analogs by the pig. Can. J. of An. Sci. 72, 185-188.

De Groote, M.A., Fang, F.C., 1995. Nitric oxide inhibitions: antimicrobial properties of nitric oxide. Clin. Infect. Dis. 21, S162-S165.

Dibner, J.J., 2003. Review of the metabolism of 2-hyroxy-4-(methylthio)butanoic acid. World's Poult. Sci. J. 59, 99-110.

Dibner, J.J., 2005. Early nutrition of zinc and copper in chicks and poults: impact on growth and immune function. Proc. 3rd Mid-Atl. Nutr. Conf., 23-32.

Dibner, J.J., Knight, C.D., 1984. Conversion of 2-hydroxy-4-(methylthio) butanoic acid to L-methionine in the chick: a sterospecific pathway. J. Nutr. 114.

Dibner, J.J., Atwell, C.A., Ivey, F.J., 1992. Effect of heat stress on 2-hydroxy-4-(methylthio) butanic acid and DL-methionine absorption measured in vitro. Poul. Sci. 71, 1900-1910.

Dibner, J.J., Durley, R.C., Kostelc, J.G., Ivey, F.J., 1990. 2-Hydroxy-4-methylthio butanoic acid (HMB) is a naturally occurring methionine precursor in the chick. J. Nutr. 120, 553-560.

Ellis, A.E., 1990. Lysozyme assays. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., van Muiswinkel, W.B. (Eds.), Techniques in Fish Immunology. SOS Publications, Fair Haven, NJ, USA, pp. 101-103.

Eloranta, T.O., 1977. Tissue distribution of S-adenosylmethionine and S-adenosylhomocysteine in the rat. Effect of age, sex, and methionine administration on the metabolism of S-adenosylmethionine, S-adenosylhomocysteine and polyamines. Biochem. J. 166, 521-529.

FAO Fisheries Department, F.I., Data and Statistics Unit, 2005. Fish Stat Plus: Universal Software for Fishery Statistical Time Series.

Feedstuffs, 2008. Grain and ingredient cash market comparisons, Feedstuffs The Weekly Newspaper For Agribusiness.

Finkelstein, J.D., 1990. Methionine metabolism in mammals. J. Nutr. Biochem. 1, 228-237.

Fournier, V., Gouillou-Coustans, M.F., Metailler, R., Vachot, C., Moriceau, J., Le Delliou, H., Desbruyeres, E., Kaushik, S.J., 2003. Excess dietary arginine affects urea excretion but does not improve N utilisation in rainbowtrout *Oncorhynchus mykiss* and turbot *Psetta maxima*. Aquaculture 217, 559-576.

Gallagher, M.L., 1994. The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass (Morone saxatilisxM. chrysops). Aquaculture 126, 119-127.

Garber, A.F., Sullivan, C.V., 2006. Selective breeding for the hybrid striped bass (*Morone chrysops x M. saxatilis*, Walbaum) industry: status and perspectives. Aquaculture Res. 37, 319-338.

Gatlin, D.M., 1997. Nutrition and feeding of striped bass and hybrid striped bass. In: Harrell, R.M. (Ed.), Striped Bass and Other Morone Culture. Elsevier Science BV, Amsterdam, The Netherlands, pp. 235-251.

Gatlin, D.M., Phillips, H.F., 1989. Dietary calcium, phytate and zinc interactions in channel catfish. Aquaculture 79, 259-266.

Gatlin, D.M., Wilson, R.P., 1984. Zinc supplementation of practical channel catfish diets. Aquaculture 41, 31-36.

Gaylord, G.T., Barrows, F.T., Teague, A.M., Johansen, K.A., Overturf, K.E., Shepherd, B., 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (Oncorhynchus mykiss). Aquaculture 269, 514-524.

Gaylord, T.G., Teague, A.M., Barrows, F.T., 2006. Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). J. World Aquaculture Soc. 37, 509-517.

Goff, J.B., Gatlin, D.M., 2004. Evaluation of different sulfur amino acid compounds in the diet of red drum, *Sciaenops ocellatus*, and sparing value of cystine for methionine. Aquaculture 241, 465-477.

Griffin, M.E., Wilson, K.A., Brown, P.B., 1994. Dietary arginine requirement of juvenile hybrid striped bass. J. Nutr. 124, 888-893.

Gummadi, R.C., Reigh, R.C., 2003. Growth performance and body composition of palmetto bass fed five levels of dietary protein at two energy-to-protein ratios. N. Am. J. of Aquaculture 65, 278-288.

Han, Y., Castanon, F., Parsons, C.M., Baker, D.H., 1990. Absorption and bioavailability of DL-methionine hydroxy analog compared to DL-methionine. Poul. Sci. 69, 281-287.

Hand, M.S., Thatcher, C.D., Remillard, R.L., Roudebush, P., 2000. Small Animal Clinical Nutrition. Walsworth Publishing Company, Topeka, USA.

Harrell, R.M., 1997. *Morone* pond production. In: Harrell, R.M. (Ed.), Striped Bass and Other *Morone* Culture. Elsevier, Amsterdam, The Netherlands, pp. 75-97.

Harter, J.M., Baker, D.H., 1977. Sulfur amino acid activity of glutathione, DL-alpha-hydroxy-methionine, and alpha-keto-methionine in chicks. Proc. Soc. Exp. Biol. Med. 156, 201-204.

Hibbs, J.B., Taintor, R.R., Vavrin, Z., 1987. Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Sci. 235, 473-476.

Hibbs, J.B., Taintor, R.R., Vavrin, Z., 1988. Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem. and Biophys. Res. Comm. 157, 87-94.

Hirsh, D.C., MacLachlan, N.J., Walker, R.L., 2004. Veterinary Microbiology. Blackwell Publishing, Victoria, Australia.

Hodson, R.G., 1989. Hybrid striped bass biology and life history. Southern Regional Aquaculture Center Publication, 4.

Huggins, A.K., Skutsch, G., Baldwin, E., 1969. Ornithine urea cycle enzyme in teleostean fish. Comp. Biochem. Physiol. 28, 587-602.

IFFO, International Fishmeal and Fish Oil Organisation, 2006. The production of fishmeal and fish oil from gulf menhaden. http://www.iffo.net/intranet/content /archivos/63.pdf.

Jorgensen, J.B., Sharp, J.E., Secombes, C.J., Robertsen, B., 1993. Effect of a yeast-cell-wall glucan on the bacterial activity of rainbow trout macrophages. Fish Shellfish Immunol. 3, 267-277.

Katz, R.S., Baker, D.H., 1975. Factors associated with utilization of the calcium salt of methionine hydroxy analogue by the young chick. Poul. Sci. 54, 584-591.

Kaushik, S.J., Fauconneau, B., Terrier, L., Gras, J., 1988. Arginine requirement and status assessed by different biochemical indices in rainbow trout (*Salmo gairdneri*). Aquaculture 70, 75-95.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 133, 257-274.

Keembiyehetty, C.N., Gatlin, D.M., 1993. Total sulfur amino acid requirement of juvenile hybrid striped bass (*Morone chrysops x M. saxatilis*). Aquaculture 110, 331-339.

Keembiyehetty, C.N., Gatlin, D.M., 1995. Evaluation of different sulfur compounds in the diet of juvenile sunshine bass (*Morone chrysops* (female) *x M. saxatilis* (male)) Comp. Physiol., 155-159.

Keembiyehetty, C.N., Gatlin, D.M., 1997. Preformance of sunshine bass fed soybean-meal-based diets supplemented with different methionine compounds. The Progressive Fish Culturist 59, 25-30.

Kelly, M., Grisdale-Helland, B., Helland, S.J., Gatlin, D.M., 2006. Refined understanding of sulphur amino acid nutrition in hybrid striped bass, *Monroe chrysops* x *M. saxatilis*. Aquaculture Res. 37, 1546-1555.

Kim, B.G., 2006. Efficacy of DL-methionine hydroxy analog free acid and DL-methionine as methionine sources for pigs. Journal of Animal Science 84, 104-111.

Kim, S.-K., Takeuchi, T., Yokoyama, M., Murata, Y., 2003. Effect of dietary supplementation with taurine, beta-alanine, and GABA on the growth of juvenile and finglering Japanese flounder *Paralichthys olivaceus*. Fisheries Sci. 69, 242-248.

Kim, S.-K., Takeuchi, T., Akimoto, A., Furuita, H., Yamamoto, T., Yokoyama, M., Murata, Y., 2005. Effect of taurine supplemented practical diet on growth performance and taurine contents in whole body and tissues of juvenile Japanese flounder *Paralichthys olivaceus*. Fisheries Sci. 71, 627-632.

Klein, R.G., Halver, J.E., 1970. Nutrition of salmonoid fishes: arginine and histidine requirements of chinook and coho salmon. Journal of Nutrition 100, 1105-1110.

Leeson, S., Summers, J., 2001. Scott's Nutrition of the Chicken. Univ. Books, Guelph, Ontario, Canada.

Li, P., Gatlin, D.M., 2003. Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* x *M. saxatilis*). Aquaculture 219, 681-692.

Lo, G.S., Settle, S.L., Steinke, F.H., Hopkins, D.T., 1981. Effect of phytate : zinc molar ratio and isolated soybean protein on zinc bioavailability. J. Nutr. 111, 2223-2235.

Lobley, G.E., 1992. Control of the metabolic fate of amino acids in ruminants: A review. J. Anim. Sci. 70, 3264-3275.

Loest, C.A., Titgemeyer, E.C., St-Jean, G., Van Metre, D.C., Smith, J.S., 2002. Methionine as a methyl group donor in growing cattle. J. Anim. Sci. 80, 2197-2206.

Lowe, J.A., Wiseman, J., Cole, D.J.A., 1994. Absorption and retention of zinc when administered as an amino-acid chelate in the dog. J. Nutr. 124, 2572S-2574.

Lunger, A.N., McLean, E., Gaylord, T.G., Kuhn, D., Craig, S.R., 2007. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (Rachycentron canadum). Aquaculture 271, 401-410.

Marletta, M.A., Yoon, P.S., Iyengar, R., Leaf, C.D., Wishnok, J.S., 1988. Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. Biochem. 27, 8706-8711.

Masuda, M., 1984. Role of taurine in neutrophil function. Nippon Yakurigaku Zasshi - Folia Pharmacologica Japonica 84, 283-292.

McCollum, M.Q., Vazquez-Anon, M., Dibner, J.J., Webb, K.E., 2000. Absorption of 2-hydroxy-4-(methylthio)butanic acid by isolated sheep ruminal and omasal epithelia. J. Anim. Sci. 78, 1078-1083.

McGoogan, B.B., Gatlin, D.M., 1997. Effects of replacing fish meal with soybean meal in diets for red drum *Sciaenops ocellatus* and potential for palatability enhancement. J. World Aquaculture Soc. 28.

Mohsen, A.A., Lovell, R.T., 1990. Partial substitution of soybean meal with animal protein sources in diets for channel catfish. Aquaculture 90, 303-311.

Morris, J.E., Kohler, C.C., Mischke, C.C., 1999. Pond culture of hybrid striped bass in the north central region. North Central Regional Aquaculture Center Publication 107, 5.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017-1024.

Nematipour, G.R., Brown, M.L., Gatlin, D.M., 1992. Effects of dietary energy: protein ratio on growth characteristics and body composition of hybrid striped bass, *Morone chrysops* [female] X *M. saxatilis* [male]. Aquaculture 107, 359-368.

Novus International, 2008. MINTREX® Next Generation Organic Trace Mineral Nutrition for Poultry. novusint.com.

NRC, The National Research Council, 1993. Nutrient Requirements of Fish. National Academy Press, Washington, D.C.

O'Dell, B.L., Yohe, J.M., Savage, J.E., 1964. Zinc availability in the chick as affected by phytate, calcium and ethylenediaminetetraacetate. Poul. Sci. 43, 415-419.

O'Neill, B., 2005. U.S. Farmed Hybrid Striped Bass *Morone chrysops x Morone saxatilis*. Monterey Bay Aquarium, Monterey, CA.

Oberleas, D., Muhrer, M.E., O'Dell, B.L., 1962. Effects of phytic acid on zinc availability and parakeratosis in swine. J. Anim. Sci. 21, 57-61.

Paripatananont, T., Lovell, R.T., 1997. Comparative net absorption of chelated and inorganic trace minerals in channel catfish *Ictalurus punctatus* diets. J. of the World Aquaculture Soc. 28.

Park, G.-S., Takeuchi, T., Yokoyama, M., Seikai, T., 2002. Optimal dietary taurine level for growth of juvenile Japanese flounder *Paralichthys olivaceus*. Fisheries Sci. 68, 824-829.

Park, S., Birkhold, S., Kubena, L., Nisbet, D., Ricke, S., 2004. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biol. Trace Element Res. 101, 147-163.

Reifsnyder, D.H., Young, C.T., Jones, E.E., 1984. The use of low protein liquid diets to determine the methionine requirement and the efficacy of methionine hydroxy analogue for the three-week-old pig. J. Nutr. 114, 1705-1715.

Reigh, R.C., Ellis, S.C., 1992. Effects of dietary soybean and fish-protein ratios on growth and body composition of red drum (*Sciaenops ocellatus*) fed isonitrogenous diets. Aquaculture 104, 279-292.

Reyes, A.A., Karl, I.E., Klahr, S., 1994. Role of arginine in health and renal disease. Am. J. of Physiol. 267, F331-F346.

Richards, J.D., Dibner, J.J., Knight, C.D., 2007. *Reply*: DL-2-hydroxy-4-(Methylthio)Butanoic Acid from any commerical source is fully available as a source of methionine activity. Poul. Sci. 86, 1613-1614.

Richards, J.D., Atwell, C.A., Vazquez-Anon, M., Dibner, J.J., 2005. Comparative in vitro and in vivo absorption of 2-hydroxy-4-(methylthio)butanoic acid and methionine in the broiler chicken. Poultry Science 84, 1397-1405.

Robinson, E.H., Wilson, R.P., Poe, W.E., 1981. Arginine requirement and apparent absence of a lysine-arginine antagonist in fingerling channel catfish. J. Nutr. 111, 46-52.

Robinson, E.H., Allen, O.W., Jr., Poe, W.E., Wilson, R.P., 1978. Utilization of dietary sulfur compounds by fingerling channel catfish: l-Methionine, dl-Methionine, methionine hydroxy analogue, taurine and inorganic sulfate. J. Nutr. 108, 1932-1936.

Sanford, P.E., 1976. Zinc-methionine supplement lowers protein requirement for broiler chicks. Poul. Sci. 55, 2087.

Santos, O.S., 2007. Performance of pigs fed diets supplemented with DL-Methionine or liquid MHA-FA from 6-25 kg. Journal of Dairy Science 90, 443-443.

SAS Institute, Inc., 1996. SAS User's Guide: Statistics, Version 5, Cary, North Carolina.

Savage, J.E., Yohe, J.M., Pickett, E.E., O'Dell, B.L., 1964. Zinc metabolism in the growing chick. Tissue concentration and effect of phytate on absorption. Poul. Sci. 43, 420-426.

Scarpa, J., Gatlin, D.M., 1992. Dietary zinc requirements of channel catfish, *Ictalurus punctatus*, swim-up fry in soft and hard water. Aquaculture 106, 311-322.

Tacon, A.G.J., Haaster, J.V., Featherstone, P.B., Kerr, K., Jackson, A.J., 1983. Studies on the utilization of full-fat soybean and solvent extracted soybean meal in a complete diet for rainbow trout. Bulletin of the Japanese Society for Scientific Fisheries 49, 1437-1443.

Takeuchi, T., 2001. A review of feed development for early life stages of marine finfish in Japan. Aquaculture 200, 203-222.

Tulli, F., Vachot, C., Tibaldi, E., Fournier, V., Kaushik, S.J., 2007. Contribution of dietary arginine to nitrogen utilisation and excretion in juvenile sea bass (*Dicentrarchus labrax*) fed diets differing in protein source. Comp. Biochem. Physiol. Part A 147, 179-188.

Tuncer, H., Harrell, R.M., Houde, E.D., 1990. Comparative energetics of striped bass (*Morone saxatilis*) and hybrid (*M. saxatilis* x *M.chrysops*) juveniles. Aquaculture 86, 387-400.

Underwood, E., Suttle, N., 2001. The Mineral Nutrition of Livestock. CABI Publishing, London, UK.

USDA, 2008. National Feedstuffs Market Review, USDA Market News, Minneapolis, MN.

Weber, M.L., 2003. What price farmed fish: a review of the environmental and social costs of farming carnivorous fish. SeaWeb Aquaculture Clearinghouse, Providence, RI, 52 pp.

Wedekind, K.J., Hortin, A.E., Baker, D.H., 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70, 178-187.

Wilson, K.R., Abney, C.S., Vasconcelos, J.T., Vazquez-Anon, M., McMeniman, J.P., Galyean, M.L., 2008. Effects of 2-hydroxy-4-(methylthio)-butanoic acid on performance and carcass caracteristics of finishing beef cattle and on fermentation in continuous culture. J. Anim. Sci. 86, 1951-1962.

Wright, P.A., Land, M.D., 1998. Urea production and transport in teleost fishes. Comp. Biochem. Physiol. Part A 119, 47-54.

Yi, G.F., Atwell, C.A., Hume, J.A., Dibner, J.J., Knight, C.D., Richards, J.D., 2007. Determining the methionine activity of mintrex organic trace minerals in broiler chicks by using radiolabel tracing or growth assay. Poul. Sci. 86, 877-887.

Yokoyama, M., 2001. Hepatic cysteinesulphinate decarboxylase activity in fish. Aquaculture Res. 32, 216-220.

Yokoyama, M., Kaneniwa, M., Sakaguchi, M., 1997. Metabolites of L-[35S]cysteine injected into the peritoneal cavity of rainbow trout. Fisheries Sci. 63, 799-801.

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