REFINED UNDERSTANDING OF SULFUR AMINO ACID NUTRITION IN HYBRID STRIPED BASS, *Morone chrysops* ♀ × *M. saxatilis* ♂

A Thesis

by

MARK C. KELLY

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

May 2005

Major Subject: Wildlife and Fisheries Sciences

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Approved as to style and content by:

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ABSTRACT

Refined Understanding of Sulfur Amino Acid Nutrition in Hybrid Striped Bass, *Morone chrysops* ♀ × *M. saxatilis* ♂. (May 2005)

Mark C. Kelly, B.S., University of North Carolina Wilmington

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

Previous studies have indicated the level of total sulfur amino acids (TSAA) (methionine + cystine) is most limiting in practical diet formulations for hybrid striped bass (HSB), especially if animal feedstuffs are replaced with plant feedstuffs. Reduction of costly animal feedstuffs such as fish meal while maintaining adequate dietary levels of TSAA may enhance cost effectiveness of production. Therefore, this study, consisting of four separate feeding trials, investigated three different aspects of sulfur amino acid nutrition of HSB including: (1) the efficacy of crystalline methionine hydroxy analog (MHA) and liquid MHA (Alimet™) relative to L-methionine in meeting the requirement for TSAA; (2) the cystine sparing value for methionine; and, (3) the influence of various sulfur amino acid supplements on ammonia excretion.

During the feeding trials, juvenile HSB were fed various diets including a basal diet deficient in TSAA (0.33 or 0.51% of diet), and experimental diets supplemented on an equal-sulfur basis with different levels of either L-methionine, Alimet™ or crystalline MHA. Diets containing TSAA at 1% of diet and different ratios of cystine to methionine (60:40, 55:45, 50:50, and 45:55) also were fed to re-evaluate sparing effects of cystine on methionine. During the ammonia excretion trial, HSB were fed diets containing either L-methionine,

Alimet^{TM} or crystalline MHA after which total ammonia nitrogen (TAN) excretion was determined 4 h postprandial.

In trial 1, Alimet^{TM} was 73% as effective in promoting growth as L-methionine at the same concentration while MHA was 83% as effective. In trial 3, fish fed Alimet^{TM} at 1.25% of diet displayed similar growth performance as those fed TSAA at 1.0% of diet while weight gain of fish fed Alimet^{TM} at 1% was only 58% of that displayed by fish fed TSAA at 1.0%. No significant differences in weight gain, feed utilization or survival were observed among fish fed diets containing various ratios of cystine to methionine although the diet with 60:40 cystine to methionine supported the lowest responses. Inclusion of MHA or Alimet^{TM} did not affect TAN excretion of HSB. These findings will aid in refining diet formulations for HSB to ensure adequate sulfur amino acid nutrition.

DEDICATION

This thesis is dedicated to my family, who has always loved and supported me, and to my friends, who have kept me smiling all the way.

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I would like to thank the Texas A&M Office of Graduate Studies for generously supporting my studies through a Graduate Merit Fellowship. Financial support for my study also was provided by the Aquaculture Protein Centre of Norway.

Special thanks go to the members of my committee, Dr. Ståle Helland, Dr. Barb Grisdale-Helland, Dr. William Neill, and Dr. Robert Stickney. I wish to express my sincere appreciation to Dr. Delbert Gatlin for his continuous support, expertise, and encouragement in this endeavor.

Finally, this study would not have been possible without the skill, foresight, and boundless patience of Jon Goff, Peng Li, Kasey Whiteman, and the ARTF staff who kept my fish alive and my experiments running in spite of my best efforts.

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INTRODUCTION

Hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂) culture represents the fourth most valuable ($28 million annually) and the fifth largest (5170 metric tons annually) aquacultural enterprise in the United States (Carlberg et al., 2000; Carlberg et al., 2004). Improvements to hybrid striped bass production, such as refinements in diet formulations, should have a large impact on this important industry. The protein component of the diet represents one of the most expensive constituents; therefore, using less expensive protein feedstuffs and/or amino acids that constitute these proteins is important in reducing production costs.

Methionine is an indispensable amino acid which along with the dispensable amino acid cystine constitute the total sulfur amino acids (TSAA) (NRC, 1993). In fish diets, these amino acids are usually the most limiting metabolically (DeSilva and Anderson, 1995; Wilson, 2002). The requirement for TSAA by several cultured species to support maximum growth has been reported to vary widely. For example, requirements for TSAA by three salmonids are between 0.7 and 0.92% of diet [Artic charr *Salvelinus alpinus*, 0.7% of diet (Simmons et al., 1999); rainbow trout *Oncorhynchus mykiss*, 0.8 % of diet (Kim et al., 1992); Atlantic salmon *Salmo salar*, 0.92% of diet (Sveier et al., 2001)], but a requirement for TSAA of 1.4 to 1.5% of diet was reported for the pleuronectid Japanese flounder *Paralichthys olivaceus* (Alam et al., 2001). Schwarz et al. (1998) found the methionine requirement of common carp (*Cyprinus carpio* L.) to be 0.86% of dry diet; whereas, Harding et al. (1977) quantified the requirement for TSAA by channel catfish *Ictalurus punctatus* at 0.56% of diet.

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

Keembiyehetty and Gatlin (1993) found that normal growth of hybrid striped bass required a minimum content of TSAA at 1% of dry diet or 2.9% of dietary protein. Fish fed methionine-deficient diets (0.6 %) showed significantly lower growth rates and lower feed efficiency as well as high levels of mortality and vulnerability to fungal disease compared to bass fed diets containing higher levels of methionine. In contrast, Griffin et al. (1994) estimated the requirement of hybrid striped bass for TSAA to be 0.7% of diet which is considerably lower than the estimate of Keembiyehetty and Gatlin (1993).

One possible method of reducing the cost of meeting the requirement for TSAA of a species involves the inclusion of methionine analogs in the diet. The efficacy of methionine 2-hydroxy-4-(methylthiol) butyric acid, or methionine hydroxy analog (MHA) in satisfying the requirement for TSAA has been studied in terrestrial animals and some fish species (Lewis, 2003). Garlich (1985) found that broiler chicks fed either liquid MHA or L-methionine showed no significant differences in growth. Knight et al. (1998) found no significant difference in growth between pigs fed diets supplemented with equal molar amounts of either DL-methionine or Alimet™, a commercially available form of liquid MHA consisting of approximately 88% MHA and 12% water.

Effects of crystalline MHA supplementation on hybrid striped bass were explored by Keembiyehetty and Gatlin (1995) who found weight gain of fish fed a diet supplemented with MHA was only 75% that of fish fed a diet containing L-methionine. Feed efficiency, expressed as g gain/g dry feed, for fish fed MHA was 0.66 compared to 0.77 for fish fed L-methionine. Thus, MHA was not as efficiently used as L-methionine by hybrid striped bass. In channel catfish, MHA was only 26% as effective as L-methionine based on weight gain (Robinson et al., 1978). However, most recently, juvenile red drum *Sciaenops ocellatus* were found to use crystalline MHA and Alimet^{TM} as proficiently as L-methionine based on growth performance (Goff and Gatlin, 2004).

Another important aspect of refining a species' requirement for TSAA is determining the cystine sparing value for methionine. Methionine can be converted to cysteine via homocysteine and serine. A cystine (Cys2) residue is formed when a disulfide bridge is formed from the sulfhydryl groups of two cysteine residues. Because cystine can be synthesized by the body, it is considered a dispensable or "nonessential" amino acid. However, the presence of cystine in a diet can limit the amount of methionine that must be converted to cystine, thereby reducing the overall amount of methionine required to meet the requirement for TSAA (Lewis, 2003). The ability of cystine to spare a portion of the dietary methionine requirement has been demonstrated in various terrestrial animals and several fish species (NRC, 1993). Chung and Baker (1992) determined that at least 50% of the requirement for TSAA by juvenile pigs' could be met by cystine. Twibell et al. (2000) found that L-cystine was capable of sparing up to 51% of the requirement for TSAA by yellow perch *Perca flavescens*. Moon and Gatlin (1991) placed cystine's ability to spare methionine in red drum *Sciaenops ocellatus* diets at approximately 40%. Griffin et al. (1994) established that cystine could spare up to 40% of the TSAA of juvenile hybrid striped bass, with similar estimates obtained by Keembiyehetty and Gatlin (1993).

The production of nitrogenous wastes by aquacultured organisms can have important influences on the water quality of a culture system as well as the surrounding environment. Because of concerns about eutrophication caused by aquaculture, research has recently focused on ways of reducing the amount of nitrogen and phosphorus produced by cultured organism (Wu, 1995). The production of ammonia (NH_3) in fish is primarily due to protein catabolism via transdeamination of dietary amino acids (Médale et al., 1995). One way of reducing NH_3 excretion is by providing diets containing high levels of digestible non-protein energy, thus limiting amino acid catabolism while reducing the amount of protein needed for optimum growth (Wu, 1995). Webb and Gatlin (2003) demonstrated that this was also the case for red drum. Because of the absence of nitrogen in the chemical structure of MHA, it may be possible to reduce NH_3 excretion while satisfying the requirement for TSAA by the organism. Römer and Abel (1999) demonstrated reduction of nitrogen excretion in both broiler chickens and pigs fed diets supplemented with MHA compared to those fed DL-methionine. Thus, MHA supplementation also may work to reduce nitrogen excretion of hybrid striped bass.

Objectives

Based on the preceding information, the purpose of this study was to evaluate both MHA and Alimet™, a liquid form of MHA, as possible substitutes for L-methionine in the diet of hybrid striped bass. In addition, this study re-evaluated the ability of cystine to spare methionine in meeting the requirement for TSAA by hybrid striped bass. This study also assessed the possibility of reducing ammonia excretion of hybrid striped bass via dietary sulfur amino acid manipulation.

METHODS

Feeding trial 1

A basal diet (Table 1) was formulated from casein, gelatin and crystalline amino acids to be deficient in sulfur amino acids with 0.3% methionine and 0.03% cystine. All experimental diets were formulated to contain 35% crude protein and 3.3 kcal estimated digestible energy/g. The methionine compounds were obtained from U.S. Biochemical Corporation (Cleveland, Ohio) except for Alimet™ which was provided by Novus International, Inc. Test diets were supplemented with L- methionine, DL-MHA, or Alimet™ on an equal-sulfur basis to provide a total of 1.0% of diet to satisfy the minimum requirement of hybrid striped bass (Keembiyehetty and Gatlin, 1993). All diet ingredients except Alimet™ were combined prior to the introduction of menhaden oil and water. During preparation, all diets were adjusted to pH 7 by the addition of 6 *N* NaOH. The mixed diets were then extruded into 3-mm pellets and air dried.

The feeding trial was conducted at the Texas A&M University Aquacultural Research and Teaching Facility in twelve, 38-l aquaria maintained indoors in a climate-controlled laboratory. Water temperature was kept constant at 27±2 °C with a salinity of 7ppt. The aquaria were part of a recirculating system in which approximately 10% of water was replaced by filtered well water daily. Photoperiod was set at 12h light/12h dark. Groups of 15 fish initially weighing an average of 5.1 g were graded by size and stocked into each aquarium. Each experimental diet was randomly assigned to three aquaria. Fish were fed their respective diets twice daily over a 10-week period. Feeding rate was initially 6% of body weight per day and was reduced uniformly across all dietary treatments to 3% of body weight as the feeding trial progressed. This approach allowed the fish to be consistently fed at a rate approaching apparent satiation without overfeeding. Collective weighing of fish and feeding rate adjustment took place each week.

Table 1
Composition of basal diet for feeding trial 1.

Ingredient	Amount (expressed as % of dry diet)
Amino acid premix [1]	24.9
Casein	9.0
Gelatin	1.8
Dextrin	25.0
Mineral premix[2]	4.0
Vitamin premix[2]	3.0
Carboxymethyl cellulose	2.0
Cellulose	24.3
Menhaden oil	6.0

[1] See Table 2 for composition.
[2] Same as Moon and Gatlin (1991).

Table 2
Composition of amino acid premix for feeding trial 1.

Amino acid [1]	Amount (% of dry diet)
Arginine HCl	1.3
Histidine	0.8
Isoleucine	1.4
Leucine	2.4
Lysine HCl	1.0
Methionine	--
Cystine	--
Phenylalanine	1.6
Tyrosine	1.6
Threonine	1.4
Tryptophan	0.4
Valine	1.7
Aspartate	3.2
Proline	--
Glutamate	3.2
Alanine	1.3
Serine	0.5
Glycine	3.2

[1] All amino acids were provided as L-isomers.

Upon conclusion of the feeding trial, five fish from each aquarium were randomly selected and subjected to biochemical analysis. Analysis of whole-body protein, lipid, dry matter, and ash were conducted according to methods described by Nematipour et al. (1992).

Feeding trials 2 and 3

Diets used in feeding trials 2 and 3 were similar to those of Keembiyehetty and Gatlin (1993) and formulated to contain 35% crude protein, 10% lipid, and 3.4 kcal estimated digestible energy/g (Table 3). Alimet ™ was once again compared with L-methionine. A basal diet without supplemental methionine or cystine was used as the negative control. It contained total sulfur amino acids (TSAA) at 0.51% of diet. Test diets were supplemented with L-methionine or Alimet™ on an equal-sulfur basis to provide TSAA at 1% of diet to satisfy the minimum requirement of hybrid striped bass (Keembiyehetty and Gatlin, 1993). Alimet ™ also was included to provide TSAA at 1.25% of diet to account for anticipated reduced efficiency of MHA utilization by hybrid striped bass as previously reported (Keembiyehetty and Gatlin, 1995) and observed in feeding trial 1. One other diet was supplemented with L-methionine to contain TSAA at 0.7% of diet to compare the previously reported requirement value of Griffin et al. (1994).

Another series of experimental diets was prepared to re-evaluate the cystine replacement value for methionine with diets containing TSAA at 1% and cystine providing either 40%, 45% 50% or 55% of the total with the remainder provided by L-methionine. The cystine: methionine ratios were selected to refine the estimated replacement value for hybrid striped bass as previously reported by Keembiyehetty and Gatlin (1993) and Griffin et al. (1994). The casein/gelatin/amino acid diet from feeding trial 1 that was supplemented with L-methionine to provide TSAA at 1% also was included in feeding trial 2 to compare with the red drum muscle-based experimental diets. Procedures for diet preparation and feeding trial execution were similar to those employed in feeding trial 1.

Table 3
Composition of experimental diets for feeding trials 2 and 3 (expressed as % of dry diet).

	Diet designation								
Ingredient	Basal	TSAA 1%	TSAA 0.7%	Alimet 1.25%	Alimet 1%	Cys2:Met[1] 45:55	Cys2:Met 50:50	Cys2:Met 55:45	Cys2:Met 60:40
Drum muscle [2]	12.38	12.40	12.40	12.40	12.40	12.40	12.40	12.40	12.40
Amino acid premix [3]	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Dextrin	27.60	27.54	27.58	26.86	27.11	27.61	27.62	27.64	27.64
Mineral premix[4]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[4]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Carboxymethyl Cellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cellulose	17.82	17.80	17.80	17.80	17.80	17.80	17.80	17.80	17.80
Menhaden oil	8.73	8.73	8.73	8.73	8.73	8.73	8.73	8.73	8.73
Aspartate	1.47	1.04	1.30	1.47	1.47	0.97	0.96	0.94	0.94
L-Methionine	0.00	0.49	0.19	0.00	0.00	0.17	0.12	0.07	0.02
L-Cystine	0.00	0.00	0.00	0.00	0.00	0.32	0.37	0.42	0.47
Alimet ™	0.00	0.00	0.00	0.74	0.49	0.00	0.00	0.00	0.00

[1] Cystine: methionine ratio.
[2] Contains 84.8% crude protein and 10.3% lipid.
[3] See Table 4 for composition.
[4] Same as Moon and Gatlin (1991).

In feeding trial 2, groups of 12 fish initially averaging 9.7 g were graded by size and stocked into each aquarium. The culture system water temperature was maintained at 27±2°C. Salinity was kept near 5 ppt. Triplicate groups of fish were fed each diet twice daily at the same rate which ranged from 6% of body weight at the start of the trial to 3% at the end of the 4-week period to maintain a level approaching satiation without overfeeding. Fish were weighed collectively each week and feeding rates were adjusted accordingly. This feeding trial was terminated after 4 weeks.

Table 4
Amino acid composition (g/100 g diet) of dietary ingredients for feeding trials 2 and 3.

Amino acid[1]	Provided by crystalline Amino acid premix	Provided by 12.1% drum muscle	Total
Arginine HCl	1.97	0.61	2.58
Glycine	1.07	0.58	1.65
Histidine	0.75	0.27	1.02
Isoleucine	1.70	0.46	2.16
Leucine	2.27	0.77	3.04
Lysine HCl	2.82	0.93	3.75
Methionine	-	0.38	0.38
Cystine	-	0.13	0.13
Phenylalanine	1.56	0.40	1.96
Tyrosine	1.12	0.32	1.44
Serine	2.26	0.38	2.64
Threonine	1.24	0.46	1.70
Tryptophan	0.42	0.13	0.55
Valine	1.99	0.50	2.49
Proline	2.01	0.38	2.39
Alanine	2.01	0.71	2.72

[1] All amino acids were provided as L-isomers.

In feeding trial 3 all dietary treatments were similar to feeding trial 2 except for exclusion of the casein/gelatin/crystalline amino acid diet. Fish in this experiment initially weighed 6.4 g with groups of 12 fish stocked in each aquarium. Each diet was fed to triplicate groups of fish using to the protocol previously described for trials 1 and 2 for a total of 6 weeks.

Weight gain as a percentage of initial weight, feed efficiency ratio (FER, gram of weight gain/gram of feed fed) and protein efficiency ratio (PER, gram of weight gain/gram of protein fed) were calculated for the replicate tanks within each dietary group.

Upon conclusion of feeding trial 3, three fish from each aquarium were randomly selected and subjected to biochemical analysis. Analysis of whole-body protein, lipid, dry matter, and ash was conducted according to methods described by Nematipour et al. (1992). Protein conversion efficiency, another measure of protein utilization, was determined by dividing whole-body protein gained from the start of the feeding trial by the amount of protein fed.

Feeding trial 4

A separate trial was conducted to determine ammonia excretion of hybrid striped bass fed different sulfur amino acid compounds. Diets evaluated in the ammonia excretion trial were the same as those formulated for a similar trial involving red drum (Goff, 2003). These diets were supplemented with L-methionine, MHA, and Alimet™ on an equal-sulfur basis but varying in total nitrogen (Table 5). Diet preparation was the same as previously described except for the pellet diameter which was 5 mm.

The culture system used in the ammonia excretion trial consisted of nine, 110-l aquaria containing 80 l of brackish (7 ppt) water operated in a recirculating mode similar to that described earlier. Water temperature was held at 27 ± 2°C by conditioning the ambient air temperature. Other environmental conditions were as previously described.

Groups of 12 fish initially averaging of 54 g were graded by size and stocked into each aquarium. Each experimental diet was randomly assigned to three aquaria. Fish were conditioned by feeding the respective diets to apparent satiation twice daily over a 2-week period. Then fish were not fed for 24 h prior to sampling to allow for complete evacuation of any diet from the gastrointestinal tracts. After this 24-h period, all aquaria were cleaned and siphoned to remove particulates. Fish then were fed their respective diets to apparent satiation. Subsequently, 30 minutes postprandial, the water flow to each aquarium was discontinued, all uneaten feed was removed, and an initial water sample was taken for total ammonia nitrogen (TAN) determination. A HACH DR/200 spectrophotometer using the

Nessler method (SMEWW, 1999) was employed to measure TAN. After the fish remained in the static aquaria for an additional 4 h, another water sample was taken and TAN production was measured as previously described (McGoogan and Gatlin, 2000; Webb and Gatlin, 2003). Once the 4-h samples were taken, another sample was removed from each aquarium and allowed to incubate for an additional 2 h in an effort to estimate the level of nitrification occurring in the static system. Upon completion of all TAN measurements, all fish in each aquarium were weighed in order to express ammonia production per unit of body weight.

Statistical analysis

One-way analysis of variance and Duncan's multiple-range test was used to analyze data from all four feeding trials. Differences were considered significant at $P<0.05$. All data also were subjected to Levene's test to confirm homogeneous variances among treatment means (SAS Institute 1985).

Table 5
Experimental diets (expressed as % of dry diet) for feeding trial 4.

Ingredient	L-Methionine	MHA	Alimet™
Red drum muscle[1]	11.84	11.84	11.84
Amino acid Premix[2]	24.00	24.00	24.00
Dextrin	26.65	26.65	26.65
Mineral premix[3]	4.00	4.00	4.00
Vitamin premix[3]	3.00	3.00	3.00
Carboxymethyl cellulose	2.00	2.00	2.00
Cellulose	17.13	17.13	17.13
Menhaden oil	10.21	10.21	10.21
Aspartate	0.58	0.58	0.58
L-Methionine	0.52	0.00	0.00
MHA	0.00	0.59	0.00
Alimet™	0.00	0.00	0.59

[1]Contains 84.1% crude protein and 10.7% lipid.

[2] Goff (2003).

[3] Moon and Gatlin (1991).

RESULTS

Feeding trial 1

At the end of 10 weeks of feeding the experimental diets, greatest weight gain was observed in fish fed the diet supplemented with L- methionine while significantly suppressed weight gain was observed in fish fed the basal diet (Table 6). No significant differences in weight gain were seen in fish fed the MHA-supplemented diet compared to those fed L-methionine although MHA was only 82% as effective as L- methionine based on weight gain. Fish fed the Alimet™- supplemented diet displayed similar weight gain as fish fed the MHA-supplemented diet but significantly lower weight gain compared to those fed L-methionine. Alimet™ was only 73% as effective in supporting weight gain as L-methionine.

FER of fish fed L- methionine were statistically similar to that of the MHA-supplemented group (Table 6). FER of fish fed the MHA-supplemented diet also was similar to that of fish fed Alimet ™ while fish fed the basal diet had significantly lower FE than any other dietary group.

The highest PER values were obtained by fish fed the L- methionine and MHA-supplemented diets (Table 6). Fish fed the Alimet™-supplemented diet had a PER value that was not significantly different from that of the MHA-fed fish while fish fed the basal diet had the lowest PER value that was statistically distinct from all other treatments (Table 6). Survival across all groups varied slightly though insignificantly at the end of 10 weeks, with lowest survivability among fish fed the basal diet (Table 6).

Table 6
Weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of juvenile hybrid striped bass fed diets with various sulfur amino acid compounds for 10 weeks during feeding trial 1[1].

Dietary supplement	Weight gain (% initial wt[2])	FER (g gain/g dry feed)	PER (g gain/g protein fed)	Survival (%)
Basal	123^{c}	0.24^{c}	0.77^{c}	80
L-methionine	266^{a}	0.40^{a}	1.24^{a}	82
MHA	220^{a,b}	0.36^{a,b}	1.12^{a,b}	95
Alimet ™	195^{b}	0.34^{b}	1.06^{b}	82
Pr>F[3]	0.0047	0.0004	0.0009	NS[4]
Pooled s.e.	33.11	0.03	0.09	9.98

[1]Means of three replicate groups. Values with the same superscript in a column are not statistically different (*P* >0.05) as determined by Duncan's multiple range test.

[2] Average initial weight was 5.1 g fish.

[3] Significance probability associated with the *F* statistic.

[4]NS = not significant.

Whole-body ash and moisture levels were independent of dietary treatment (Table 7). Carcass protein levels also did not vary significantly among the dietary treatments although fish fed the basal diet displayed slightly lower levels of protein than the other groups. The same observation was made for carcass lipid composition, with no significant differences among any of the four dietary groups.

Table 7
Proximate composition of whole-body tissue from hybrid striped bass fed diets with various sulfur amino acid compounds for 10 weeks during feeding trial 1[1].

Dietary supplement	Protein	Lipid	Ash	Moisture
		(g/100 g fresh weight)		
Basal	14.6	7.3	3.7	70.7
L-methionine	17.2	8.6	3.4	68.3
MHA	17.1	8.6	3.5	68.2
Alimet ™	17.0	8.5	4.2	68.4
Pr>F[3]	NS[3]	NS	NS	NS
Pooled s.e.	1.21	.602	.56	1.35

[1] Values are means of composite samples of three whole fish from each of three replicate groups.
[2] Fish initially contained 13.6% protein, 6.8% lipid, 4.5% ash, and 74.0% moisture.
[3]NS = not significant.

Feeding trials 2 and 3 at week 4

Comparison of Different Sulfur Amino Acid Compounds and Levels. Because feeding trial 2 was terminated after 4 weeks, data from both feeding trials 2 and 3 at week 4 are presented for comparison. Fish fed the diets containing Alimet™ or L-methionine exhibited significantly higher weight, FER, and PER values compared to fish fed the basal diet (Table 8). Although not significantly different, fish fed the Alimet™ diets tended to perform better than those fed TSAA at 1% or 0.7% of diet during feeding trial 2. Fish fed the casein/gelatin diet from feeding trial 1 had generally reduced weight gain compared to that of fish fed the other diets based on red drum muscle.

Table 8

Weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of juvenile hybrid striped bass fed diets with various sulfur amino acid compounds for 4 weeks during feeding trials 2 and 3 [1].

Diet	Weight gain (% initial wt)		FER (g gain/g dry feed fed)		PER (g gain/g protein fed)		Survival (%)	
	Trial 2[2]	Trial 3[3]	Trial 2	Trial 3	Trial 2	Trial 3	Trial 2	Trial 3
Basal	45[c]	64[c]	0.21[b]	0.37[c]	0.58[b]	1.13[c]	94	89
TSAA 0.7%	99[a,b]	97[a,b]	0.42[a]	0.50[a,b]	1.23[a]	1.50[a,b]	97	78
TSAA 1%	106[a,b]	117[a]	0.44[a]	0.57[a]	1.34[a]	1.71[a]	97	89
Alimet™ 1%	109[a,b]	88[b,c]	0.44[a]	0.46[b]	1.39[a]	1.33[b,c]	100	75
Alimet™ 1.25%	125[a]	96[a,b]	0.48[a]	0.47[b]	1.65[a]	1.46[a,b]	100	64
Casein/gelatin L-methionine 1%	86[b]	--	0.39[a]	--	1.44 [a]	--	100	--
Pr>F[4]	0.004	0.014	0.0094	0.0049	0.0043	0.0042	NS[5]	NS
Pooled s.e.	18.77	14.50	0.0744	0.045	0.25	0.14	4.91	19.01

[1]Means of three replicate groups. Values with the same superscript in a column are not statistically different (*P* >0.05) as determined by Duncan's multiple range test.

[2] Average initial weight was 9.7 g/fish.

[3] Average initial weight was 6.4 g/fish.

[4] Significance probability associated with the *F* statistic.

[5] NS = not significant.

Differential responses of fish fed the diets containing different levels of Alimet^{TM} and TSAA were not readily apparent at week 4 when the experiment was terminated.

Results of feeding trial 2 were in slight contrast to data from week 4 of feeding trial 3 in which fish fed TSAA at 1% of diet had higher weight gain, FER, and PER values compared to fish fed Alimet^{TM} at 1% of diet but not those fed Alimet^{TM} at 1.25% of diet (Table 8). In feeding trials 2 and 3, the diet containing TSAA at 0.7% was 7 and 17 % less effective in promoting weight gain than the diet with TSAA at 1%. Growth performance of fish fed Alimet^{TM} at 1% of diet was similar to that of fish fed TSAA at 1% of diet during feeding trial 2 but was 25% less effective in feeding trial 3. Alimet^{TM} at 1.25% of diet generally provided similar responses as fish fed TSAA at 1% of diet in both feeding trials 2 and 3.

<u>Cystine: methionine ratio manipulations</u>. Results from feeding trials 2 and 3 at week 4 showed no significant differences in responses of fish fed the various dietary treatments consisting of different cystine to methionine ratios (Table 9). With the exception of fish fed the diet with a cystine to methionine ratio of 45:55, growth rates where lower at week 4 of feeding trial 3 compared to week 4 of feeding trial 2. In both trials, fish fed the diet with a cystine to methionine ratio of 60:40 exhibited the lowest weight gain, FER, and PER values but differences were not statistically distinguishable by the end of 4 weeks (Table 9).

Table 9

Weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of hybrid striped bass fed diets with various cystine: methionine ratios during feeding trials 2 and 3 after 4 weeks[1].

Cys2: Met Ratio[2]	Weight gain (% initial wt[3])		FER (g gain/g dry feed fed)		PER (g gain/g protein fed)		Survival (%)	
	Trial 2[3]	Trial 3[4]	Trial 2[3]	Trial 3[4]	Trial 2[3]	Trial 3[4]	Trial 2[3]	Trial 3[4]
60:40	102	87	0.40	0.47	1.20	1.44	100	67
55:45	110	109	0.46	0.53	1.34	1.55	100	89
50:50	120	98	0.46	0.49	1.62	1.50	100	72
45:55	114	117	0.46	0.58	1.34	1.76	100	83
Pr>F[5]	NS[6]	NS	NS	NS	NS	NS	NS	NS
Pooled s.e.	13.78	18.07	0.04	0.05	0.27	0.16	0	16.68

[1]Means of three replicate groups. Values with the same superscript in a column are not statistically different (*P* >0.05) as determined by Duncan's multiple range test.

[2] Cystine:methionine ratio.

[3] Average initial weight was 9.7 g.

[4] Average initial weight was 6.4 g.

[5]Significance probability associated with the *F* statistic.

[6]NS = not significant.

Feeding trial 3

Comparison of Different Sulfur Amino Acid Compounds and Levels At the end of 6 weeks, significant differences were seen in weight gain of fish fed the various diets, with those fed TSAA at 1% of diet displaying the largest overall gain (142% of initial weight) which was similar only to that of fish fed TSAA at 0.7% and Alimet™ at 1.25% of diet (Table 10). Fish fed Alimet™ at 1% of diet and those fed TSAA at 0.7% of diet had no significant differences in weight gain values compared to fish fed the basal diet which exhibited the lowest weight gain.

Highest FER was observed in fish fed TSAA at 1% of diet, which was not significantly different from fish fed Alimet™ at 1.25%. FER of fish fed TSAA at 0.7% was slightly higher than that of fish fed Alimet™ at 1% of diet but was lower than that of fish fed TSAA at 1% (Table 10). The range of PER values followed the same general tendencies of both weight gain and FER with fish fed TSAA at 1% exhibiting the most efficient protein usage (Table 10). Similar trends in survival as seen in weight gain, FER and PER also were observed at the end of 6 weeks, although survival rates did not vary significantly across dietary groups (Table 10). Fish fed higher concentrations of TSAA or Alimet ™ fared better than those fed the other diets, especially the basal diet that was deficient in TSAA. Survival of fish in all treatments was affected by a *Mycobacterium* infection that became evident during week 4 of the trial and continued to persist through week 6.

Neither whole-body composition nor protein conversion efficiency of fish fed the various diets differed significantly at the end of the 6-week period (Table 11). The values were within the ranges typically reported for hybrid striped bass.

Table 10

Weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of hybrid striped bass fed diets with various sulfur amino acid compounds for 6 weeks during feeding trial 3[1].

Diet	Weight gain (% initial wt[3])	FER (g gain/g dry feed fed)	PER (g gain/g protein fed)	Survival (%)
Basal	53[c]	0.27[c]	0.82[c]	30.6
TSAA 0.7%	95[a,b,c]	0.37[b,c]	1.12[b,c]	64.9
TSAA 1%	142[a]	0.48[a]	1.44[a]	70.1
Alimet™ 1%	83[b,c]	0.34[b,c]	0.97[b,c]	55.6
Alimet™ 1.25%	110[a,b]	0.40[a,b]	1.23[a,b]	69.9
Pr>F[3]	0.0177	0.0092	0.0071	NS[4]
Pooled s.e.	25.38	0.054	0.16	24.37

[1]Means of three replicate groups. Values with the same superscript in a column are not statistically different (P >0.05) as determined by Duncan's multiple range test.

[2] Average initial weight was 6.4 g/fish.

[3] Significance probability associated with the F statistic.

[4]NS = not significant.

Table 11

Whole-body composition and protein conversion efficiency (PCE) of hybrid striped bass fed diets with various sulfur amino acid compounds for 6 weeks during feeding trial 3[1].

Diet	Protein	Lipid (g/100 g fresh weight)	Ash	Moisture	PCE (g protein gain x 100/g protein fed)
Basal	15.1	9.1	3.5	73.0	12.8
TSAA 0.7 %	14.5	9.3	3.5	73.6	15.2
TSAA 1 %	14.7	10.3	3.4	72.2	20.5
Alimet™ 1 %	14.7	8.2	3.2	73.4	13.4
Alimet™ 1.25 %	14.0	12.4	3.6	72.4	15.8
Pr>F[2]	NS[3]	NS	NS	NS	NS
Pooled s.e.	1.69	2.0	0.61	2.86	6.61

[1] Means of three individual fish from each of the three replicate groups. Values with the same superscript in a column are not statistically different (*P* >0.05) as determined by Duncan's multiple range test.

[2]Significance probability associated with the *F* statistic.

[3]NS = not significant.

Cystine: methionine ratio manipulations. While fish fed the diet with a cystine: methionine ratio of 55:45 exhibited the highest weight gain, FER, and PER values, no significant differences in these responses were observed among fish fed the four diets with various cystine: methionine ratios (Table 12). No significant difference was observed in survival of fish fed the different diets, although fish fed the 45:55 cystine: methionine diet exhibited the highest survival (77.8%), and fish fed the 60:40 diet had the lowest (30.6%)

(Table 12). Fish fed the 60:40 diet also had the lowest weight gain, FER, and PER values compared to fish fed the other diets, although the differences were not significant.

No significant differences were observed in whole-body protein, ash, or moisture of fish fed the various dietary treatments (Table 13). Only whole-body lipid differed significantly, as fish fed the diet with a cystine: methionine ratio of 55:45 possessed the highest concentration of lipid (11.2%). A reason for this difference in whole-body lipid was not readily apparent although fish fed this diet also had the greatest weight gain. Fish fed the diet with a cystine: methionine ratio of 45:55 demonstrated the highest protein conversion efficiency (19.1%), although values for fish fed all four diets were statistically indistinguishable from one another (Table 13).

Feeding trial 4

Ammonia production by fish fed either L-methionine, MHA, or Alimet™ did not differ significantly (Table 14). However, fish fed Alimet™ and L-methionine excreted similar levels of total ammonia nitrogen over the 4-h period which were slightly lower than observed for fish fed MHA. Thus, the hydroxyl analog of methionine in the form of Alimet™ or MHA did not significantly reduce ammonia excretion of hybrid striped bass relative to L-methionine.

Table 12

Weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of hybrid striped bass fed diets with various cystine: methionine ratios for 6 weeks during feeding trial 3[1].

Cys2: Met Ratio[2]	Weight gain (% initial wt[2])	FER (g gain/g dry feed fed)	PER (g gain/g protein fed)	Survival (%)
60:40	78	0.35	0.92	30.6
55:45	131	0.46	1.35	61.1
50:50	103	0.39	1.18	58.3
45:55	119	0.41	1.25	77.8
Pr>F[3]	NS[4]	NS	NS	NS
Pooled s.e.	30.52	0.08	0.23	23.94

[1]Means of three replicate groups. Values with the same superscript in a column are not statistically different (*P* >0.05) as determined by Duncan's multiple range test.

[2] Cystine:methionine ratio.

[3] Average initial weight was 6.4 g.

[4]Significance probability associated with the *F* statistic.

[5]NS = not significant.

Table 13

Whole-body composition and protein conversion efficiency (PCE) of hybrid striped bass fed diets with various cystine: methionine ratios for 6 weeks during feeding trial 3[1].

Cys2: Met Ratio[2]	Protein	Lipid	Ash	Moisture	PCE (g protein gain x 100/g protein fed)
	(g/100 g fresh weight)				
60:40	14.1	8.2[b]	3.6	73.7	13.8
55:45	14.1	11.2[a]	3.2	71.6	17.8
50:50	15.0	8.8[b]	3.4	71.2	17.3
45:55	15.2	8.8[b]	3.6	70.7	19.1
Pr>F[2]	NS[3]	0.0078	NS	NS	NS
Pooled s.e.	1.33	0.8092	.45	3.00	4.85

[1] Means of three individual fish from each of the three replicate groups. Values with the same superscript in a column are not statistically different (*P* >0.05) as determined by Duncan's multiple range test.

[2] Cystine: methionine ratio.

[3] Significance probability associated with the *F* statistic.

[4] NS = not significant.

Table 14

The effects of dietary supplementation of various sulfur amino acid compounds on total ammonia nitrogen (TAN) excretion of hybrid striped bass during feeding trial 4[1].

Dietary supplement	Ammonia production (mg TAN/(kg·h))
L-methionine	22.0
MHA[2]	28.4
Alimet™	22.2
Pr>F[3]	NS[4]
Pooled s.e.	5.86

[1]Means of three replicate groups.

[2]DL- methionine hydroxy analog.

[3]Significance probability associated with the *F* statistic.

[4]NS = not significant.

DISCUSSION

Efficacy of sulfur amino acid compounds

Research involving terrestrial animals such as broiler chicks, turkeys, and pigs has shown variable results with regard to the efficacy of MHA in both liquid and dry forms relative to L-methionine. Garlich (1985) found no significant differences in weight gain of broiler chickens fed diets supplemented with either L-methionine or crystalline MHA at various levels. In contrast, Van Weerden et al. (1982) compared weight gain of chicks fed diets supplemented with DL-methionine, crystalline MHA, or liquid MHA-free acid (Alimet^{TM}) and reported that MHA promoted growth 81% as effectively as DL-methionine; whereas, Alimet^{TM} supported growth 66% as effectively. Harms et al. (1977) reported that MHA supported growth of turkeys approximately 82% as effectively as DL-methionine. Knight et al. (1998) indicated that MHA was 95% as effective as DL-methionine in promoting growth of young swine.

Similar results have not consistently been reported for various fish species, although red drum fed L-methionine, crystalline MHA, or Alimet^{TM} showed no significant differences in weight gain, FE, or PER values (Goff and Gatlin, 2004). In contrast, channel catfish were able to use MHA only 26% as effectively as L-methionine based on weight gain (Robinson et al., 1978). Poston (1986) observed responses in rainbow trout relatively similar to those of HSB in the present study. MHA and Alimet were 86% and 63% as effective, respectively, as L-methionine at promoting growth on an equal sulfur basis. Cheng et al. (2003) reported that rainbow trout performance was improved when diets containing soybean meal and distiller's dried grains in place of 50% fish meal were supplemented with MHA. Responses of hybrid striped bass fed L-methionine and MHA during feeding trial 1 of the present study were similar to those reported in previous studies with hybrid striped bass (Keembiyehetty and

Gatlin, 1995; Keembiyehetty and Gatlin, 1997). On average, crystalline MHA was only 83% as effective in growth promotion as L-methionine in feeding trial 1 of the present study; whereas, Alimet™ was 73% as effective as L-methionine based on weight gain responses.

Overall FER as well as PER values in feeding trial 1 were much lower than those reported by Keembiyehetty and Gatlin (1995) and Keembiyehetty and Gatlin (1997). Similar experimental procedures and environmental conditions were employed in all these trials except for differences in the composition of the experimental diets. The casein/gelatin/amino acid-based diets used in feeding trial 1 generally have not been used as efficiently as red drum muscle or fishmeal based diets employed in previous studies. However, in another recent trial in this laboratory, comparable performance of hybrid striped bass was achieved with both fishmeal and casein/gelatin/amino acid-based diets (Jaramillo and Gatlin, 2004). Therefore, the casein/gelatin/amino acid-based diets were chosen in feeding trial 1 due the ease with which amino acid composition could be altered. Comparison of fish performance at week 4 in feeding trial 2 confirmed that fish fed the casein/gelatin diet also exhibited inferior performance compared to fish fed the other diets based on red drum muscle. It is also possible that the lower weight gain, FER and PER values observed in feeding trial 1 were due to the genetic constitution of fish used in that experiment or other unidentified factors.

Overall weight gain, FER, PER and survival values observed for hybrid striped bass at the end of week 6 in feeding trial 3 were generally lower than those reported in other studies involving juvenile hybrid striped bass fed similar diets (Keembiyehetty and Gatlin 1993, 1995, 1997). Routine water quality analyses insured optimal conditions were maintained and thus the *Mycobacterium* infection was the most likely cause of reduced performance. Nonetheless, significant differences in weight gain were observed among fish fed diets with various amounts of L-methionine and Alimet™. Comparison of fish performance at week 4 of feeding trials 2 and 3 also reflected generally similar responses although significant differences were not completely resolved at that time. At the end of week 6 in feeding trial 3, the diet containing Alimet™ of 1.25% was 77% as effective in supporting growth of hybrid striped bass as TSAA at 1% of diet, while fish fed Alimet™ at 1% of diet grew only 58% of those fed TSAA at 1% of diet. These results were generally consistent with those observed in feeding trial 1. Alimet™ supplementation at 1.25% of diet resulted in statistically similar weight gain, FER, and PER values as that of fish fed TSAA at 1% of diet; therefore, substitution of Alimet™ at 1.25% of the total sulfur amino acid requirement may result in comparable performance as seen in fish fed diets containing TSAA at the minimum requirement.

The differences in growth efficiency between L-methionine and MHA may possibly be explained by their different methods of absorption. As seen in broiler chickens, L-methionine is actively transported across the cell membrane against a concentration gradient; whereas, MHA is passively diffused from areas of higher to lower concentration (Meirelles et al., 2003). Potential differences in absorption may have affected the availability of these various methionine compounds to the fish although specific characteristics of L-methionine and MHA absorption and metabolism have not been studied in any fish species.

Total sulfur amino acid requirement of hybrid striped bass

Different growth rates between fish fed TSAA at 1% and 0.7% of diet suggest that Keembiyehetty and Gatlin's (1993) minimum TSAA requirement estimate of 1% of diet for normal growth is more accurate than the estimated requirement of 0.7% of diet reported by Griffin et al. (1994). This requirement for TSAA by hybrid striped bass is therefore marginally higher than requirements reported for Artic charr (0.7%; Simmons et al., 1999) and Atlantic salmon (0.92%; Sveier et al., 2001), and much higher than that of channel catfish (0.56%) according to Harding et al. (1977). While higher than many aquacultured species, the requirement for TSAA by hybrid striped bass at 1% of diet for normal growth is lower than that of the pleuronectid Japanese flounder *Paralichthys olivaceus* (1.4 to 1.5%; Alam et al. [2001]).

Cystine: methionine ratio manipulations

The similar growth responses of hybrid striped bass fed diets with various cystine to methionine ratios in both feeding trials 2 and 3 indicated that cystine could spare a considerable amount of methionine is satisfying their requirement for TSAA. Weight gain of hybrid striped bass fed diets with various ratios of cystine to methionine did not differ significantly although fish fed the diet with a cystine to methionine ratio of 60:40 consistently displayed the lowest weight gain, FER, and PER values in both trials. Survival of fish fed the cystine to methionine 60:40 diet was almost 50% lower than all other groups. Therefore, based on these various responses, it is estimated that cystine could conservatively satisfy 55% of the requirement for TSAA by hybrid striped bass. Weight gain and feed efficiency ratio values in trials 2 and 3 of the present study were somewhat lower than those reported by Griffin et al. (1994) in which methionine sparing was evaluated with cysteine. In that study, hybrid striped bass fed a diet with a cysteine to methionine ratio of 60:40 gained 122% of initial weight compared to 150% for fish fed a diet with cysteine to methionine at 40:60 (Griffin et al., 1994). These results are generally consistent with those observed in the present study.

The cystine sparing value of 55% suggested in the present study with hybrid striped bass is relatively high but similar to values reported for some other fish species. Harding et al. (1977) found a 60% sparing value for channel catfish, while Hidalgo et al. (1987) reported a value of 64% for European sea bass *Dicentrarchus labrax*. A 50% replacement value was reported for chinook salmon *Oncorhynchus tshawytscha* (NRC, 1993) and red drum (Goff and Gatlin, 2004). Lower cystine sparing values have been reported for several other fish including 44% for blue tilapia *Oreochromis aureus* (Liou, 1989), 42% for rainbow trout (Kim et al., 1992), and 40% for red drum (Moon and Gatlin, 1991) and hybrid striped bass (Griffin et al., 1994).

Prior research involving terrestrial animals indicates that the dietary inclusion of cystine can successfully lower the amount of methionine required in the diet. By observing hepatic homocysteine metabolism, the intermediate through which methionine is converted to cystine, Finkelstein et al. (1988) concluded that up to 70% of the methionine requirement of Sprague-Dawley rats could be spared with cystine. Chung and Baker (1992) determined that cystine could supply up to 50% of the TSAA requirement in pigs. As in the present study, a cystine to methionine ratio of 60:40 resulted in the lowest growth of pigs with a trend of reduced weight gain with increasing levels of cystine. Shoveller et al. (2003) found that cystine can spare 40% of the requirement for TSAA by neonatal pigs. Baker et al. (1996) also reported that cystine could furnish nearly 52% of the requirement for TSAA by 3 to 6-week-old chicks.

Body composition

Whole-body composition and protein conversion efficiency of hybrid striped bass in the various feeding trials of the present study were similar to values reported in other studies although Keembiyehetty and Gatlin (1995, 1997) reported slightly higher levels of protein and ash in hybrid striped bass fed comparable diets. No consistent differences were observed in the present study between protein, lipid, ash, or moisture content as a function of diet. Only fish fed the cystine: methionine 55:45 diet had significantly higher levels of body lipid compared to those fed the other cystine: methionine diets. This was likely attributable to the fact that fish fed the 55:45 diet tended to be larger than those fed the other diets. However, due to the lack of significant differences in weight gain of fish fed these various diets, body composition was not analyzed with fish size as a covariate.

Ammonia excretion

While TAN excretion rates of hybrid striped bass were lower in the present study than those presented for red drum by Webb and Gatlin (2003) and Goff (2003), the supplementation of Alimet^{TM} and MHA did not significantly affect the level of nitrogen excreted by hybrid striped bass. Goff (2003) found that red drum fed L-methionine and MHA had similar levels of postprandial ammonia excretion (38.7 and 38.5 mg TAN/kg/ h) compared to that of red drum fed Alimet^{TM} (31.9 mg TAN/kg/h). In the current study, hybrid striped bass fed L-methionine and Alimet^{TM} had more comparable levels of ammonia excretion (22.0 and 22.15 mg TAN/kg/h) than those fed MHA (28.38 mg TAN/kg/h) although these differences were not significant. In terrestrial animals such as pigs, reductions in nitrogen excretion with the supplementation of MHA and Alimet^{TM} have been more pronounced. Römer and Abel (1999) found that pigs fed diets supplemented with MHA had 16% lower urine nitrogen levels compared to pigs fed diets containing DL-methionine. While not significant, pigs fed MHA-supplemented diets had a 4% higher level of nitrogen retention. However, within the same study, Römer and Abel (1999) found little difference between nitrogen excretion and retention in broiler chickens fed diets supplemented with either MHA or DL-methionine.

SUMMARY AND CONCLUSIONS

Results from the various feeding trials in this study indicate that hybrid striped bass did not utilize Alimet™ and crystalline MHA as effectively as L-methionine. However, supplementation of Alimet™ to provide a TSAA level of 1.25% resulted in similar weight gain, FER and PER values as fish fed L-methionine to provide a TSAA level of 1% of diet. Reduced performance of fish fed a diet supplemented with L-methionine to provide TSAA at 0.7% of diet suggested this level was insufficient to meet the requirement for TSAA by hybrid striped bass.

Comparison of growth, FER, and PER values of fish fed diets with different ratios of cystine to methionine demonstrate that the non-essential amino acid cystine could conservatively spare up to 55% of the requirement for TSAA by hybrid striped bass. This value is higher than the 40% sparing value previously reported for hybrid striped bass (Keembiyehetty and Gatlin 1993; Griffin et al., 1994).

The dietary substitution of Alimet™ and MHA for L-methionine did not significantly impact levels of nitrogen excreted by hybrid striped bass and thus does not appear to be an effective strategy to reduce nitrogen excretion while satisfying the requirement for TSAA by this fish.

However, the results of this study support Alimet™ as a viable alternative to L-methionine for supplementation in hybrid striped bass diets. The level of dietary methionine required for optimal growth also may be reduced by the sparing effects of cystine without reducing fish growth or quality. Therefore, information provided by the present research will allow cost-effective refinements of diet formulations to meet the requirement for TSAA by hybrid striped bass.

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APPENDIX A

Feeding Trial 1

Obs	diet	wt	fe	pe	surv
1	Basal	127.15	0.24	0.79	80.00
2	Basal	96.27	0.20	0.64	93.33
3	Basal	144.73	0.27	0.88	66.67
4	Lmet	226.66	0.37	1.15	73.00
5	Lmet	287.01	0.42	1.31	81.25
6	Lmet	284.61	0.41	1.27	93.00
7	MHA	201.93	0.34	1.08	93.00
8	MHA	228.55	0.37	1.15	100.00
9	MHA	228.41	0.37	1.14	93.00
10	Alimet	150.38	0.33	1.01	73.00
11	Alimet	246.83	0.37	1.16	93.00
12	Alimet	185.41	0.33	1.02	80.00

The GLM Procedure

Level of		------------wt------------		------------fe------------		------------pe------------	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Alimet	3	194.206667	48.8230134	0.34333333	0.02309401	1.06333333	0.08386497
Basal	3	122.716667	24.5322998	0.23666667	0.03511885	0.77000000	0.12124356
Lmet	3	266.093333	34.1713452	0.40000000	0.02645751	1.24333333	0.08326664
MHA	3	219.630000	15.3288095	0.36000000	0.01732051	1.12333333	0.03785939

Level of		-------------surv------------	
diet	N	Mean	Std Dev
Alimet	3	82.0000000	10.1488916
Basal	3	80.0000000	13.3300000
Lmet	3	82.4166667	10.0509121
MHA	3	95.3333333	4.0414519

Obs	diet	pro	lip	ash	mois
1	Basal	14.80	7.40	4.0	72.0
2	Basal	13.20	6.60	2.7	71.2
3	Basal	15.90	7.90	4.5	68.9
4	Lmet	18.68	9.30	3.2	68.8
5	Lmet	16.60	8.30	4.1	66.4
6	Lmet	16.40	8.20	2.9	69.9
7	MHA	15.90	7.93	3.6	67.1
8	MHA	17.00	8.51	3.4	68.1
9	MHA	18.60	9.27	3.3	68.6
10	Alimet	16.40	8.17	4.2	69.7
11	Alimet	17.04	8.50	4.1	67.6
12	Alimet	17.74	8.90	4.3	68.0

Level of		pro		lip		ash	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Alimet	3	17.0600000	0.67022384	8.52333333	0.36555893	4.20000000	0.10000000
Basal	3	14.6333333	1.35769412	7.30000000	0.65574385	3.73333333	0.92915732
Lmet	3	17.2266667	1.26258993	8.60000000	0.60827625	3.40000000	0.62449980
MHA	3	17.1666667	1.35769412	8.57000000	0.67201190	3.43333333	0.15275252

Level of		mois	
diet	N	Mean	Std Dev
Alimet	3	68.4333333	1.11504858
Basal	3	70.7000000	1.60934769
Lmet	3	68.3666667	1.78978583
MHA	3	67.9333333	0.76376262

Feeding Trial 2, Week 4

Obs	diet	wt	fe	pe	surv
1	Basal	76.040	0.35	0.97	100
2	Basal	26.700	0.12	0.33	83
3	Basal	32.900	0.15	0.44	100
4	Lmet1	113.540	0.48	1.47	100
5	Lmet1	90.786	0.37	1.18	92
6	Lmet1	113.040	0.47	1.36	100
7	Lmet7	117.640	0.47	1.37	100
8	Lmet7	65.950	0.30	0.93	91
9	Lmet7	113.620	0.47	1.38	100
10	Alimet1	89.920	0.38	1.13	100
11	Alimet1	119.500	0.47	1.41	100
12	Alimet1	116.200	0.46	1.64	100
13	Alimet12	122.060	0.48	1.81	100
14	Alimet12	122.270	0.47	1.42	100
15	Alimet12	130.270	0.51	1.72	100

The GLM Procedure

Level of		wt		fe		pe	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Alimet1	3	108.540000	16.2095898	0.43666667	0.04932883	1.39333333	0.25540817
Alimet12	3	124.866667	4.6806018	0.48666667	0.02081666	1.65000000	0.20420578
Basal	3	45.213333	26.8760587	0.20666667	0.12503333	0.58000000	0.34219877
Lmet1	3	105.788667	12.9950954	0.44000000	0.06082763	1.33666667	0.14640128
Lmet7	3	99.070000	28.7531024	0.41333333	0.09814955	1.22666667	0.25696952

Level of		surv	
diet	N	Mean	Std Dev
Alimet1	3	100.000000	0.00000000
Alimet12	3	100.000000	0.00000000
Basal	3	94.333333	9.81495458
Lmet1	3	97.333333	4.61880215
Lmet7	3	97.000000	5.19615242

Obs	diet	wt	fe	pe	surv
1	cm6040	96.20	0.3733	1.17	100
2	cm6040	110.43	0.4400	1.25	100
3	cm6040	100.34	0.4000	1.18	100
4	cm5545	106.50	0.4600	1.28	100
5	cm5545	93.00	0.3900	1.03	100
6	cm5545	130.70	0.5200	1.71	100
7	cm5050	101.93	0.4000	1.19	100
8	cm5050	138.43	0.5100	2.00	100
9	cm5050	120.99	0.4700	1.66	100
10	cm4555	116.65	0.4700	1.66	100
11	cm4555	112.71	0.4600	1.60	100
12	cm4555	111.29	0.4500	1.59	100

The GLM Procedure

Level of		wt		fe		pe	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
cm4555	3	113.550000	2.7769768	0.46000000	0.01000000	1.61666667	0.03785939
cm5050	3	120.450000	18.2559908	0.46000000	0.05567764	1.61666667	0.40673497
cm5545	3	110.066667	19.1013961	0.45666667	0.06506407	1.34000000	0.34394767
cm6040	3	102.323333	7.3193875	0.40443333	0.03357027	1.20000000	0.04358899

Level of		surv	
diet	N	Mean	Std Dev
cm4555	3	100.000000	0
cm5050	3	100.000000	0
cm5545	3	100.000000	0
cm6040	3	100.000000	0

Feeding Trial 3 Week 4

Obs	diet	wt	fe	pe	surv
1	Basal	59.82	0.33	1.02	67
2	Basal	60.71	0.40	1.21	42
3	Basal	70.24	0.38	1.16	83
4	Lmet1	95.92	0.52	1.57	67
5	Lmet1	141.47	0.64	1.93	100
6	Lmet1	115.71	0.54	1.64	100
7	Lmet7	84.48	0.47	1.41	50
8	Lmet7	106.93	0.54	1.62	83
9	Lmet7	100.87	0.49	1.48	100
10	Alimet1	71.44	0.42	1.20	58
11	Alimet1	103.48	0.51	1.48	83
12	Alimet1	88.69	0.45	1.30	83
13	Alimet12	84.50	0.43	1.32	75
14	Alimet12	101.18	0.49	1.51	100
15	Alimet12	103.02	0.50	1.54	92

The GLM Procedure

Level of		------------wt------------		------------fe------------		------------pe------------	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Alimet1	3	87.870000	16.0357320	0.46000000	0.04582576	1.32666667	0.14189198
Alimet12	3	96.233333	10.2029277	0.47333333	0.03785939	1.45666667	0.11930353
Basal	3	63.590000	5.7762358	0.37000000	0.03605551	1.13000000	0.09848858
Lmet1	3	117.700000	22.8401116	0.56666667	0.06429101	1.71333333	0.19087518
Lmet7	3	97.426667	11.6143460	0.50000000	0.03605551	1.50333333	0.10692677

Level of		------------surv------------	
diet	N	Mean	Std Dev
Alimet1	3	74.6666667	14.4337567
Alimet12	3	89.0000000	12.7671453
Basal	3	64.0000000	20.6639783
Lmet1	3	89.0000000	19.0525589
Lmet7	3	77.6666667	25.4230866

Obs	diet	wt	fe	pe	surv
1	cm6040	65.16	0.39	1.21	50
2	cm6040	87.45	0.49	1.52	58
3	cm6040	108.74	0.51	1.57	92
4	cm5545	105.25	0.52	1.52	83
5	cm5545	117.89	0.54	1.58	100
6	cm5545	104.38	0.53	1.55	83
7	cm5050	70.91	0.41	1.25	50
8	cm5050	122.49	0.56	1.70	92
9	cm5050	101.86	0.51	1.54	75
10	cm4555	107.31	0.55	1.68	83
11	cm4555	118.46	0.60	1.83	75
12	cm4555	127.25	0.58	1.78	92

The GLM Procedure

Level of		------------wt------------		------------fe------------		------------pe------------	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
cm4555	3	117.673333	9.9932494	0.57666667	0.02516611	1.76333333	0.07637626
cm5050	3	98.420000	25.9614965	0.49333333	0.07637626	1.49666667	0.22810816
cm5545	3	109.173333	7.5613777	0.53000000	0.01000000	1.55000000	0.03000000
cm6040	3	87.116667	21.7919121	0.46333333	0.06429101	1.43333333	0.19502137

Level of		-------------surv------------	
diet	N	Mean	Std Dev
cm4555	3	83.3333333	8.5049005
cm5050	3	72.3333333	21.1266025
cm5545	3	88.6666667	9.8149546
cm6040	3	66.6666667	22.3009716

Feeding Trial 3

Obs	diet	wt	fe	pe	surv
1	Basal	36.74	0.19	0.57	16.7
2	Basal	55.90	0.33	1.00	58.3
3	Basal	67.22	0.29	0.89	16.7
4	Lmet1	102.19	0.45	1.35	33.3
5	Lmet1	189.76	0.54	1.64	100.0
6	Lmet1	134.03	0.44	1.34	76.9
7	Lmet7	75.94	0.35	1.06	36.4
8	Lmet7	101.54	0.37	1.13	83.3
9	Lmet7	106.96	0.38	1.14	75.0
10	Alimet1	78.82	0.37	1.06	50.0
11	Alimet1	104.39	0.38	1.08	75.0
12	Alimet1	64.46	0.26	0.76	41.7
13	Alimet12	92.75	0.38	1.17	50.0
14	Alimet12	129.08	0.44	1.37	75.0
15	Alimet12	106.96	0.37	1.16	84.6

The GLM Procedure

Level of		wt		fe		pe	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Alimet1	3	82.556667	20.2255589	0.33666667	0.06658328	0.96666667	0.17925773
Alimet12	3	109.596667	18.3079555	0.39666667	0.03785939	1.23333333	0.11846237
Basal	3	53.286667	15.4071325	0.27000000	0.07211103	0.82000000	0.22338308
Lmet1	3	141.993333	44.3247925	0.47666667	0.05507571	1.44333333	0.17039171
Lmet7	3	94.813333	16.5679248	0.36666667	0.01527525	1.11000000	0.04358899

Level of		surv	
diet	N	Mean	Std Dev
Alimet1	3	55.5666667	17.3338782
Alimet12	3	69.8666667	17.8620641
Basal	3	30.5666667	24.0177712
Lmet1	3	70.0666667	33.8709807
Lmet7	3	64.9000000	25.0281841

Obs	diet	pro	lip	ash	mois
1	Basal	12.8	8.5	3.1	74.2
2	Basal	17.7	9.2	4.5	68.6
3	Basal	14.8	9.6	3.1	76.4
4	Lmet1	14.4	12.6	3.6	72.9
5	Lmet1	14.5	7.7	4.0	70.9
6	Lmet1	15.1	10.6	2.5	72.8
7	Lmet7	14.5	12.0	3.9	72.0
8	Lmet7	14.2	7.5	3.2	75.4
9	Lmet7	14.8	8.4	3.3	73.6
10	Alimet1	14.3	5.5	3.9	68.8
11	Alimet1	13.9	10.8	2.9	77.3
12	Alimet1	15.7	8.2	3.0	74.2
13	Alimet12	15.3	11.1	3.6	71.5
14	Alimet12	11.0	12.8	3.2	74.0
15	Alimet12	15.8	13.3	4.1	71.7

The GLM Procedure

Level of diet	N	pro Mean	pro Std Dev	lip Mean	lip Std Dev	ash Mean	ash Std Dev
Alimet1	3	14.6333333	0.94516313	8.1666667	2.65015723	3.26666667	0.55075705
Alimet12	3	14.0333333	2.63881286	12.4000000	1.15325626	3.63333333	0.45092498
Basal	3	15.1000000	2.46373700	9.1000000	0.55677644	3.56666667	0.80829038
Lmet1	3	14.6666667	0.37859389	10.3000000	2.46373700	3.36666667	0.77674535
Lmet7	3	14.5000000	0.30000000	9.3000000	2.38117618	3.46666667	0.37859389

Level of diet	N	mois Mean	mois Std Dev
Alimet1	3	73.4333333	4.30155011
Alimet12	3	72.4000000	1.38924440
Basal	3	73.0666667	4.02160830
Lmet1	3	72.2000000	1.12694277
Lmet7	3	73.6666667	1.70098011

Obs	diet	wt	fe	pe	surv
1	cm6040	42.360	0.256	0.79	75.0
2	cm6040	72.130	0.390	1.20	0.0
3	cm6040	120.860	0.410	0.79	16.7
4	cm5545	128.300	0.470	1.37	66.7
5	cm5545	164.340	0.540	1.57	50.0
6	cm5545	99.875	0.380	1.11	66.7
7	cm5050	109.337	0.490	1.49	33.3
8	cm5050	107.631	0.360	1.08	66.7
9	cm5050	92.175	0.320	0.96	75.0
10	cm4555	113.224	0.410	1.26	75.0
11	cm4555	90.897	0.350	1.06	66.7
12	cm4555	153.950	0.470	1.44	91.7

The GLM Procedure

Level of		------------wt------------		------------fe------------		------------pe------------	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
cm4555	3	119.357000	31.9707752	0.41000000	0.06000000	1.25333333	0.19008770
cm5050	3	103.047667	9.4545634	0.39000000	0.08888194	1.17666667	0.27790886
cm5545	3	130.838333	32.3073739	0.46333333	0.08020806	1.35000000	0.23065125
cm6040	3	78.450000	39.6297779	0.35200000	0.08373769	0.92666667	0.23671361

Level of		------------surv------------	
diet	N	Mean	Std Dev
cm4555	3	77.8000000	12.7330279
cm5050	3	58.3333333	22.0731360
cm5545	3	61.1333333	9.6417495
cm6040	3	30.5666667	39.3759233

Obs	diet	pro	lip	ash	mois
1	cm6040	14.2	8.2	3.7	76.5
2	cm6040	15.3	7.6	3.2	74.7
3	cm6040	12.9	8.7	3.9	69.8
4	cm5545	14.7	12.2	2.8	69.8
5	cm5545	11.6	10.6	3.4	74.3
6	cm5545	16.0	10.8	3.4	70.8
7	cm5050	14.7	9.6	2.7	73.3
8	cm5050	14.7	8.4	4.1	69.3
9	cm5050	15.6	8.5	3.2	71.0
10	cm4555	14.6	7.9	3.5	66.7
11	cm4555	15.8	8.5	3.5	74.2
12	cm4555	15.4	9.9	3.8	71.4

The GLM Procedure

Level of		pro		lip		ash	
diet	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
cm4555	3	15.2666667	0.61101009	8.7666667	1.02632029	3.60000000	0.17320508
cm5050	3	15.0000000	0.51961524	8.8333333	0.66583281	3.33333333	0.70945989
cm5545	3	14.1000000	2.26053091	11.2000000	0.87177979	3.20000000	0.34641016
cm6040	3	14.1333333	1.20138809	8.1666667	0.55075705	3.60000000	0.36055513

Level of		mois	
diet	N	Mean	Std Dev
cm4555	3	70.7666667	3.78989886
cm5050	3	71.2000000	2.00748599
cm5545	3	71.6333333	2.36290781
cm6040	3	73.6666667	3.46746786

Feeding Trial 4

Obs	diet	TAN
1	Lmet	28.24
2	Lmet	18.96
3	Lmet	18.82
4	MHA	29.99
5	MHA	21.58
6	MHA	33.57
7	Alimet	18.56
8	Alimet	18.82
9	Alimet	29.10

The GLM Procedure

Level of		-------------TAN-------------	
diet	N	Mean	Std Dev
Alimet	3	22.1600000	6.01162208
Lmet	3	22.0066667	5.39867885
MHA	3	28.3800000	6.15500609

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