Development of Alternative-protein-based Diets for the Intensive Production of Florida pompano *Trachinotus carolinus* L.

by

Waldemar Rossi Junior

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 6, 2011

Keywords: Trachinotus carolinus, Florida pompano,
Fishmeal Replacement

Approved by

Donald Allen Davis, Chair, Professor of Fisheries and Allied Aquacultures
Ronald P. Phelps, Associate Professor of Fisheries and Allied Aquacultures
Chhorn E. Lim, Affiliate Professor of Fisheries and Allied Aquacultures

Abstract

The Florida pompano industry is a promising enterprise in the United States and with commercial production progressively increasing. However, the success of such an enterprise is dependent on the supply of environmentally sustainable and cost-effective diets able to promote optimum growth with reduced dependence on fishmeal as the primary dietary protein source. To facilitate the development of sustainable and cost-effective diets for Florida pompano, a series of three studies evaluating poultry by-product meal, and a meat and bone meal blend as alternative protein sources to fishmeal in diets for this species was conducted. Primary objectives of the first and second studies were to evaluate the isonitrogenous replacement of fishmeal (initially comprising 15% of the diets) by poultry by-product meal or meat and bone meal blend in pompano diets (40% crude protein and 8% lipid), containing 50% soybean meal as the primary protein source. Based on the results from the first two studies, the third study was conducted to evaluate the potential requirement for the free amino acid taurine in the fishmeal-free diets for Florida pompano.

The results of the first two studies demonstrated that poultry by-product and meat and bone meals are suitable for replacing 66% (10 g/100g) of dietary fishmeal without significant effects on fish performance. However, the total replacement of dietary fishmeal led to significantly lower diet digestibility and poorer pompano performance. In

the third study, the supplementation of taurine to the fishmeal-free diets based on poultry by-product meal or meat and bone meal blend from the first and second studies led to a significant improvement of fish performance. Pompano fed diets with at least 0.25% or higher taurine content demonstrated improved growth and feed efficiency relative to fish fed diets without taurine supplementation.

The results of these studies demonstrated that Florida pompano can accept and assimilate fishmeal-free diets based on high levels of soybean meal in combination with poultry by-product meal or meat and bone meal blend and that taurine is likely dietary indispensable for this species.

Acknowledgements

Firstly I thank to God for the gift of life, for his blessings and for his protection in every moment of my life.

I give especial thanks to the loving memory of my mother, Maria Celia, to my father, Waldemar Rossi, for their love, kindness, encouragement and support. My special thanks also go to my whole family, whose support and encouragement was indispensable for this accomplishment.

I thank to my dear brother, Giovani Rossi, my best friend. We are always together! I owe you this accomplishment in my life! Thank for you presence, for the invaluable moments together, for your encouragement and kindness.

I thank to my lovely fiancée, Elaine Fernandes de Souza "Linda", for blessing me with her love, kindness, support and encouragement from the first moment together. You are the gift of my life and will have my love forever! Yes, I found you!

I extend my special thanks to Dr. Allen Davis for accepting me as his student, for his guidance, professionalism and for his encouragement and support during this project. I would also like to thank my committee members, Dr Ronald P. Phelps and Dr Chhorn E. Lim, for their participation and invaluable contribution.

I thank to Dr. Leonard "Len" Lovshin, for his support and example of determination, enthusiasm and leadership. Without your support, I would not be writing this thesis! I would never forget to thank to his lovely wife, Nancy, for her support and kindness. I give a special thanks to my friend Dr. Fernando Kubitza, for his trust and help on the accomplishment of my dream.

My thanks are also extended to Michael Miller, my boss and friend at Southfresh, LLC, for accepting me as a trainee. Thanks for the trust, for the learning opportunities provided, for all the encouragement and help on my path towards Auburn University.

I give special thanks to all those who supported me and helped with the field work during this study, especially Melanie Rhodes, Daranee Sookying, Patricio Paz, Bouchao Hu, Jessica Jacquay, and the brothers Fabio and Andre Soller. I would also like to thank all the bright faculty, staff and fellow graduate students at the Department of Fisheries and Allied Aquacultures for their contribution and help.

Finally, I would like to express my appreciation for the support and funding of this project from the National Sea Grant College Program of the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Grants NA080AR4170840, administered by Mississippi-Alabama Sea Grant Consortium. Additional support was provided by Auburn University and the Alabama Department of Marine Resources at the Claude Peteet Mariculture Center, Gulf Shores, AL.

Table of Contents

Abstract ... ii

Acknowledgements ... iv

List of Tables ... viii

List of Figures ... xi

Chapter

I. Introduction ... 1

Figures ... 10

II. Evaluation of the Replacement of Fishmeal by Poultry By-product Meal in Diets for Florida pompano *Trachinotus carolinus* L. ... 12

Introduction ... 12

Material and Methods ... 14

Results ... 20

Discussion ... 23

References ... 28

Tables ... 32

III. Evaluation of the Replacement of Fishmeal with Meat and Bone Meal Blend in Diets for Florida pompano *Trachinotus carolinus* L. ... 42

Introduction ... 42

Material and Methods ... 45

Results ... 51

Discussion ... 54

References ... 59

Tables ... 64

IV. Evaluation of the Supplementation of Taurine to Fishmeal-free Diets for Florida pompano *Trachinotus carolinus* L. 74

Introduction 74

Material and Methods 77

Results 82

Discussion..86

References 91

Tables 94

Figures..104

V. Summary and Conclusions..107

Literature Cited 109

List of Tables

1. Formulation of experimental diets (g/100g as is basis) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (12 weeks). 32

2. Proximate analysis and amino acid composition of major dietary ingredients (fishmeal, FM; poultry by-product meal, PBM; soybean meal, SBM; and whole wheat, WW) used in the diets (g/100g as is basis). 34

3. Amino acid composition of experimental diets (40% crude protein) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (12 weeks), and of the 40% crude protein pompano muscle (g/100g dry matter basis)....35

4. Water quality parameters during experiment 1 (10 weeks) and experiment 2 (12 weeks). 36

5. Apparent digestibility coefficients for dry matter, crude protein and gross energy of diets with 50% soybean meal, 15% fishmeal (FM15) or 14.7% poultry by-product meal (FM0), fed to juveniles (3.48 ± 019 g mean initial weight) Florida pompano *T. carolinus* L. (Experiment 1). 37

6. Performance and nutrient retention of juveniles (3.48 ± 019 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean meal and graded levels of fishmeal (15 – 0%) and poultry by-product meal (0 – 14.7%) after 10..................38

7. Proximate composition (dry matter basis) of whole body of juveniles (3.48 ± 019 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean

meal and graded levels of fishmeal (15 – 0%) and poultry by-product meal (0 – 14.7%) after 10 weeks of feeding (Experiment 1)...39

8. Performance and nutrient retention of juveniles (4.37 ± 0.29 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 50% soybean meal, 14.7% poultry by-product meal and supplemental amino acids after 12 weeks of feeding (Experiment 2). 40

9. Proximate composition of whole body of juveniles (4.37 ± 0.29 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 50% soybean meal, 14.7% poultry by-product meal and supplemental amino acids after 12 weeks of feeding (Experiment 2). 41

10. Formulation of experimental diets (g/100g as is basis) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (8 weeks). ..64

11. Proximate analysis and amino acid composition of major dietary ingredients (fishmeal, FM; meat and bone meal blend, MBM; soybean meal, SBM; and whole wheat, WW) used in the diets (g/100g as is basis). 66

12. Amino acid composition of experimental diets (40% crude protein) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (8 weeks), and of the 40% crude protein pompano muscle (g/100g dry matter basis)....67

13. Water quality parameters during experiment 1 (10 weeks) and experiment 2 (8 weeks). 68

14. Apparent digestibility coefficients for dry matter, crude protein and gross energy of diets with 50% soybean meal, 15% fishmeal (FM15) or 14.7% meat and bone meal blend (FM0), fed to juveniles (2.99 ± 0.28 g mean initial weight) Florida pompano *T. carolinus* L. (Experiment 1). 69

15. Performance and nutrient retention of juveniles (2.99 ± 0.28 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean meal and graded levels of fishmeal (15 – 0%) and meat and bone meal blend (0 – 14.7%) after 10 weeks of feeding (Experiment 1). 70

16. Proximate composition of whole body of juveniles (2.99 ± 0.28 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean meal and graded levels of fishmeal (15 – 0%) and meat and bone meal blend (0 – 14.7%) after 10 weeks of feeding (Experiment 1). 71

17. Performance and nutrient retention of juveniles (14.39 ± 0.58 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 41.2% soybean meal, 14% meat and bone meal blend and supplemental methionine after 8 weeks of feeding (Experiment 2). 72

18. Proximate composition of whole body of juveniles (14.39 ± 0.58 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 41.2% soybean meal, 14% meat and bone meal blend and supplemental methionine after 8 weeks of feeding (Experiment 2). 73

19. Formulation of experimental diets (g/100 g as is basis) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (8 weeks) and experiment 2 (12 weeks). ..94

20. Proximate analysis and amino acid composition of major dietary ingredients (fishmeal, FM; meat and bone meal blend, MBM; soybean meal, SBM; and whole wheat, WW) used in the diets (g/100g as is basis). 96

21. Amino acid composition of experimental diets (40% crude protein) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (8 weeks) and experiment 2 (12 weeks), and of the 40% crude protein pompano muscle (g/100g dry matter basis)....97

22. Water quality parameters during experiment 1 (8 weeks) and experiment 2 (12 weeks). 98

23. Performance of juveniles (6.25 ± 0.22 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.27% poultry by-product meal and graded levels of supplemental taurine after 8 weeks of feeding (Experiment 1)...99

24. Proximate composition (dry matter basis) of whole body of juveniles (6.25 ± 0.22 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.27% poultry by-product meal and graded levels of supplemental taurine after 8 weeks of feeding (Experiment 1)...............................100

25. Serum osmolality of juveniles Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.27% poultry by-product meal or 14.16% meat and bone meal blend and graded levels of supplemental taurine in experiment 1 (8 weeks, PT diets) and experiment 2 (12 weeks, MT diets). 101

26. Performance of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.16% meat and bone meal blend and graded levels of supplemental taurine after 12 weeks of feeding (Experiment 2). 102

27. Proximate composition (dry matter basis) of whole body of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.16% meat and bone meal blend and graded levels of supplemental taurine after 12 weeks of feeding (Experiment 2). 103

List of Figures

1. Florida pompano *T. Carolinus* L. – Fish and wildlife Research Institute, Florida Fish and Wildlife Conservation Commission; (http://myfwc.com/research/saltwater/fish/florida-pompano)....................................10

2. Annual commercial landings of Florida pompano from 1990 to 2009 [National Oceanic and Atmospheric Administration, Fisheries and Statistics Division (www.st.nmfs.noaa.gov)]..11

3. Growth response of juveniles (6.25 ± 0.22 g mean initial weight) Florida pompano *T. carolinus* L. fed poultry by-product meal based diets with supplemental taurine after 8 weeks of feeding (Experiment 1)..104

4. Dietary taurine and postprandial serum taurine levels of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed meat and bone meal blend based diets during 12 weeks of experiment 2. Vertical bars represent the standard error of the mean serum taurine (SEM, n = 3)..105

5. Growth response of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed meat and bone meal blend based diets with supplemental taurine after 12 weeks of feeding (Experiment 2)...106

CHAPTER I

INTRODUCTION

Florida pompano *Trachinotus carolinus* L., also referred as “pompano”, was targeted as a potential species for mariculture since the early 1960s when research was initially conducted with this species (Berry & Iversen 1967; Moe Jr *et al*. 1968; Finucane 1969a). More recently, the pompano mariculture in United States is progressively expanded as a result of extensive efforts of many research groups, including research institutions and private commercial operations geared towards the development of a feasible exploration of this highly prized marine fish. Albeit much of the past failures on the commercial exploration of this species have been overcome, especially regarding maturation in captivity, seed production and management practices, there is still a need to develop nutritional parameters for production

Biology

Florida pompano is a member of the jack family (Carangidae) and is native to the western Atlantic Ocean. Its distribution range extends from Massachusetts to Brazil, including the Gulf of Mexico and the Caribbean coasts of central and South America (Jordan & Evermann 1898). The body is short, deep (depth contained 2.0 to 2.8 times in fork length in adults) and compressed, with upper and lower profiles similar and head profile sloping to a blunt snout (Figure 1). Eye is small, the upper jaw very narrow at the end and extending to below mid-eye. The color is bluish green on back, shading into silvery on sides; stomach area and parts of head sometimes yellowish; fins mostly yellowish, the elevated part of the dorsal dusky and pelvic fins are white (Gilbert 1986).

The maximum size of Florida pompano in nature is about 7.5 pounds and individuals over four pounds are considered rare. Two other species of *Trachinotus* occurring in the western Atlantic Ocean and Gulf of Mexico regions are the permit *T. falcatus* and palometa *T. goodei*. These species are similar to Florida pompano with the permit reaching larger sizes (20 – 30 pounds) (Gilbert 1986) which is attributed in part to its greater body depth (Fields 1962).

Pompano are considered opportunistic feeders when young (10 – 25 mm in length) , feeding on a variety of organisms including polychaets, amphipods, gastropod larvae and insects, becoming more selective in their diet as they attain larger sizes (Finucane 1969b; Bellinger & Avault Jr 1971). Their stomach is well-defined and sac-shaped (Gilbert 1986), the gut is considered short (Gothreaux 2008) and the feces evacuation was observed to occur in three hours after feeding (Williams *et al.* 1985; Riche 2009).

Pompano appear to prefer water temperatures ranging from 28 to 32 ºC (Finucane 1969b; Gilbert 1986), while the critical low and high temperatures may be 10 and 38 ºC, respectively. Salinity preferences for adult pompano is considered to be within the 28 to 37 g/L range (Gilbert 1986), albeit fish were observed in waters with salinities as low as 9 g/L (Gunter & Hall, 1963) and as high as 50 g/L (Perret *et al.* 1971). More recently studies have demonstrated that pompano can tolerate a wide range of salinity (Watanabe 1995) and can be reared in low salinity conditions (Weirich *et al.* 2009). Pompano become stressed when dissolved oxygen levels are equal or below 3 mg/L and pH levels below 4 or higher than 12 is lethal (Moe Jr *et al.*1968). Pompano are relatively sensitive to environmental unionized ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N);

the LC_{50} for pompano in 24 hours of exposure at the temperature of 28 ºC and pH around 8 ranged from 1.05 to 1.12 mg/L, and from 67.4 to 220.1 mg/L, respectively (Weirich & Riche 2006).

Aquaculture

Florida pompano aquaculture is gaining interest in United States and commercial production of this species from initial stages to marketable-sized fish is already possible. Private operations are beginning to produce pompano in cages and intensive recirculating systems targeting production to provide marketable sized fish year-round.

Pompano's readily adaptation to high densities, acceptance of formulated feeds, relatively fast growth rate, non-cannibalistic behavior, tolerance to a wide range of salinity and no unusual requirements regarding water quality parameters are traits highly desired for aquaculture. Additionally, the increasing interest in commercially production of pompano is also market driven as Florida pompano is rated among the most highly prized food fish in United States commanding high market prices (Weirich 2011).

In the a twenty year period (1990 – 2009) an overall decline in pompano landings has occurred accompanied by a rise in ex-vessel prices (Figure 2), indicating that the demand consistently exceeded supply. In 2009, the average wholesale price of whole pompano was US$ 6.93/kg and the price of pompano fillets can range from US$ 35 to US$ 45/kg, depending on the time of the year and availability (Weirich 2011). The historically high market value and high demand has prompted research with Florida pompano in the United States since the early 1960s (Berry & Iversen 1967; Moe Jr *et al.* 1968; Finucane 1969a) primarily focusing on the development of a cost-effective pompano aquaculture. In general, the supply of juveniles, diseases, temperature tolerance and lack of specific diets were considered as the major constraints leading to unsuccessful results of the past studies.

More recently, the intensification of research concerning maturation and induced spawning (Weirich & Riley 2007), proper systems design and feeding management (Heilman & Spieler 1999; Weirich *et al.* 2006) has surmounted a great majority of the problems encountered in the past. Today, Florida pompano can be artificially spawned and reared in intensive systems successfully.

Despite of these achievements in pompano aquaculture, extensive research on nutrition is necessary to optimize feed formulations. Poor food conversion ratios and slow growth rates are still limiting factors for the development of a sustainable and profitable pompano industry. Jory *et al.* (1985) suggested that the determination of nutritional requirements of pompano as the most important research priority for its production.

Nutrition Research

A significant number of studies have been conducted with Florida pompano and important contributions have been provided. These studies have contemplated feeding strategies (Heilman & Spieler 1999; Weirich *et al.* 2006), optimum dietary protein and/or lipid levels (Lazo *et al.*1998; Williams *et al.* 1985; Riche 2009), digestibility and nutrient availability of feedstuffs (Riche & Williams 2010; González-Félix *et al.* 2010; Gothreaux *et al.* 2010), and the utilization of alternative feed ingredients to replace fishmeal in aquatic feeds (Davis *et al* 2009; Riche & Williams 2011).

Timing of feeding affects pompano. Heilman & Spieler (1999) found a better growth performance when fed in the evening than when fed in the morning, indicating that the preferred feeding time (morning) may not be the best time for feeding on a production setting. Weirich *et al.*(2006) observed that feed frequency and rate significantly influenced mean weight and weight gain but caused no effects on feed conversion efficiency or whole pompano composition.

Lazo *et al.*(1998) used graded levels of protein (from 30 to 45% crude protein) in four isolipidic (8% lipid) diets and observed significant improvement in growth and feed efficiency as dietary protein increased when using fishmeal and soybean meal as major protein sources. They concluded that a minimum of 45% dietary crude protein is required for maximize pompano growth. Riche (2009) reported that diets with digestible protein between 35.6 and 36.6%, and digestible energy of at least 368.1 kcal/100g provided the maximum growth of Florida pompano. In a study evaluating the value of fish oil in diets for Florida pompano, Williams *et al.* (1985) concluded that the most productive diets contained 4 and 8% fish oil and a digestible energy to digestible protein ratio from 7.4 to 8.1 kcal/g.

Gothreaux *et al.* (2010) reported apparent energy and protein digestibility values of soybean meal for Florida pompano of 67.4 and 84.3%, respectively. High amino acid availability of this ingredient was observed with exception of lysine and total sulfur amino acids. Soybean meal provided a good approximation of the whole fish (17 – 34 g mean weight) essential amino acid profile. Similar nutrient digestibility and amino acid availability in soybean meal for Florida pompano were observed in the study conduct by Riche & Williams (2010). The apparent energy digestibility of various vegetable feed ingredients in Florida pompano was evaluated by González-Félix *et al.* (2010). The apparent energy digestibility ranged from 8.2% in wheat meddling to 55.4% in whole wheat flour. These studies demonstrate that soybean meal is a good ingredient to be employed in pompano diets and that this species do not digest carbohydrates very efficiently.

The replacement of fishmeal with alternative ingredients has been investigated in Florida pompano by several authors. Davis *et al*. (2009) replaced fishmeal with soybean meal and soy protein concentrate in pompano diets and observed depressions in fish performance as dietary fishmeal was removed. Dietary fishmeal level was decreased from 30 to 5%, and was isonitrogenously replaced with soy protein concentrate while soybean levels were maintained constant (37%) among test diets. The replacement of 50% of fishmeal (to 15% of the diet) with soy protein concentrate did not affect fish performance; however, further fishmeal replacement impaired fish performance. In the fishmeal replacement study conducted by Riche &Williams (2011), the isonitrogenous replacement of more than 60% of fishmeal in the control diet (31.2%) with soybean meal led to depression in pompano performance. These authors suggested a conservative value of 36% of fishmeal protein substitution with soybean meal could be acceptable.

Studies on the replacement of fishmeal in aquatic feeds have received considerable attention in the last few years. Fishmeal is widely employed in aquatic feeds, especially those used for shrimp and marine finfish, because of its high protein content, excellent amino acid profile, high nutrient digestibility and the lack of antinutrients (NRC 1993; Gatlin *et al.*, 2007). However, as aquaculture industry continues to growth, feed companies will progressively have less access to this finite resource. Therefore, as a result of increasing costs, limited supply of marine resources and the risk for potential contaminants, the evaluation of other alternative feedstuffs becomes imperative.

Soybean meal has been evaluated as a potential alternative for fishmeal in aquatic feeds and its utilization has been increasing in recent years. Soybean meal has been the predominant form of soy products used in aquatic feeds, being considered as the most widely studied and may become a model for other plant resources (Barrows *et al.* 2008). Soybean meal is regarded as a highly nutritious feedstuff with a high crude protein content and reasonable amino acid profile. However, lower levels of the essential amino acids lysine, methionine and threonine may result in amino acid deficiency in high soybean diets. Additionally, the presence of antinutritional factors comprises another constraint that may limit the utilization of this ingredient. Therefore, the evaluation of other commercially available and potential alternative ingredients is necessary to insure future availability of sustainable and cost-effective aquatic feeds for the aquaculture industry.

Rendered animal products such as poultry by-product meal and meat and bone meal are readily available in United States and have been evaluated as alternative ingredients in aquatic feeds. The poultry by-product meal is rendered from the waste generated from poultry processing plants and it is composed of rendered parts of slaughtered poultry. It typically consists of heads, feet, underdeveloped eggs, gizzards and intestines. The meat and bone meal is rendered from dried mammalian tissue and it is comprised of hair, hooves, horn, hide trimmings, manure and stomach trimmings (Hardy & Barrows 2002).

These by-products are potential alternative feedstuffs to fishmeal and have already been evaluated in a fairly large number of nutritional studies (Kikuchi *et al.* 1997; Kureshy *et al.* 2000; Webster *et al*. 2000; Gaylord & Rawles 2005; Hedrick *et al.* 2005; Turker *et al.* 2005; Ai *et al.* 2006; Zhang *et al.* 2006; Wang *et al.* 2006; Rawles *et al.* 2006b; Williams 2008; Nguyen *et al.* 2009). Protein and energy digestibilities and the amino acid availability of poultry by-product meal and meat and bone meal have been reported for Florida pompano (Williams 2008). However, the replacement of fishmeal in pompano diets utilizing these two potential feed ingredients have not been accomplished to date.

Several attempts to completely replace fish meal in aquatic feeds for many species have failed. Suggested causes are typically related to potential limitations in essential amino acids such as lysine and methionine and poor palatability. However, taurine deficiency in low fish meal diets has also been considered as the cause of poor performance of fish fed low fishmeal diets with high content of vegetable-based ingredients. Some fish species (most marines) have demonstrated positive responses to supplemental taurine indicating that taurine may be essential (Park *et al*. 2002; Matsunari *et al*. 2005; Takagi *et al.* 2006b; Lunger *et al*. 2007). Differences in taurine biosynthesis have been demonstrated in several fish species by Goto *et al.* (2001) and Yokoyama *et al.* (2001) indicating that the biosynthesis of this organic compound varies among fishes and thus may be indispensable in the diets.

If the commercial production of Florida pompano is to be expanded then species specific diets must be formulated that limit the use of fishmeal. Consequently the objectives of this research were to further improve our knowledge on the use of poultry by-product meal and meat and bone meal as alternative ingredients to fishmeal in Florida pompano diets.

FIGURES

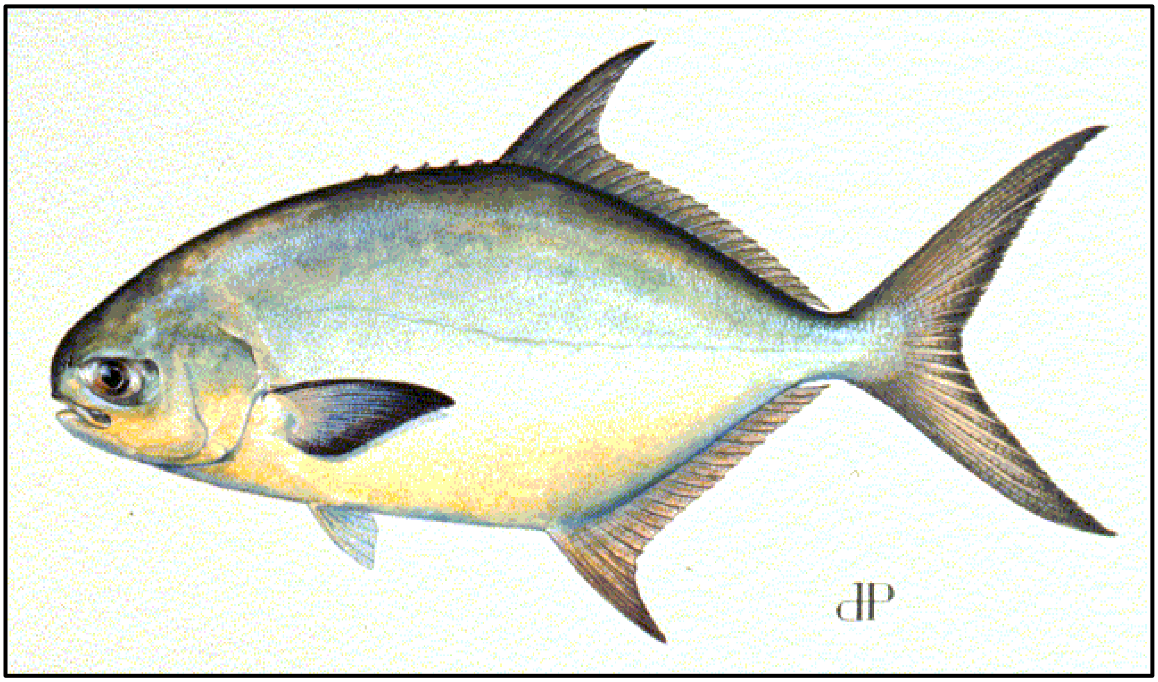

Figure 1. Florida pompano *T. Carolinus* L. – Fish and wildlife Research Institute, Florida Fish and Wildlife Conservation Commission; (http://myfwc.com/research/saltwater/fish/florida-pompano)

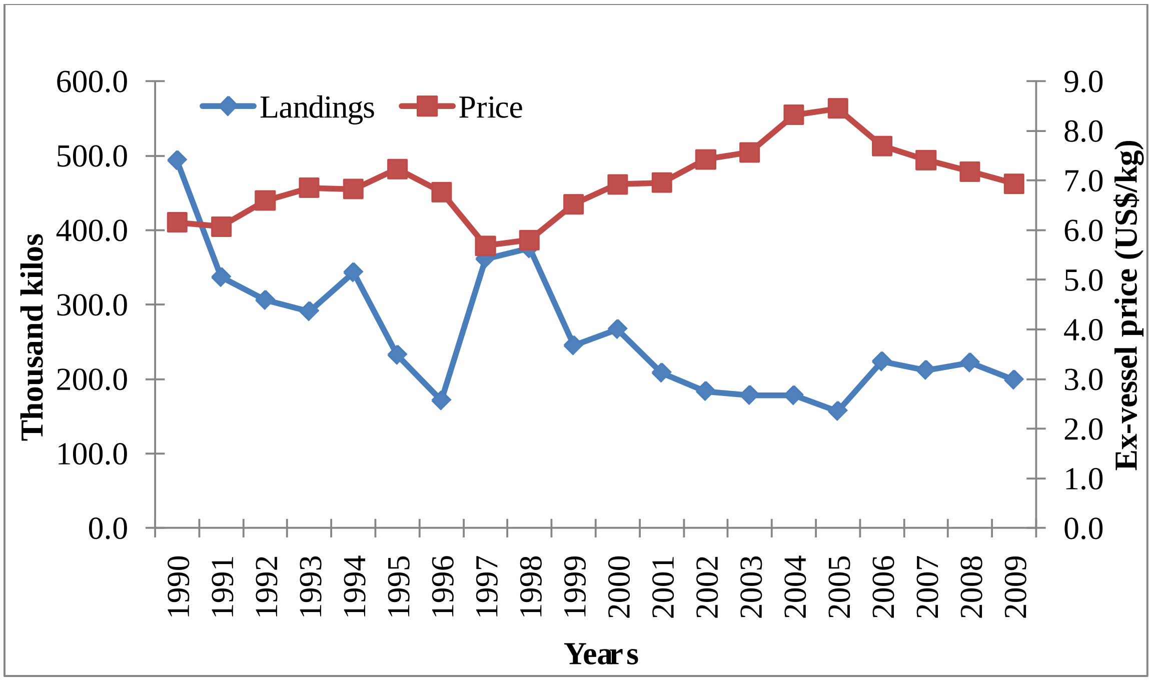

Figure 2. Annual commercial landings of Florida pompano from 1990 to 2009 [National Oceanic and Atmospheric Administration, Fisheries and Statistics Division (www.st.nmfs.noaa.gov)].

CHAPTER II

EVALUATION OF THE REPLACEMENT OF FISHMEAL BY POULTRY BY-PRODUCT MEAL IN DIETS FOR FLORIDA POMPANO *Trachinotus carolinus* L.

INTRODUCTION

Florida pompano *Trachinotus carolinus* L. is becoming an important species for marine aquaculture in the United States. With a strong consumer acceptance and demand, the pompano is considered to be among the highest prized food fishes in many areas of the world. The demand for Florida pompano in the United States is consistently higher than what the fishery sector can supply, resulting in a strong market-driven interest on the commercial cultivation of this species. The development of a sustainable pompano aquaculture will rely, among other factors, on the design of feeds that are less dependent in fishmeal as the primary protein source. Generally, fishmeal is incorporated in feeds for marine fishes at levels ranging from 30 to 60% (Wang *et al.* 2006). Research efforts have consistently reduced the levels of fishmeal used in aquatic feeds with the inclusion of plant based ingredients such as soybean meal. However, the levels of the essential amino acids lysine, methionine and threonine are low in soybean and consequently, may be limiting in high soybean diets. Moreover, the presence of antinutritional factors, nutrient density of the meal and palatability problems may play important roles on limiting high inclusion levels of soybean in diets of aquatic animals (Gatlin *et al.* 2007). The utilization of high levels of soy-based products to replace fishmeal has resulted in depressed growth of pompano (Lazo *et al*. 1998; Davis *et al*. 2009; Riche &Williams 2011). Therefore, further research on potential alternative ingredients is necessary.

Poultry by-product meal has long been tested as an alternative animal ingredient to replace fishmeal in aquatic feeds (Fowler 1991). It consists of rendered material from the waste generated from poultry processing plants and it is composed of rendered parts of slaughtered poultry (Hardy & Barrows 2002). It has a protein level and amino acid profile relatively similar to fishmeal (NRC 1993) and is considered a valuable protein source for many species.

Positive results on the utilization of poultry by-product meal to replace fishmeal in aquatic feeds have been reported for species such as black sea turbot *Scophthalmus maeoticus* (Turker *et al.* 2005), cuneate drum *Nibea miichthioides* (Wang *et al.* 2006), and hybrid striped bass *Morone chrysops* x *M. saxatilis* (Webster *et al.* 2000; Gaylord & Rawles 2005; Rawles *et al.* 2006b). In pompano, poultry by-product meal has been evaluated as a potential replacement for fishmeal and the results demonstrated that it is well digested by pompano, indicating the potential aplicability of this ingredient in pompano diets (Williams 2008).

The poultry by-product meal is widely available in United States and its utilization in diets for Florida pompano would reduce feed costs and contribute to a more economically feasible pompano aquaculture. Therefore, the major goal of this study is to evaluate the replacement of fishmeal with poultry by-product meal on the performance and body composition of Florida pompano.

MATERIAL AND METHODS

Fish

Juvenile pompano, 1.73 g mean weight, were obtained from Harbor Branch Oceanographic Institute, Fort Pierce, FL. Fish were loaded into a hauling tank equipped with a supplemental oxygen supply system and transported to the Alabama Department of Conservation and Natural Resources Marine Resource Division, Claude Peteet Mariculture Center (CPMC), in Gulf Shores, Alabama. At CPMC, fish were acclimated to the facilities' water parameters by slowly replacing the hauling water with local brackish water (12.0 g/L salinity, 6.6 mg/L dissolved oxygen and 7.75 pH) and subsequently stocked into a $5m^3$ fiberglass recirculating tank equipped with independent biological filter, air lift pumps and supplemental aeration provided by a regenerative air blower and air diffusers. Fish remained in this tank acclimating to local conditions until adequate size for the growth trials was achieved. During this period fish were fed to apparent satiation with a 40% crude protein and 9% crude fat commercial diet (EXTR 400, Rangen Inc).

Diets

Experimental diets were manufactured at Auburn University, Department of Fisheries and Allied Aquacultures, Auburn, Al, USA. They were prepared by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer (Hobart A200FT, Troy, OH, USA) for 15 minutes. Boiling water was blended into the mixture to promote appropriate consistency for pelleting. Subsequently, the moist mash from each diet was passed through a 3.0 mm die in the grinder. Wet diets were then placed into a forced air drying oven (<45 °C) for approximately 24 h attaining a moisture content of less than 10%. Dry diets were stored at -20 °C, and prior to use each diet was crumbled and sieved to an appropriate size. All experimental diets were formulated to be isonitrogenous and isolipidic, containing 40% crude protein crude protein and 8.0% lipids, using 50% soybean meal as the major protein source.

Experiment 1. Replacement of fishmeal by poultry by-product meal in pompano diets

The objective of this experiment was to evaluate the effects of the replacement of fishmeal with poultry by-product meal on the production performance and body composition of pompano. The duration of the experiment was 10 weeks. Four diets were designed to produce a gradual replacement of fishmeal with poultry by-product meal to a complete fishmeal free diet (Table 1). The basal diet (FM15) contained 15% fishmeal and 50% soybean meal as the major protein source.

In each of the test diets, 5% fishmeal was isonitrogenously replaced with poultry by-product. With the gradual fishmeal replacement, the three test diets contained 10% (FM10), 5% (FM05) and 0% (FM0) fishmeal. The test diets were supplemented with methionine to obtain the same level as the basal diet. With exception of the test ingredient, other ingredient levels were maintained constant.

Digestibility

Apparent digestibility coefficients for dry matter (ADMD), protein (APD) and energy (AED) for the FM15 and FM0 diets were determined. Digestibility diets consisted of a portion of approximately 3.0 kg of each diet to which chromic oxide (Cr_2O_3) was added to obtain a final marker level of 1%. The Pompano used for the digestibility trial were larger fish (mean weight 150.0 g) overwintered from the previous year. These fish were kept in a 5.0 m^3 circular fiberglass tank and fed a 40% crude protein, 9% crude fat commercial extruded diet (EXTR 400, Rangen, Inc) until the commencement of the digestibility trial. Fish were fed at 5% of the body weight per day and daily ration was provided at 0800 and 1600 h. A seven day dietary acclimation period was allowed prior to the collection of fecal material. In the following day, fecal collections were conducted within 3 – 4 hours after morning feeding using methods similar to those of Riche (2009).

Groups of five fish were removed from the holding tank and placed into a plastic container where they were anesthetized with tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA) at 80 mg/L. Upon showing disoriented swimming, fish were handled and fecal samples collected by gentle stripping the lower intestine as described by Austreng (1978). Fecal samples were then dried at 105 °C for 24 h before being stored at – 60 °C. Sampled fish were recovered and kept in a secondary tank until the termination of fecal collection and then returned to the original tank. Fish rested for six days before the next fecal collection on the following day. Three consecutive fecal collections were conducted per each diet.

Experiment 2. Evaluation of the potential amino acid limitations in the poultry by-product meal based diet (FM0)

The second experiment was conducted over a 12 week period to evaluate potential limitations of dietary levels of methionine, lysine or taurine in the poultry by-product meal based diet (FM0) of experiment 1. Four diets were formulated to have the same formulation of the FM0 diet. The TLM diet was the basal diet to which methionine (M), lysine (L) and taurine (T) were supplemented (Table 1). The final methionine and lysine levels in this diet were similar to those in the FM15 diet used in experiment 1. Each of the subsequent test diets was not supplemented with methionine (TL), lysine (TM), or taurine (LM).

Experimental systems and husbandry

The experiments were conducted in two separate semi-recirculating systems, each comprised of 12 (0.9 m^3) circular fiberglass culture tanks, a reservoir tank of approximately the same volume, biological filter, water pump and supplemental aeration supplied by a regenerative blower and air diffusers. The experimental systems were located in a greenhouse which provides a natural 14 h L/10 h D cycle. Ventilation was provided to reduce water temperatures during the summer. Routine systems maintenance consisted of partial water exchanges and of siphoning of solids as necessary.

Water quality parameters were monitored routinely. Dissolved oxygen, temperature, salinity and pH were monitored twice daily using an YSI 556 multi probe meter (Yellow Spring Instruments Co., Yellow Springs, OH, USA). Total ammonia nitrogen was analyzed on a weekly basis using an ion selective electrode (Orion EA 940, Thermo Electron Corporation, Beverly - MA, USA).

At the commencement of growth trials, fish were manually graded to reduce size variations. Size sorted fish (mean weight 3.48 ± 0.19 g) were stocked into culture tanks of experiment 1 at a density of 25 fish per tank (28 fish/m^3). The remaining fish (mean weight 4.37 ± 0.29 g) were stoked into experiment 2 tanks several days later at the same density. Stocking procedure was done by manually counting and weighing fish groups, followed by a two minute immersion in chloroquine phosphate (Marex, Aquatronics, Oxnard, CA) prophylactic treatment against *Amyloodinium ocellateum*, which consisted of the immersion of fish groups into a solution at 21.1 mg/ L followed by a fresh water bath. This prophylactic treatment was employed every time fish were handled.

Fish in experimental tanks were fed twice daily one of the randomly assigned test diets. An initial daily feed input was set at 5% of fish's biomass and was reduced as the biomass in the tank increased. Three replicates were used per treatment. Experimental fish were sampled every two weeks for growth and survival evaluations and for feed input readjustment.

Data acquisition and analytical procedures

The following parameters were used to evaluate fish performance in each of the experiments:

- Weight gain (%) = [(final body weight – initial body weight)/ initial body weight) × 100]
- Average Daily Gain (g/fish/day) = (weight gain/days of experiment)
- Food conversion ratio; FCR = (as is feed intake/wet weight gain)
- Daily protein gain (g/fish/day) = [(final body weight × final body protein) – (initial body weight × initial crude protein)]/days of the experiment
- Daily energy gain (kcal/fish/day) = [(final body weight × final body energy) – (initial body weight × initial body energy)]/days of the experiment.
- Protein retention efficiency (%); PR = [(final body weight × final body protein) – (initial body weight × initial body protein)]/total protein intake (g)] × 100

- Energy retention efficiency (%); ER = [(final body weight × final body energy) – (initial body weight × initial body energy)]/total energy intake (g) × 100

At the end of each experiment, four fish from each experimental tank were randomly collected, euthanized with 200 mg/L of tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA), and homogenized in a food processor and frozen at – 60 °C for proximate analyses. Dry matter was determined by placing representative portions of each sample in an oven at 105 °C until constant weight was obtained. Protein content of whole fish body, diets and fecal material was determined by the micro-Kjeldahl method (Ma & Zuazaga 1942). Gross energy was analyzed with a semimicro-bomb calorimeter (Model 1425, Parr Instrument Co. Moline, IL, USA).The chromic oxide concentrations were determined by the method of McGinnis & Kasting, (1964). After colorimetric reaction, absorbance was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy Co., Rochester, NY, USA) at a 540 nm. All analytical procedures were conducted in triplicate.

The apparent digestibility coefficients for dry matter (ADMD), protein (APD) and energy (AED) were calculated according to Mainard & Loosli (1969) and Hardy & Barrows (2002), as follow:

$$\text{ADMD (\%)} = 100 - \left[100 \times \left(\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}}\right)\right]$$

$$\text{APD and AED (\%)} = 100 - \left[100 \times \left(\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}} \times \frac{\%\text{ nutrient feces}}{\%\text{ nutrient feed}}\right)\right]$$

Statistical analyses

All data were analyzed using one-way analysis of variance to determine significant ($P<0.05$) differences among treatments. When significant differences were identified the Student – Neuman Keul's multiple –range test was used to determine significant differences between treatment means. Linear regression analysis was used to evaluate the relation between independent and dependent variables for select data. The Statistical analyses were performed using the SAS® software package (SAS Institute Inc., Cary, NC USA).

RESULTS

Florida pompano showed complete acclimation to facility's water and experimental systems and accepted both commercial and experimental diets with no noticeable palatability problems. The prophylactic preventive treatments were applied to prevent any disease outbreaks and to promote good health of the fish during the experiments. The water quality for both experiments 1 and 2 is presented in Table 4. The values of all water quality parameters are consistent and within acceptable ranges for pompano production (Watanabe 1995; Weirich & Riche 2006).

Experiment 1. Replacement of fishmeal by poultry by-product meal in pompano diets

The analyzed values of gross energy and crude protein of experimental diets are presented in Table1. Gross energy levels ranged from 459.5 to 489.7 kcal/100g, showing to be fairly constant among experimental diets. A reduction in digestible energy from diet FM15 (352.6 kcal/100g) to the diet FM0 (296.4 kcal/100g) was observed. Protein analyses of the experimental diets showed lower crude protein values compared to calculated values. Test diets were formulated to contain 40% crude protein, the analyzed values ranged from 36.7 to 40.8% crude protein and the digestible protein were 32.2% and 30.6% for the FM15 and FM0 diets, respectively.

The proximate analysis and amino acid composition of major dietary ingredients and the calculated amino acid composition of experimental diets are presented in Tables 2 and 3, respectively. The essential amino acid composition among experimental diets was very similar. With the exception of lysine and valine, all amino acid levels were fairly similar or higher than those of pompano muscle adjusted to 40% protein.

Diet digestibility was affected by the total replacement of fishmeal with poultry by-product meal (Table 5). Pompano fed the FM0 diet had lower ADMD, APD and AED compared to fish fed the FM15 diet. The ADMD decreased from 62.9% in the FM15 diet to 51.2% in the FM0 diet. The same diets had significant depressions in APD and AED from 87.7 to 79.5% and from 73.6 to 61.9%, respectively.

Concerning fish performance (Table 6), no significant differences were found when pompano were fed diets containing a minimum of 5% fishmeal. However, significant reductions in weight gain (form 338.1to 260.4%), PR (from 19.6 to 12.4%) and ER (from 20.0 to14.0%) and a significant increase in FCR (from 2.03 to 2.42) were observed in fish fed the FM15 and FM0 diets, respectively. Regression analysis on final weight, weigh gain, PR and ER as a response to fishmeal replacement in the diets indicated a significant negative response between fish performance and fishmeal replacement.

The proximate composition of whole body of fish is presented in Table 7. Significant differences in dry matter were observed when fishmeal was completely replaced by poultry by-product meal. Pompano fed the FM15 diet had higher dry matter (29.3%) relative to fish fed the FM0 diet (25.9%). No differences in crude protein and gross energy values were found among treatments.

Experiment 2. Evaluation of the potential amino acid limitations in the poultry by-product meal based diet (FM0)

The gross energy and crude protein of experimental diets are presented in Table 1. Gross energy values were very similar among diets and ranged from 485.9 to 499.9 kcal/100g. Likewise, the analyzed crude protein values were similar to the formulated values and ranged from 39.4 to 41.0%.

The amino acid composition of test diets in experiment 2 is listed in Table 3. As a result of amino acid supplementation, the calculated methionine and lysine levels differed among experimental diets. The methionine level of the TL diet was 0.74 g/100g, lower relative to the other test diets and the pompano muscle (0.85 g/100g). Likewise, the TM diet had the lowest level of lysine (2.20 g/100g) as opposite for the other test diets in which dietary lysine levels were similar. In all diets the lysine level remained lower than in the 40% crude protein pompano muscle. Dietary taurine levels ranged from less than 0.1 to 0.57 g/100g as a result of supplementation.

The nutrition performance of pompano fed amino acid supplemented diets is presented in Table 8. The daily energy gain was significantly lower for fish fed the LM diet (0.42 kcal/fish/day) but no significant differences were found for the other performance parameters. Concerning proximate analysis, the supplementation of amino acids did not affect whole pompano dry matter. However, significant differences were observed for crude protein and gross energy (Table 9). Fish fed the TLM diet had significant higher crude protein (51.0%) compared to fish fed other diets and a significant lower gross energy value was observed in fish fed the LM diet (581.5 kcal/100g).

DISCUSSION

The removal of fish meal from the pompano diets evaluated in this study led to depressions in performance. The lower digestibility and potential limitations in one or more essential amino acid might have been the cause of the poorer performance observed in fish fed diets devoid of fishmeal. Another potential reason for the depression in pompano performance was the low taurine levels in the diets, since pompano in experiment 2 fed diets without fishmeal appeared to respond positively to supplemental taurine. Some fish species have demonstrated a limited ability to biosynthesize this amino acid (Yokoyama *et al.* 2001, Goto *et al.* 2003).

In general, the partial replacement of fishmeal with alternative protein sources has been accomplished for many fish species; however, the total replacement of this high quality nutrient source in fish diets is still a challenge. In pompano, the removal of 50% of dietary fishmeal from a 30% fishmeal diet led to depression in fish performance as observed by Davis *et al*. (2009). In the present study, the replacement of fishmeal in pompano diets was evaluated with the utilization of poultry by-product meal as the alternative ingredient. The results demonstrated that a further reduction in dietary fishmeal was possible without detrimental effects on pompano performance and nutrient retention when a minimum of 5% fishmeal was in the diet. However, the complete replacement of fishmeal with poultry by-product meal resulted in poorer fish performance.

Similar results have been observed in other marine species utilizing poultry by-product to replace fishmeal. Rawles *et al.* (2006b) observed a reduction on the performance of hybrid striped bass *M. chrysops x M. saxatilis* when 75% of fishmeal (25% initial dietary level) digestible protein was replaced by that of poultry by-product meal. Webster *et al.* (1999) found that the total replacement of fishmeal by poultry by-product meal was not possible without negative effects on the performance of sunshine bass *M. chrysops x M. saxatilis.* Yigit *et al.* (2006) was able to use poultry by-product meal to replace more than 25% of the fishmeal protein in diets for the black sea turbot when fishmeal comprised 77.3% of the diet. The replacement of 67% fishmeal (30% initial level) with poultry by-product meal in diets for red drum *Sciaenops ocellatus* containing 24.8% soybean meal was possible without impairment in fish performance (Kureshy 2000). In another study with red drum fed 44% crude protein and 10% lipid diets with 20% poultry by-product meal, the replacement of 87.5% of fishmeal (from 40% in the control diet to 5%) with solvent-extracted soybean meal was accomplished without detrimental effects on fish performance (Davis & Arnold 2004). These differences in response to low fishmeal levels in diets utilizing poultry by-product meal and soy products as substitutes may be related to differences in diet formulation, differences in tolerances to changes in diet palatability among different species and the variations in quality of test ingredients.

Despite similar values found for crude protein and gross energy among diets, DP and DE were lower in the FM0 diet. Although digestibility was not determined for the

FM10 and FM05 diets, it is suggestive that diet digestibility decreased gradually along with fishmeal replacement. The differences in diet digestibility may have contributed to the reduction in performance observed in fish fed the FM0 diet. The reduction in ADMD, APD and ADE observed between the diets FM15 and FM0 are indicative of lower digestibility of the poultry by-product compared to fishmeal or interactions among ingredients leading to lower diet digestibility following fishmeal replacement.

High variability in chemical composition and APD of poultry by-product meal from seven different manufactures was observed by Dong *et al.* (1993), reporting in vivo APD of poultry by-product in salmonids ranging from 64.4 to 77.7%. Rawles *et al.* (2006a) reported APD and AED of 55 and 66%, respectively, for poultry by-product meal in sunshine bass *M. chrysops* x *M. saxatilis.* Nengas *et al.* (1995) reported AED of poultry by-product and herring fishmeal of 44.7 and 94.1%, respectively in gilthead sea bream *Sparus auratus* L. For pompano held in water with a salinity of 28 g/L were observed APD and AED values for poultry by-product meal of 67.4% and 72.1%, respectively (Williams 2008). These values are generally lower than the digestibility values reported for fishmeal in most fish species.

Despite the supplementation of methionine, the occurrence of possible limitations in this amino acid, alone or coupled with other essential amino acids, was possible. The supplementation of methionine and lysine to low fishmeal diets with increasing levels of soybean meal and poultry by-product are normally implemented to cope with natural limitations in these amino acids (Steffens 1994; Gaylord and Rawles 2005; Rawles *et al.* 2006b). The results observed in experiment 2 did not show conclusive evidence of limitations in methionine and lysine in the FM0 diet. The reductions of dietary methionine and lysine to levels lower than those used in the FM0 diet of experiment 1 did not significantly affect pompano performance. These findings suggested that the observed reduction in fish performance when fed the FM0 diet was not due to limitations in these amino acids.

A limited number of studies have evaluated taurine supplementation in fish diets and found positive responses in different species such as Japanese Flounder *Paralichthys olivaceus* (Kim *et al.* 2005a; Kim *et al.* 2005b), yellowtail *Seriola quinqueradiata* (Matsunari *et al.* 2005; Takagi *et al.* 2008) and red sea bream *Pagrus major* (Goto *et al.* 2001). However, no studies with taurine supplementation in pompano have been reported. Furthermore, while taurine is found in fairly high levels in fishmeal, it is present in low concentrations in poultry by-product and practically nonsexist in soybean meal and other plant based ingredients (NRC 2006). Consequently, the calculated dietary levels of taurine in experiment 1were very low, becoming questionable whether taurine could have been limiting.

The results observed for fish performance and nutrient retention in experiment 2 were indicative of a potential dietary requirement for taurine by pompano. Despite no significant differences found, pompano performance was numerically lower when fed the LM diet. Lowest values of final weight (22.97g), weight gain (445.3%), average daily gain (0.24 g/day), daily protein gain (0.03 g/day), protein (16.3%) and energy (30.7%) retention efficiency along with highest FCR (1.95) were observed when taurine was not present in the diet. On the other hand, pompano fed the diets TLM, TL and TM performed similarly.

Different abilities of taurine biosynthesis have been observed in other fish species, especially marine species (Yokoyama *et al.* 2001, Goto *et al.* 2003). Therefore, fish species with low ability of taurine biosynthesis more likely have a requirement for taurine and its supplementation to specie specific diets may be necessary to support maximum growth.

This study demonstrated that high quality poultry by-product meal can be used as an alternative ingredient to reduce fishmeal content in high soybean meal based diets. An inclusion of at least 5% fishmeal in similar formulations is encouraged to avoid depressions in pompano performance. Pompano appeared to respond to supplemental taurine when fed low fishmeal, high soybean meal based diets. Therefore, further investigation on the potential requirement for taurine in pompano is warranted.

REFERENCES

Austreng, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of gastrointestinal-tract. *Aquaculture,* 13, 265-272.

Davis, D. A., & Arnold, C. R. (2004) Red Drum, *Sciaenops ocellatus* Production Diets: Replacement of Fish Meal with Soybean Meal. *J. Appl. Aquacult.*, 15, 173-181.

Davis, D. A., Rhodes, M. & Quintero, H.E. (2009) Update on nutrition research with florida pompano *(Trachinotus carolinus)*. *Abstracts of the 25th Annual Meeting of the Alabama Fisheries Association.*

Dong, F.M., Hardy, R.W., Haard, N.F., Barrows, F.T., Rasco, B.A., Fairgrieve, W.T. & Forster, I.P. (1993) Chemical-composition and protein digestibility of poultry by-product meals for salmonid diets. A*quaculture,* 116, 149-158.

Fowler, L.G. (1991) Poultry by-product meal as a dietary-protein source in fall chinook salmon diets. *Aquaculture,* 99, 309-321.

Gatlin, D.M., Barrows, F.T., Brown, P. *et al*. (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res.*, 38, 551-579.

Gaylord, T.G. & Rawles, S.D. (2005) The modification of poultry by-product meal for use in hybrid striped bass *Morone chrysops* X *M-saxatilis* diets. *J. World Aquac. Soc.*, 36, 363-374.

Gothreaux, C.T., Reigh, R.C., Williams, M.B. & Chesney, E.J. (2010) Amino Acid Availability of Soybean Meal for Florida Pompano. *N. Am. J. Aquacult.*, 72, 309-313.

Goto, T., Matsumoto, T., Murakami, S., Takagi, S. & Hasumi, F. (2003) Conversion of cysteate into taurine in liver of fish. *Fish. Sci.*, 69, 216-218.

Goto, T., Takagi, S., Ichiki, T., Sakai, T., Endo, M., Yoshida, T., Ukawa, M. & Murata, H. (2001) Studies on the green liver in cultured red sea bream fed low level and non-fish meal diets: Relationship between hepatic taurine and biliverdin levels. *Fish. Sci.*, 67, 58-63.

Hardy, R.W. & Barrows, F.T. (2002) Diet formulation and manufacture. In: *Fish Nutrition* (Halver, J.E. ed.), 3nd edn, Vol. 1, pp. 506-596. Academic Press Inc., San Diego, CA, USA.

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

Kim, S.K., Takeuchi, T., Yokoyama, M., Murata, Y., Kaneniwa, M. & Sakakura, Y. (2005b) Effect of dietary taurine levels on growth and feeding behavior of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquaculture,* 250, 765-774.

Kureshy, N., Davis, D.A. & Arnold, C.R. (2000) Partial replacement of fish meal with meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. *N. Am. J. Aquacult.*, 62, 266-272.

Lazo, J.P., Davis, D.A. & Arnold, C.R. (1998) The effects of dietary protein level on growth, feed efficiency and survival of juvenile Florida pompano (*Trachinotus carolinus*). *Aquaculture*, 169, 225-232.

Ma, T. & Zuazaga, G. (1942) Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. *Industrial & Engineering Chemistry Analytical Edition*, 14, 280-282.

Mainard, L.A.W. & Loosli, J.K. (1969) Animal Nutrition (6th Edn.), pp. 613. McGraw-Hill, New York, NY.

Matsunari, H., Takeuchi, T., Takahashi, M. & Mushiake, K. (2005) Effect of dietary taurine supplementation on growth performance of yellowtail juveniles *Seriola quinqueradiata*. *Fish. Sci.*, 71, 1131-1135.

McGinnis, A. & Kasting, R. (1964) Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. *J. Agric. Food Chem.*, 12, 259-262.

Nengas, I., Alexis, M., Davies, S. & Petichakis, G. (1995) Investigation to determine digestibility coefficients of various raw materials in diets for gilthead sea bream, *Sparus auratus* L. *Aquacult. Res.*, 26, 185-194.

NRC (Natural Research Council). (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, DC., USA, 114 p.

NRC (Natural Research Council). (2006) *Nutrient requirements of dogs and cat*. National Academy Press, Washington, DC., USA, 398 p.

Rawles, S.D., Gaylord, T.G. & Gatlin, D.M. (2006a) Digestibility of gross nutrients by sunshine bass in animal by products and commercially blended products used as fish meal replacements. *N. Am. J. Aquacult.*, 68, 74-80.

Rawles, S.D., Riche, M., Gaylord, T.G., Webb, J., Freeman, D.W. & Davis, M. (2006b) Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* female X *M. saxatilis* male) in recirculated tank production. *Aquaculture*, 259, 377-389.

Riche, M. & WILLIAMS, T. (2011) Fish meal replacement with solvent extracted soybean meal or soy protein isolate in a practical diet formulation for Florida pompano (*Trachinotus carolinus*, L.) reared in low salinity. *Aquacult. Nutr.*, 17: no. doi: 10.1111/j.1365-2095.2010.00808.x ...2011.

Riche, M. (2009) Evaluation of Digestible Energy and Protein for Growth and Nitrogen Retention in Juvenile Florida Pompano, *Trachinotus carolinus*. *J. World Aquac. Soc.*, 45-57.

Steffens, W. (1994) Replacing fish-meal with poultry by-product meal in diets for rainbow-trout, *Oncorhynchus mykiss*. *Aquaculture*, 124, 27-34.

Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H. & Ukawa, M. (2008) Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. *Aquaculture*, 280, 198-205.

Turker, A., Yigit, M., Ergun, S., Karaali, B. & Erteken, A. (2005) Potential of poultry by-product meal as a substitute for fishmeal in diets for Black Sea turbot *Scophthalmus* *maeoticus*: Growth and nutrient utilization in winter. *Isr. J. Aquacult./Bamidgeh*., 57, 49-61.

Wang, Y., Guo, J.L., Bureau, D.P. & Cui, Z.H. (2006) Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibea miichthioides*). *Aquaculture,* 252, 476-483.

Watanabe, W.O. (1995) Aquaculture of the Florida pompano and other jacks (Family Carangidae) in the western Atlantic, Gulf of Mexico and Caribbean basin: status and potential. In: *Culture of High-Value Marine Fishes* (Main, K.L. & Rosenfeld, C. eds), pp. 185-205. Oceanic Institute, Honolulu, HI, USA.

Webster, C.D., Thompson, K.R., Morgan, A.M., Grisby, E.J. & Gannam, A.L. (2000) Use of hempseed meal, poultry by-product meal, and canola meal in practical diets without fish meal for sunshine bass (*Morone chrysops* X *M. saxatilis*). *Aquaculture,* 188, 299-309.

Webster, C.D., Tiu, L.G., Morgan, A.M. & Gannam, A. (1999) Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone chrysops x M. saxatilis* fed practical diets. *J. World Aquac. Soc.,* 30, 443-453.

Weirich, C. & Riche, M. (2006) Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia and nitrite at various salinities. *Aquacult. Res*., 855-861.

Williams, T. (2008) An Assessment of Alternative Feed Ingredients in Practical Diets for Florida Pompano (*Trachinotus carolinus*) Held in Low Salinity Recirculating Systems. Master’s thesis.The University of Maine Orono.

Yigit, M., Erdem, M., Koshio, S., Ergun, S., Turker, A. & Karaali, B. (2006) Substituting fish meal with poultry by-product meal in diets for black Sea turbot *Psetta maeotica*. *Aquacult. Nutr*., 12, 340-347.

Yokoyama, M., Takeuchi, T., Park, G. & Nakazoe, J. (2001) Hepatic cysteinesulphinate decarboxylase activity in fish. *Aquacult. Res.,* 32, 216-220.

TABLES

Table 1. Formulation of experimental diets (g/100g as is basis) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (12 weeks).

	Experiment 1				Experiment 2			
Ingredients	FM15	FM10	FM05	FM0	TLM	TL	TM	LM
Fishmeal[1]	15.0	10.0	5.00	0.00	-	-	-	-
Poultry by product meal[2]	0.00	4.90	9.80	14.70	14.70	14.70	14.70	14.70
Soybean meal[3]	50.0	50.0	50.0 0	50.00	50.00	50.00	50.00	50.00
Menhaden fish oil[1]	5.30	5.25	5.20	5.15	5.15	5.15	5.15	5.15
Corn starch[4]	5.65	5.56	5.47	5.38	4.71	4.83	4.88	5.21
Whole wheat[4]	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Corn gluten meal[5]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lecithin (soy refined)[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ASA mineral premix[6]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA vitamin premix[7]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride[4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 250 mg/kg[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
$CaPO_4$ [4]	1.00	1.20	1.40	1.60	1.60	1.60	1.60	1.60
Lysine[9]	-	-	-	-	0.17	0.17	0.00	0.17
DL-methionine[9]	0.00	0.04	0.08	0.12	0.12	0.00	0.12	0.12
Taurine[9]	-	-	-	-	0.50	0.50	0.50	0.00
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein[10]	40.1	40.1	40.0	40.2	40.1	40.0	40.2	40.2
(Crude protein)[11]	(36.7)	(37.8)	(37.9)	(40.8)	(39.4)	(41.0)	(39.6)	(39.6)
Lipid[10]	8.1	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Gross energy (kcal/100g)[11]	479.3	465.1	459.5	489.7	499.7	499.9	485.9	485.9
Digestible protein[11]	32.2	-	-	30.6	-	-	-	-
Digestible energy (kcal/100g)[11]	352.6	-	-	296.4	-	-	-	-

FM = Fishmeal; T = Taurine; L = Lysine; M = Methionine.

[1] Omega Protein Inc., Reedville, Virginia, USA.

[2] Griffin Industries, Inc., Mobile, Alabama, USA.

[3] De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Alabama, USA.

[4] MP Biochemicals Inc., Solon, Ohio, USA.

[5] Grain Processing Corporation, Muscatine, IA, USA.

[6] ASA Premix (g $100g^{-1}$ premix) : cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250, ferrous sulfate heptahydrate, 4.0, manganous sulfate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193, and α cellulose 81.826.

[7] ASA Premix (g/kg Premix): thiamin HCL, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU g^{-1}), 2.40; vitamin D_3 (400,000 IU g^{-1}), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.

[8] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[9] Aldrich-Sigma, St. Louis, MO.

[10] Calculated.

[11] Analyzed.

Table 2. Proximate analysis and amino acid composition of major dietary ingredients (fishmeal, FM; poultry by-product meal, PBM; soybean meal, SBM; and whole wheat, WW) used in the diets (g/100g as is basis).

Ingredients	FM[1]	PBM[1]	SBM[1]	WW[2]
Proximate analysis				
Dry matter	90.34	94.97	86.58	88.50
Crude protein	62.80	66.00	47.60	15.80
Crude fat	9.47	16.23	1.62	1.59
Fiber	0.31	1.08	2.70	2.78
Ash	20.19	10.72	5.94	1.53
Amino acids				
Methionine	1.75	1.19	0.82	0.21
Cystine	0.54	0.68	0.88	0.27
Lysine	4.91	4.21	2.81	0.36
Phenylalanine	2.38	2.44	2.16	0.63
Leucine	4.32	4.92	3.36	0.89
Isoleucine	2.36	2.41	1.86	0.51
Threonine	2.26	2.29	1.56	0.37
Valine	2.71	2.73	1.78	0.59
Histidine	1.32	1.37	1.16	0.30
Arginine	3.87	4.47	3.26	0.64
Glycine	5.07	6.83	1.89	-
Aspartic acid	6.63	5.90	6.55	-
Serine	1.94	2.22	1.95	-
Glutamic acid	8.78	9.19	9.64	-
Proline	3.32	3.89	2.36	-
Hydroxyproline	0.89	1.90	0.02	-
Alanine	3.78	4.02	1.72	-
Tyrosine	2.08	1.92	1.49	0.43
Taurine[3]	0.32	0.31	-	-
Total essential amino acids[4]	*25.88*	*26.50*	*18.77*	*4.50*

[1] New Jersey Feed Laboratory, Inc. 1686 Fifth Street Trenton, NJ 08638.
[2] NRC 1993.
[3] NRC 2006.
[4] Except Tryptophan.

Table 3. Amino acid composition of experimental diets (40% crude protein) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (12 weeks), and of the 40% crude protein pompano muscle (g/100g dry matter basis).

	Experiment 1				Experiment 2				*Fish*
Amino acid[1]	FM15	FM10	FM05	FM0	TLM	TL	TM	LM	*Muscle*[2]
Methionine	0.85	0.87	0.88	0.87	0.86	0.74	0.85	0.85	*0.85*
Cystine	0.66	0.68	0.68	0.68	0.67	0.67	0.66	0.68	*0.78*
Lysine	2.39	2.39	2.34	2.25	2.40	2.41	2.20	2.43	*2.57*
Phenylalanine	1.84	1.87	1.87	1.83	1.81	1.81	1.79	1.83	*0.96*
Leucine	3.16	3.24	3.27	3.22	3.18	3.19	3.15	3.22	*2.51*
Isoleucine	1.58	1.61	1.61	1.57	1.55	1.56	1.54	1.57	*1.61*
Threonine	1.36	1.38	1.38	1.35	1.33	1.34	1.32	1.35	*1.37*
Valine	1.64	1.67	1.66	1.62	1.60	1.61	1.59	1.62	*1.99*
Histidine	0.95	0.96	0.96	0.94	0.93	0.93	0.92	0.94	*0.65*
Arginine	2.56	2.63	2.66	2.59	2.60	2.57	2.57	2.62	*1.41*
Tyrosine	1.37	1.38	1.37	1.31	1.32	1.30	1.30	1.33	*0.49*
Taurine	0.08	0.05	0.05	0.05	0.57	0.56	0.56	0.05	*ND*

ND = not determined; FM = Fishmeal; T = Taurine; L = Lysine; M = Methionine.

[1] Calculated from NRC 2006.

[2] Calculated from a 60% crude protein Florida pompano muscle (Gothreaux *et al.*, 2010).

Table 4. Water quality parameters during experiment 1 (10 weeks) and experiment 2 (12 weeks).

Parameter	Experiment 1 Mean (± SD)	Experiment 2 Mean (± SD)
Temperature (°C)	28.1 (1.4)	27.6 (1.6)
Salinity (g/L)	16.5 (2.6)	14.5 (2.5)
Dissolved oxygen (mg/L)	6.8 (0.9)	6.9 (0.8)
pH	7.7 0.2)	7.6 (0.2)
Total ammonia nitrogen (mg/L)	0.1 (0.2)	0.1 (0.1)

Table 5. Apparent digestibility coefficients for dry matter, crude protein and gross energy of diets with 50% soybean meal, 15% fishmeal (FM15) or 14.7% poultry by-product meal (FM0), fed to juveniles (3.48 ± 019 g mean initial weight) Florida pompano *T. carolinus* L. (Experiment 1).

Variable	FM15	FM0	P - value	PSE[1]
Dry matter (%)	62.9[a]	51.2[b]	0.0006	0.8265
Crude protein (%)	87.7[a]	79.5[b]	0.0035	0.9353
Gross energy (%)	73.6[a]	61.9[b]	0.0001	0.5402

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

Table 6. Performance and nutrient retention of juveniles (3.48 ± 019 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean meal and graded levels of fishmeal (15 – 0%) and poultry by-product meal (0 – 14.7%) after 10 weeks of feeding (Experiment 1).

Variable	FM15	FM10	FM05	FM0	P – value	PSE[1]
Final weight (g)	15.40	14.78	13.98	12.54	0.1720	0.8418
Weight gain (%)	338.1[a]	331.5[a]	297.9[ab]	260.4[b]	0.0364	16.5407
Average daily gain (g/fish/day)	0.17	0.16	0.15	0.13	0.1045	0.0108
Food conversion ratio	2.03[b]	2.06[b]	2.21[ab]	2.42[a]	0.0134	0.0687
Daily protein gain (g/fish/day)	0.02	0.02	0.02	0.01	0.0940	0.0020
Daily energy gain (kcal/fish/day)	0.29	0.25	0.24	0.19	0.0634	0.0216
Survival (%)	97.3	97.3	94.7	92.0	0.2579	2.0000
Nutrient Retention						
Protein retention efficiency (%)	19.6[a]	17.4[ab]	16.7[ab]	12.4[b]	0.0214	1.2660
Energy retention efficiency (%)	20.04[a]	18.4[a]	18.1[a]	14.0[b]	0.0125	0.9682

FM = Fishmeal.

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

Table 7. Proximate composition (dry matter basis) of whole body of juveniles (3.48 ± 019 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean meal and graded levels of fishmeal (15 – 0%) and poultry by-product meal (0 – 14.7%) after 10 weeks of feeding (Experiment 1).

Variable	Initial	FM15	FM10	FM05	FM0	P - value	PSE[1]
Dry matter (%)	25.0	29.3[a]	26.9[ab]	27.3[ab]	25.9[b]	0.0393	0.6553
Crude protein (%)	46.6	45.1	44.7	45.1	41.8	0.7086	2.3547
Gross energy (kcal/100g)	546.9	576.9	576.4	584.3	569.2	0.6296	7.8939

FM = Fishmeal.

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

Table 8. Performance and nutrient retention of juveniles (4.37 ± 0.29 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 50% soybean meal, 14.7% poultry by-product meal and supplemental amino acids after 12 weeks of feeding (Experiment 2).

Performance	TLM	TL	TM	LM	P - value	PSE[1]
Final weight (g)	26.39	28.25	28.31	22.97	0.1273	1.5597
Weight gain (%)	521.4	520.3	530.8	445.3	0.2176	29.2726
Average daily gain (g/fish/day)	0.28	0.30	0.30	0.24	0.1296	0.01826
Food conversion ratio	1.80	1.77	1.81	1.95	0.2218	0.0582
Daily protein gain (g/fish/day)	0.04	0.04	0.04	0.03	0.0984	0.0031
Daily energy gain (kcal/fish/day)	0.52^{ab}	0.59^{a}	0.62^{a}	0.42^{b}	0.0321	0.0404
Survival (%)	97.3	93.3	92.0	96.0	0.1189	1.4907
Nutrient Retention						
Protein retention efficiency (%)	18.5	17.7	18.0	16.3	0.4033	0.8805
Energy retention efficiency (%)	34.1	35.8	38.2	30.7	0.1198	1.9386

T = Taurine, L = Lysine, M = Methionine.

[1] Pooled standard error of treatment means (*n=3*).

Mean values across a row with different superscripts are statistically different (P<0.05).

Table 9. Proximate composition of whole body of juveniles (4.37 ± 0.29 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 50% soybean meal, 14.7% poultry by-product meal and supplemental amino acids after 12 weeks of feeding (Experiment 2).

Variable	Initial	TLM	TL	TM	LM	P - value	PSE[1]
Dry matter (%)	25.0	30.4	31.2	32.6	29.9	0.2359	0.8800
Crude protein (%)	46.4	51.0[a]	47.0[b]	45.9[b]	47.6[b]	0.0107	0.8131
Gross energy (kcal/100g)	546.9	609.5[a]	621.5[a]	624.7[a]	581.5[b]	0.0134	7.5175

T = Taurine, L = Lysine, M = Methionine.

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

CHAPTER III

EVALUATION OF THE REPLACEMENT OF FISHMEAL WITH MEAT AND BONE MEAL BLEND IN DIETS FOR FLORIDA POMPANO *Trachinotus carolinus* L.

INTRODUCTION

The contribution of the aquaculture sector to the total production of capture fisheries and aquaculture production increased from 34.5% in 2006 to 36.9% in 2008 (FAO 2010). Since the production of aquaculture is mostly destined to human consumption, in 2008 the aquaculture accounted for 45.7% of global production for human consumption (FAO 2010). Fish protein represents less than 10% of global population's protein intake (6.1% in 2007, FAO 2010) allowing room for expansion.

With world population constantly increasing (1.7% per year in the last five decades) FAO (2010), the increase in demand for high quality fish protein is incontestable. However, the production capacity of the ocean seems to have stabilized showing slightly declines over the years. Consequently, the supply of high quality fish protein can only come from aquaculture. Aquaculture is the key to meet present and future fish supply, however, the high dependency of fishmeal and fish oil for compounded aquatic feeds inhibits the development of this sector. Fishmeal has long been considered of the best ingredients in animal feeds based on a number of qualities including: high protein content, good amino acid profile, high nutrient digestibility, lack of antinutrients and wide availability. The demand for this protein source is expect to exceed the annual world supply in the following decade. Records demonstrate that fishmeal supply has been static for some time and appears to have reached its maximum. In 2008, around 20.8 million tonnes of world's fish production was reduced to fishmeal and such production has been maintained over the last three years (FAO 2010). Therefore, efforts to reduce fishmeal utilization in aquatic feeds have been considered a priority. This has resulted in considerable interest for the utilization of plant products (Gatlin *et al.* 2007; Barrows *et al.* 2008) in feed formulations.

Among terrestrial feedstuffs, soybean meal has been the predominant form of soy products used, being considered as the most widely studied feed ingredient and may become a model for other plant resources (Barrows *et al.* 2008). Accordingly, the use of soybean meal in aquatic feeds has markedly increased in recent years (Gatlin *et al.* 2007). However, the presence of antinutritional factors and generally lower essential amino acid content relative to fishmeal (especially concerning methionine and lysine) may limit soybean meal utilization in feed formulations for some species. Consequently, it is necessary to evaluate other potential alternative ingredients to fishmeal that in combination with plant based products as soybean meal could support optimum growth rates of farmed fish.

Rendered animal products such as poultry by-product meal (Fowler 1991; Steffens 1994; Nengas *et al.* 1999; Gaylord & Rawles 2005; Yigit *et al.* 2006; Rawles *et al.* 2006; Williams 2008; Wang *et al.* 2010) and various meat and bone meal products (Kikuchi *et al.* 1997; Kureshy *et al.* 2000; Hedrick *et al.* 2005; Ai *et al.* 2006; Wang *et al.* 2006; Zhang *et al.* 2006; Nguyen *et al.* 2009) have been considered potential alternative ingredients for aquatic feeds. In United States, the production of rendered animal products is approximately 11.2 billion pounds (Swisher 2006). The utilization of such products can substantially reduce the use of fishmeal in aquatic feeds and their applicability for aquaculture needs to be investigated. Therefore, this study was designed to evaluate the utilization of a meat and bone meal blend to replace fishmeal in high soybean meal based diets for Florida pompano *Trachinotus carolinus* L.

MATERIAL AND METHODS

Fish

Juvenile pompano, 1.73 g mean weight, were obtained from Harbor Branch Oceanographic Institute, Fort Pierce, FL, and transported to the Alabama Department of Conservation and Natural Resources Marine Resource Division, Claude Peteet Mariculture Center (CPMC), in Gulf Shores, Alabama. At CPMC, fish were acclimated to the facilities' water parameters by slowly replacing the hauling water with local brackish water (12 g/L). Fish were subsequently stocked in a 5m^3 fiberglass culture tank equipped with independent biological filter, air lift pumps and supplemental aeration provided by a regenerative air blower and air diffusers. Fish remained in this tank acclimating to local conditions until growing to an adequate size for the growth trials. During this period fish were fed to apparent satiation with a 40% crude protein and 9% crude fat commercial diet (EXTR 400, Rangen, Inc, Angleton, TX).

Diets

Experimental diets were manufactured at Auburn University, Department of Fisheries and Allied Aquacultures, Auburn, Al, USA. They were prepared by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer and grinder (Hobart A200FT, Troy, OH, USA) for 20 minutes. Boiling water was blended into the mixture to promote appropriate consistency for pelleting. Subsequently, the moist mash from each diet was passed through a 3.0 mm die in the grinder. Wet diets were then placed into a forced air drying oven (<45 °C) for approximately 24 h attaining a moisture content of less than 10%. Dry diets were stored at -20 °C and prior to use each diet was crumbled and sieved to an appropriate size. All experimental diets were formulated to be isonitrogenous and isolipidic, containing 40% crude protein and 8.0% lipids, using soybean meal as the major protein source.

Experiment 1. Replacement of fishmeal with a meat and bone meal blend in pompano diets

This experiment was carried out during 10 weeks seeking to evaluate the effects of the replacement of fishmeal by a meat and bone meal blend (Meat and bone meal with blood) on the production performance and body composition of pompano. Diet formulations were made to obtain a gradual replacement of fishmeal by meat and bone meal blend (Table 10). The basal diet (FM15) contained 15% fishmeal as the sole animal protein source.

In each of the test diets, 5% of the fishmeal was isonitrogenously replaced with meat and bone meal blend. With the gradual fishmeal replacement, three test diets were designed to contain 10% (FM10), 5% (FM05) and 0% (FM0) fishmeal. Test diets were supplemented with methionine to obtain the same dietary level as the basal diet. With exception of the test ingredient, the other ingredients levels were maintained constant.

Digestibility

The apparent digestibility coefficients for dry matter (ADMD), protein (APD) and energy (AED) for the FM15 and FM0 diets were determined. Digestibility diets consisted of a portion of approximately 3.0 kg of each diet to which chromic oxide was added to obtain a final marker level of 1%. The pompano used for the digestibility trial were larger fish (mean weight 150.0 g) overwintered from the year before. These fish were kept in a 5.0 m^3 circular fiberglass tank receiving a 40% crude protein, 9% crude fat commercial extruded diet (EXTR 400, Rangen, Inc) until the commencement of the digestibility trial. Fish were fed at 5% of the body weight per day and daily ration was provided at 0800 and 1600 h. A seven days dietary acclimation period was established prior to the collection of fecal material. In the following day, fecal collections were conducted within the 3 – 4 hours after morning feeding using methods similar to those of Riche (2009).

Groups of five fish were removed from the holding tank and placed into a plastic container where they were anesthetized with tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA) at 80 mg/L. Upon showing disoriented swimming, fish were handled and fecal samples collected by gentle stripping the lower intestine as described by Austreng (1978). Fecal samples were then dried at 105 °C for 24 h before being stored at – 60 °C. Sampled fish were recovered and kept in a secondary tank until the termination of fecal collection and then returned to the original tank. Fish rested for six days before the next fecal collection on the following day. Three consecutive fecal collections were conducted per each diet.

Experiment 2. Evaluation of the potential methionine limitation in the meat and bone meal blend based diet (FM0)

The experiment 2 was carried out over 8 weeks to evaluate potential methionine (M) limitations as suggested in the FM0 diet of experiment 1. The FM0 diet was used as the basal diet in experiment 2. The basal diet (M0.6) was formulated to contain a dietary methionine level of 0.6 g/100g. Three additional test diets were formulated to contain increasing levels of methionine: 0.7 (M0.7), 0.8 (M0.8), and 0.9 (M0.9) grams of methionine per 100 g of diet (Table 10).

Experimental systems and husbandry

The experiments were conducted in semi-recirculating systems, each comprised of 12 circular fiberglass culture tanks, a reservoir tank of approximately same volume, biological filter, water pump and supplemental aeration supplied by a regenerative blower and air diffusers. The culture tanks used for experiment 1 and 2 differed in volume, with 0.7 and 0.9 m^3, respectively. The experimental systems were placed in a greenhouse which provided a natural 14 h L/10 h D cycle. Ventilation was provided to reduce water temperatures during the summer. Routine system maintenance consisted of partial water exchanges and of siphoning of solids as necessary.

Water quality parameters were monitored routinely. Dissolved oxygen, temperature, salinity and pH were monitored twice daily using an YSI 556 multi probe meter (Yellow Spring Instruments Co., Yellow Springs, OH, USA). Total ammonia nitrogen was analyzed on a weekly basis using an ion selective electrode (Orion EA 940, Thermo Electron Corporation, Beverly - MA, USA).

At the commencement of growth trials, fish were manually graded to reduce size variations. Size sorted fish (mean weight 2.99 ± 0.28 g) were stocked into culture tanks of experiment 1 at a density of 15 fish per tank (21 fish/m^3). The remaining fish (mean weight 14.39 ± 0.58 g) were stocked into experiment 2 tanks several days later at a density of 25 fish per tank (28 fish/m^3). Stocking procedure was done by manually counting and weighing fish groups, followed by a two minutes prophylactic treatment against *Amyloodinium ocellateum*, which consisted on the immersion of fish groups into a chloroquine phosphate (Marex, Aquatronics, Oxnard, CA) solution at 21.1 mg/L followed by a fresh water bath. This prophylactic treatment was employed every time fish were handled.

Fish in experimental tanks were fed twice daily one of the randomly assigned test diets. Daily feed input was 5% of fish's biomass at the beginning of the experiments and declined as biomass in the tank increased. Three replicates were used per treatment. Experimental fish were sampled every two weeks for growth and survival evaluations and for feed input readjustment.

Data acquisition and analytical procedures

The following parameters were used to evaluate fish performance in each of the experiments:

- Weight gain (%) = [(final body weight – initial body weight)/ initial body weight) × 100]
- Average Daily Gain (g/fish/day) = (weight gain/days of experiment)
- Food conversion ratio; FCR = (as is feed intake/wet weight gain)
- Daily protein gain (g/fish/day) = [(final body weight × final body protein) – (initial body weight × initial crude protein)]/days of the experiment
- Daily energy gain (kcal/fish/day) = [(final body weight × final body energy) – (initial body weight × initial body energy)]/days of the experiment.
- Protein retention efficiency (%); PR = [(final body weight × final body protein) – (initial body weight × initial body protein)]/total protein intake (g)] × 100

- Energy retention efficiency (%); ER = [(final body weight × final body energy) – (initial body weight × initial body energy)]/total energy intake (g) × 100

At the end of each experiment, four fish from each experimental tank were randomly collected and homogenized in a food processor and frozen at – 60 °C for proximate analyses. Dry matter was determined by placing representative portions of each sample in an oven at 105 °C until constant weight be obtained. Protein content of whole fish body, diets and fecal material was determined by the micro-Kjeldahl method (Ma & Zuazaga 1942). Gross energy was analyzed with a semimicro-bomb calorimeter (Model 1425, Parr Instrument Co. Moline, IL, USA). The chromic oxide concentrations were determined by the method of McGinnis & Kasting (1964). After colorimetric reaction, absorbance was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy Co., Rochester, NY, USA) at a 540 nm. All analytical procedures were conducted in triplicate.

The apparent digestibility coefficients for dry matter (ADMD), protein (APD) and energy (AED) were calculated according to Mainard & Loosli (1969) and Hardy & Barrows (2002), as follow:

$$\text{ADMD (\%)} = 100 - \left[100 \times \left(\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}}\right)\right]$$

$$\text{APD and AED (\%)} = 100 - \left[100 \times \left(\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}} \times \frac{\%\text{ nutrient feces}}{\%\text{ nutrient feed}}\right)\right]$$

Statistical analyses

All data were analyzed using one-way analysis of variance to determine significant ($P<0.05$) differences among treatment means. When significant differences were identified the Student – Neuman Keul's multiple –range test was used to determine significant differences between treatment means. Linear regression analysis was used to evaluate the relation between independent and dependent variables for select data. All Statistical analyses were performed using the SAS® software package (SAS Institute Inc., Cary, NC USA).

RESULTS

Florida pompano showed complete acclimation to facility's water and experimental systems and accepted both commercial and experimental diets with no noticeable palatability problems. The prophylactic preventive treatment was thoroughly applied to prevent any disease outbreaks and to promote health condition of the fish during the experiments. Concerning water quality, despite the salinity in both experiments 1 and 2 was lower than full-scale seawater (37 - 40 g/L), the water quality was maintained within adequate ranges for pompano production (Watanabe 1995; Weirich & Riche 2006) throughout the experiments (Table 13).

Experiment 1. Replacement of fishmeal by a meat and bone meal blend in pompano diets

The formulation of experimental diets of experiment 1 is presented in Table 10. Soybean meal constituted the major dietary ingredient (50%). Formulated crude protein values were very similar among diets and ranged from 40.0 to 40.1%; however analyzed crude protein values were lower ranging from 35.2 to 37.4% with lowest value observed in the FM0 diet.

The proximate analysis and amino acid composition of major dietary ingredients is reported in Table 11 and the amino acid composition of experimental diets and the 40% crude protein pompano muscle is presented in Table 12. The amino acid composition was very similar among diets. Relative to pompano muscle values, the amino acids lysine, isoleucine and valine were generally lower in all diets. Gross energy values ranged from 482.2 to 487.7 kcal/100g and were similar among experimental diets. In contrast, digestible energy was higher in the FM15 diet (350.0 kcal/100g) compared to the FM0 diet (301.6 kcal/100g).

Diet digestibility decreased when fishmeal was totally replaced by the meat and bone meal blend (Table 14). The apparent digestibility coefficients for the FM15 diet were higher compared to the FM0 diet. The ADMD significantly decreased from 60.8 to 48.4% when fish were fed the FM15 and FM0 diets, respectively. Likewise, significant reductions in APD (from 87.6 to 77.7%) and AED (from 71.8 to 61.9%) were found between diets FM15 and FM0.

The fishmeal replacement with meat and bone meal blend affected FCR and survival of pompano (Table 15). The FCR significantly increased from 2.25 in the FM05 diet to 2.96 in the FM0 diet. Lower survival occurred in fish fed the FM0 diet (84.5%), while pompano fed diets with at least 5% fishmeal did not differ in survival. No significant differences were found for the other performance parameters. The whole pompano composition was not affected by the fishmeal replacement with meat and bone meal blend (Table 16). Dry matter ranged from 23.7 (FM10) to 25.9% (FM15), crude protein from 48.7 (FM15) to 49.6% (FM10), and gross energy from 541.5 kcal/100g (FM10) to 570.1 kcal/100g (FM05) however not significant differences were found.

Experiment 2 Evaluation of the potential methionine limitation in the meat and bone meal blend based diet (FM0)

The formulations of experimental diets for experiment 2 are presented in Table 10. All diets in this experiment had equal levels of meat and bone meal blend, which was very similar to the level used in the FM0 diet of experiment 1. Taurine was supplemented at 0.75% in all diets and gelatin was used to facilitate reduced levels of methionine. The major dietary ingredients (fishmeal, soybean meal, meat and bone meal blend and whole wheat) used in this experiment were the same used in experiment 1 and their proximate analysis and amino acid composition are presented in Table 11.

Formulated crude protein values were constant (40.1%) for all experimental diets and the analyzed crude protein values were found to be fairly similar among diets ranging from 39.1 (M0.6) to 40.9% (M0.9). The amino acid composition of experimental diets and the 40% crude protein pompano muscle is presented in Table 12. Supplementation of methionine resulted in different concentrations in the diets. Compared to concentration in muscle, methionine level in the diets were lower in the M0.6 (0.67 g/100g) and M0.7 (0.75 g/100g) diets, similar in the M0.8 diet (0.83 g/100g) and higher in the M0.9 diet (0.95 g/100g). Relative to muscle values, the other essential amino acids lysine, threonine, valine, and isoleucine were generally lower. Gross energy values of the experimental diets are presented in Table 10. A much wider variation in gross energy values was observed for experimental 2 diets relative to experiment 1. The gross energy values ranged from 479.5 to 512.9 kcal/100g in the M0.8 and M0.9 diets, respectively.

The performance of pompano fed diets supplemented with methionine is presented in Table 17. Fish fed the M0.6 diet had significant higher daily energy gain (2.01 kcal/fish/day) compared to fish fed the M0.9 diet (1.07 kcal/fish/day). Likewise, fish receiving the M0.6 diet had significant higher ER (21.47%) compared to fish fed the M0.7 (15.97%), M0.8 (14.54%) and M0.9 (10.99%) diets. Marginally significant probability values were observed for final weight (P = 0.0521), weight gain (P = 0.0531), and average daily gain (P = 0.0536). The linear regression analysis indicated a significant negative effect of supplemental methionine on fish performance. Final weight, weight gain, PR and ER tended to decrease as supplemental methionine increased in the diets. No significant differences were observed for pompano proximate composition presented in Table 18.

DISCUSSION

Reduction in the use of fishmeal in pompano diets is dependent on the suitability of alternative protein sources. Based on prior observations in this laboratory and from other studies, the reduction of dietary fishmeal to levels was low as 15% in practical diets did not statistically affect pompano performance (Davis *et al*. 2009, Riche and Williams 2011). However, they reported that further fishmeal replacement caused depressions in fish performance even though total protein concentrations were unchanged. Thus, 15% fishmeal in combination with soybean meal and meat and bone meal blend consisted of the starting point of this study.

Our results indicated that even further fishmeal replacement could be accomplished without negative effects on pompano performance. Approximately 66% of fishmeal protein (10g/100g of diet) was replaced by that of meat and bone meal blend

(FM05) without any impact on performance and body composition relative to fish fed the FM15 diet. However, the complete replacement of fishmeal with meat and bone meal blend (FM0) resulted in depression of pompano performance.

The potential of meat and bone meal products to replace fishmeal in aquatic feeds varies among different species. Meat and bone meal could successfully replace 100% of dietary fishmeal without detrimental effects on the performance of channel catfish *Ictalurus punctatus* (Li *et al.* 2002; Hedrick *et al.* 2005) and sunshine bass *Morone chrysops* x *M. saxatilis* (Webster *et al.* 1999). In other species, the inclusion of meat and bone meal even at low dietary levels (5.34%) caused reduced fish performance and was not recommended (Kureshy *et al.* 2000); or satisfactorily replace only part of the dietary fishmeal without negatively affect the performance of Japanese flounder *Paralichthys olivaceus* (Kikuchi *et al.* 1997), gibel carp *Carassius auratus gibelio* (Zhang *et al.* 2006), cuneate drum *Nibea miichthioides* (Wang *et al.* 2006), yellow croaker *Pseudosciaena crocea* (Ai *et al.* 2006) and African catfish *Clarias gariepinus* (Goda *et al.* 2007).Unlike the present study, most studies have evaluated the partial but not total replacement of fishmeal.

Lower digestibility and the imbalance in essential amino acids are suggested as the main reasons for poor fish response to increasing dietary levels of meat and bone meal. The statistically inferior FCR (2.96) and survival (84.5%) of fish fed the FM0 diet in experiment 1 was accompanied by numerical depressions in fish performance. This response may be related with lower digestibility of the FM0 diet. As observed, the FM0 diet had a reduction in APD and AED compared to the FM15 diet. The ADMD, APD and AED were approximately 10% lower in the FM0 diet relative to the FM15 diet.

Zhang *et al.* (2006) reported similar reductions in ADMD and APD of fishmeal-free diets containing meat and bone meal for gibel carp *C. auratus gibelio*, while no significant differences were observed for AED. The reduction in diet digestibility could have limited nutrient availability, resulting in the depression of fish performance observed in experiment 1 when all fishmeal was removed from the diet and replaced with the meat and bone meal blend.

Methionine and lysine may be limiting in diets utilizing high levels of soybean meal and supplementation of these amino acids may be necessary to optimize fish performance. Riche and Williams (2011) utilizing diets with 46% protein reported the lysine requirement for pompano to be 2.4% of the diet (5.2% of crude protein), and a predicted methionine requirement of 1.1% of the diet (2.4% of crude protein). However, the total sulphur amino acid requirement was not reported. Patro *et al* (2011) used the broken-line regression model to determine methionine requirement in pompano and reported the optimum dietary methionine requirement of 1.17% of the diet (2.54% of crude protein) in the presence of 0.22% cystine.

Dietary lysine and methionine in experiment 1 were 5.75% and 2.1% of crude protein, respectively. While lysine was higher than the suggested requirement at 5.7% of crude protein, methionine was lower at 2.1%. Despite some methionine requirement can be spared by cystine, the lower digestibility of the FM0 diet could have limited methionine availability reducing pompano performance in experiment 1. However, methionine supplementation to the FM0 diet did not improve fish performance suggesting that methionine was not limiting. Similar results were observed in tilapia *Oreochromis spp* (Nguyen *et al.* 2009) where methionine did not appear to be limiting when high levels of soybean meal in combination with meat and bone meal were use. Since fish have a total sulphur amino acid requirement rather than a especific methionine requirement (Wilson 1989), methionine sparing effect mediated by cystine was likely important in the FM0 diet to prevent sulphur amino acids deficiency.

In contrast to other studies in which supplemental methionine improved fish performance (Coyle *et al.* 2000; Takagi *et al.* 2001), in this study the supplementation of methionine was detrimental to fish performance. The reduction in daily energy gain (from 2.01 to 1.07 kcal/fish/day) and ER (from 21.47 to 10.99%), to fish fed the M0.6 and M0.9 diets, respectivelly, followed increasing methionine levels. In addition, there was a negative correlation between methionine levels in the diets and fish performance, indicating that the supplementation of methionine negatively affect pompano performance ($P<0.05$). Other studies have reported depressions in fish performance when dietary methionine exceeded optimum requirement levels for species such as tilapia *Sarotherodon mossambicus* (Jackson & Capper 1982), milkfish *Chanos chanos* F. (Borlongan & Coloso 1993), indian major carp *Labeo rohita* (Hamilton), also named *Cirrhinus mrigala* (Hamilton) (Murthy & Varghese 1998; Ahmed *et al.* 2003), yellow croaker *P. crocea* R. (Mai *et al.* 2006) and hybrid striped bass *M. saxatilis x M. chrysops* (Griffin *et al.* 1994).

Platability problems (Griffin *et al.* 1994) and toxicidy due to the accumulation of metabolities such as ketones (Murthy & Varghese 1998) have been suggested as possible causes of negative responses to excessive levels of dietary methionine. However, in this study, there were not evidence of diet-induced feeding behavior in pompano fed diets with supplemental methionine.

Our results demonstrated that a further replacement of fishmeal in pompano diets can be accomplished with a high quality meat and bone meal blend and that methionine does not appears to be limiting in these formulations. With a combination of soybean meal and the meat and bone meal blend, fishmeal utilization was reduced in pompano diets without apparent limitations in production performance. The reason why total replacement of fishmeal with the meat and bone meal blend was not possible in this study could not be fully elucidated. Differences in digestibility as well as limitations of some nutrient may have been part of this issue. Further investigations on the use of alternative ingredients and possible nutrient limitations are suggested in order to mitigate the nutritional aspects related to pompano nutrition.

REFERENCES

Ahmed, I., Khan, M.A. & Jafri, A. (2003) Dietary methionine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquacult. Int.*, 11, 449-462.

Ai, Q.H., Mai, K.S., Tan, B.P., Xu, W., Duan, Q.Y., Ma, H.M. & Zhang, L. (2006) Replacement of fish meal by meat and bone meal in diets for large yellow croaker, *Pseudosciaena crocea*. *Aquaculture*, 260, 255-263.

Austreng, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of gastrointestinal-tract. *Aquaculture,* 13, 265-272.

Barrows, F.T., Bellis, D., Krogdahl, A. *et al*. (2008) Report of the plant products in aquafeed strategic planning workshop: An integrated, interdisciplinary research roadmap for increasing utilization of plant feedstuffs in diets for carnivorous fish. *Rev Fish Sci.* 2008;16:449–455.

Borlongan, I.G. & Coloso, R.M. (1993) Requirements of juvenile milkfish (*Chanos-chanos forsskal*) for essential amino-acids (vol 123, pg 125, 1993). *J. Nutr.*, 123, 972-972.

Coyle, S.D., Tidwell, J.H. & Webster, C.D. (2000) Response of largemouth bass *Micropterus salmoides* to dietary supplementation of lysine, methionine, and highly unsaturated fatty acids. *J. World Aquac. Soc.*, 31, 89-95.

Davis, D. A., Rhodes, M. & Quintero (2009) Update on nutrition research with florida pompano *(Trachinotus carolinus)*. *Abstracts of the 25th Annual Meeting of the Alabama Fisheries Association.*

FAO, 2010. The state of World fisheries and aquaculture 2010. FAO Fisheries and aquaculture department. Rome, 2010.

Fowler, L.G. (1991) Poultry by-product meal as a dietary-protein source in fall chinook salmon diets. *Aquaculture,* 99, 309-321.

Gatlin, D.M., Barrows, F.T., Brown, P. *et al.* (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res.,* 38, 551-579.

Gaylord, T.G. & Gatlin, D.M. (1996) Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture*, 139, 303-314.

Gaylord, T.G. & Rawles, S.D. (2005) The modification of poultry by-product meal for use in hybrid striped bass *Morone chrysops X M-saxatilis* diets. *J. World Aquac. Soc.,* 36, 363-374.

Goda, A.M., El-Haroun, E.R. & Chowdhury, M.A.K. (2007) Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. *Aquac. Res.,* 38, 279-287.

Gothreaux, C.T., Reigh, R.C., Williams, M.B. & Chesney, E.J. (2010) Amino Acid Availability of Soybean Meal for Florida Pompano. *N. Am. J. Aquacult.*, 72, 309-313.

Griffin, M.E., White, R.W. & Brown, P.B. (1994) Total sulfur amino-acid requirement and cysteine replacement value for juvenile hybrid striped bass (*Morone saxatilis* x *Morone chrysops*). *Comp. Biochem. Physiol., A-Physiology,* 108, 423-429.

Hardy, R.W. & Barrows, F.T. (2002) Diet formulation and manufacture. In: *Fish Nutrition* (Halver, J.E. ed.), 3nd edn, Vol. 1, pp. 506-596. Academic Press Inc., San Diego, CA, USA.

Hedrick, R.L., Popma, T.J. & Davis, D.A. (2005) Pond production and fatty acid profiles of the fillets of channel catfish reared on diets with different protein sources. *N. Am. J. Aquacult.*, 67, 304-311.

Jackson, A.J. & Capper, B.S. (1982) Investigations into the requirements of the tilapia sarotherodon-mossambicus for dietary methionine, lysine and arginine in semi-synthetic diets. *Aquaculture,* 29, 289-297.

Kikuchi, K., Sato, T., Furuta, T., Sakaguchi, I. & Deguchi, Y. (1997) Use of meat and bone meal as a protein source in the diet of juvenile Japanese flounder. *Fish. Sci.*, 63, 29-32.

Kureshy, N., Davis, D.A. & Arnold, C.R. (2000) Partial replacement of fish meal with meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. N. Am. J. Aquacult., 62, 266-272.

Li, M.G.H., Manning, B.B. & Robinson, E.H. (2002) Comparison of various animal protein sources for growth, feed efficiency, and body composition of juvenile channel catfish *Ictalurus punctatus*. *J. World Aquac. Soc.*, 33, 489-493.

Ma, T. & Zuazaga, G. (1942) Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. *Industrial & Engineering Chemistry Analytical Edition*, 14, 280-282.

Mai, K., Wan, J., Ai, Q., Xu, W., Liufu, Z., Zhang, L., Zhang, C. & Li, H. (2006) Dietary methionine requirement of large yellow croaker, *Pseudosciaena crocea* R. *Aquaculture*, 253, 564-572.

Mainard, L.A.W. & Loosli, J.K. (1969) Animal Nutrition (6th Edn.), pp. 613. McGraw-Hill, New York, NY.

McGinnis, A. & Kasting, R. (1964) Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. *J. Agric. Food Chem*., 12, 259-262.

Murthy, H. & Varghese, T. (1998) Total sulphur amino acid requirement of the Indian major carp, *Labeo rohita* (Hamilton). *Aquacult. Nutr*., 4, 61-65.

Nengas, I., Alexis, M.N. & Davies, S.J. (1999) High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream *Sparus aurata* L. *Aquaculture*, 179, 13-23.

Nguyen, T.N., Davis, D.A. & Saoud, I.P. (2009) Evaluation of Alternative Protein Sources to Replace Fish Meal in Practical Diets for Juvenile Tilapia, *Oreochromis spp*. *J. World Aquac. Soc.*, 40, 113-121.

NRC (Natural Research Council). (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, DC., USA, 114 p.

NRC (Natural Research Council). (2006) *Nutrient requirements of dogs and cat*. National Academy Press, Washington, DC., USA, 398 p.

Patro, B., Reigh, R.C., Williams, M.B. (2011) Dietary methionine requirement of Florida pompano. *Aquacultre America 2011 – Meeting Abstract 344.*

Rawles, S.D., Riche, M., Gaylord, T.G., Webb, J., Freeman, D.W. & Davis, M. (2006b) Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* female x *M. saxatilis* male) in recirculated tank production. *Aquaculture,* 259, 377-389.

Riche, M. & Williams, T. (2011) Fish meal replacement with solvent extracted soybean meal or soy protein isolate in a practical diet formulation for Florida pompano (*Trachinotus carolinus*, L.) reared in low salinity. *Aquacult. Nutr.*, 17: no. doi: 10.1111/j.1365-2095.2010.00808.x ...2011.

Riche, M. (2009) Evaluation of Digestible Energy and Protein for Growth and Nitrogen Retention in Juvenile Florida Pompano, *Trachinotus carolinus*. *J. World Aquac. Soc.*, 45-57.

Steffens, W. (1994) Replacing fish-meal with poultry by-product meal in diets for rainbow-trout, *Oncorhynchus mykiss*. *Aquaculture,* 124, 27-34.

Swisher, K. (2006) The global market for rendered products. In: *Essential rendering: all about the animal by-products industry* (Meeker, D.L., ed.), pp. 213–228. Kirby Lithographic Company, Inc., Arlington, Virginia.

Takagi, S., Shimeno, S., Hosokawa, H. & Ukawa, M. (2001) Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream *Pagrus major*. *Fish. Sci.,* 67, 1088-1096.

Wang, Y., Guo, J.L., Bureau, D.P. & Cui, Z.H. (2006) Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibea miichthioides*). *Aquaculture,* 252, 476-483.

Wang, Y., Kong, L., Li, C. & Bureau, D.P. (2010) The potential of land animal protein ingredients to replace fish meal in diets for cuneate drum, *Nibea miichthioides*, is affected by dietary protein level. *Aquacult. Nutr,* 16, 37-43.

Watanabe, W.O. (1995) Aquaculture of the Florida pompano and other jacks (Family Carangidae) in the western Atlantic, Gulf of Mexico and Caribbean basin: status and potential. In: *Culture of High-Value Marine Fishes* (Main, K.L. & Rosenfeld, C. eds), pp. 185-205. Oceanic Institute, Honolulu, HI, USA.

Webster, C.D., Tiu, L.G., Morgan, A.M. & Gannam, A. (1999) Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone chrysops x M. saxatilis* fed practical diets. *J. World Aquac. Soc.*, 30, 443-453.

Weirich, C. & Riche, M. (2006) Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia and nitrite at various salinities. *Aquacult. Res.*, 855-861.

Williams, T. (2008) An Assessment of Alternative Feed Ingredients in Practical Diets for Florida Pompano (*Trachinotus carolinus*) Held in Low Salinity Recirculating Systems. Master's thesis.The University of Maine Orono.

Wilson R.P. (1989) Protein and amino acid requirements of fishes. In: Progress in Fish Nutrition (Shiau, S.ed.) pp.51 – 76. National Taiwan Ocean University, Keelung, Taiwan.

Yigit, M., Erdem, M., Koshio, S., Ergun, S., Turker, A. & Karaali, B. (2006) Substituting fish meal with poultry by-product meal in diets for black Sea turbot *Psetta maeotica*. *Aquacult. Nutr.*, 12, 340-347.

Zhang, S., Xie, S., Zhu, X., Lei, W., Yang, Y. & Zhao, M. (2006) Meat and bone meal replacement in diets for juvenile gibel carp (*Carassius auratus gibelio*): effects on growth performance, phosphorus and nitrogen loading. *Aquacult. Nutr.*, 12, 353-362.

TABLES

Table 10. Formulation of experimental diets (g/100g as is basis) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (8 weeks).

	Experiment 1				Experiment 2			
Ingredients	FM15	FM10	FM05	FM0	M0.6	M0.7	M0.8	M0.9
Fishmeal[1]	15.0	10.0	5.00	0.00	-	-	-	-
Meat and bone meal blend [2]	0.0	4.9	9.80	14.70	14.00	14.00	14.00	14.00
Soybean meal[3]	50.0	50.0	50.00	50.00	41.20	41.20	41.20	41.20
Menhaden fish oil[1]	5.30	5.25	5.20	5.15	5.20	5.20	5.20	5.20
Corn starch[4]	5.65	5.56	5.47	5.38	-	-	-	-
Whole wheat[4]	16.00	16.00	16.00	16.00	27.50	27.50	27.50	27.50
Corn gluten meal[5]	5.00	5.00	5.00	5.00	1.00	1.00	1.00	1.00
Lecithin (soy refined)[4]	1.00	1.00	1.00	1.00	1.50	1.50	1.50	1.50
ASA mineral premix[6]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA vitamin premix[7]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride[4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 250 mg/kg[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
$CaPO_4$ [4]	1.00	1.20	1.40	1.60	1.50	1.50	1.50	1.50
Gelatin[4]	-	-	-	-	6.00	6.00	6.00	6.00
Glutamic acid[9]	-	-	-	-	0.30	0.20	0.10	0.00
Taurine[9]	-	-	-	-	0.75	0.75	0.75	0.75
DL-methionine[9]	0.00	0.04	0.08	0.12	0.00	0.10	0.20	0.30
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein[10]	40.1	40.1	40.0	40.0	40.1	40.1	40.1	40.1
(Crude protein)[11]	(36.3)	(37.4)	(37.1)	(35.2)	(39.1)	(39.3)	(40.2)	(40.9)
Lipid[10]	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Gross energy (kcal/100g)[11]	487.7	482.2	482.7	487.4	493.1	489.9	479.5	512.9
Digestible protein[11]	31.8	-	-	27.4	-	-	-	-
Digestible energy (kcal/100g)[11]	350.0	-	-	301.6	-	-	-	-

FM = Fishmeal; M = Methionine.

[1] Omega Protein Inc., Reedville, Virginia, USA.

[2] Meat and bone meal with blood, 65 RDB, Mid-South Milling Company, Inc., Memphis, Tennessee, USA.

[3] De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Alabama, USA.

[4] MP Biochemicals Inc., Solon, Ohio, USA.

[5] Grain Processing Corporation, Muscatine, IA, USA.

[6] ASA Premix (g $100g^{-1}$ premix) : cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250, ferrous sulfate heptahydrate, 4.0, manganous sulfate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193, and α cellulose 81.826.

[7] ASA Premix (g/kg Premix): thiamin HCL, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU g^{-1}), 2.40; vitamin D_3 (400,000 IU g^{-1}), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.

[8] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[9] Aldrich-Sigma, St. Louis, MO.

[10] Calculated.

[11] Analyzed.

Table 11. Proximate analysis and amino acid composition of major dietary ingredients (fishmeal, FM; meat and bone meal blend, MBM; soybean meal, SBM; and whole wheat, WW) used in the diets (g/100g as is basis).

Ingredients	FM[1]	MBM[1]	SBM[1]	WW[2]
Proximate analysis				
Dry matter	90.34	94.63	86.58	88.50
Crude protein	62.80	66.50	47.60	15.80
Crude fat	9.47	7.85	1.62	1.59
Fiber	0.31	0.88	2.70	2.78
Ash	20.19	18.85	5.94	1.53
Amino acids				
Methionine	1.75	0.97	0.82	0.21
Cystine	0.54	0.75	0.88	0.27
Lysine	4.91	4.50	2.81	0.36
Phenylalanine	2.38	2.88	2.16	0.63
Leucine	4.32	5.38	3.36	0.89
Isoleucine	2.36	1.36	1.86	0.51
Threonine	2.26	2.23	1.56	0.37
Valine	2.71	3.19	1.78	0.59
Histidine	1.32	2.11	1.16	0.30
Arginine	3.87	3.88	3.26	0.64
Glycine	5.07	7.78	1.89	-
Aspartic acid	6.63	5.76	6.55	-
Serine	1.94	2.47	1.95	-
Glutamic acid	8.78	7.42	9.64	-
Proline	3.32	4.58	2.36	-
Hydroxyproline	0.89	2.12	0.02	-
Alanine	3.78	4.74	1.72	-
Tyrosine	2.08	1.83	1.49	0.43
Taurine[3]	0.32	-	-	-
Total essential amino acids[4]	*25.88*	*26.50*	*18.77*	*4.50*

[1] New Jersey Feed Laboratory, Inc. 1686 Fifth Street Trenton, NJ 08638.
[2] NRC 1993.
[3] NRC 2006.
[4] Except Tryptophan

Table 12. Amino acid composition of experimental diets (40% crude protein) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (10 weeks) and experiment 2 (8 weeks), and of the 40% crude protein pompano muscle (g/100g dry matter basis).

	Experiment 1				Experiment 2				*Fish*
Amino Acid[1]	FM15	FM10	FM05	FM0	M0.6	M0.7	M0.8	M0.9	*Muscle*[2]
Methionine	0.83	0.84	0.83	0.84	0.67	0.75	0.83	0.95	*0.85*
Cystine	0.65	0.67	0.66	0.68	0.62	0.60	0.58	0.59	*0.78*
Lysine	2.34	2.35	2.27	2.26	2.11	2.02	1.97	2.00	*2.57*
Phenylalanine	1.80	1.86	1.83	1.87	1.67	1.60	1.57	1.58	*0.96*
Leucine	3.10	3.20	3.17	3.23	2.76	2.65	2.58	2.61	*2.51*
Isoleucine	1.55	1.52	1.43	1.39	1.25	1.20	1.17	1.18	*1.61*
Threonine	1.33	1.35	1.31	1.29	1.20	1.15	1.12	1.13	*1.37*
Valine	1.61	1.65	1.64	1.66	1.52	1.46	1.43	1.45	*1.99*
Histidine	0.93	0.98	1.00	1.04	0.96	0.93	0.90	0.91	*0.65*
Arginine	2.51	2.55	2.48	2.49	2.31	2.22	2.16	2.19	*1.41*
Tyrosine	1.34	1.35	1.30	1.29	1.13	1.09	1.06	1.08	*0.49*
Taurine	0.05	0.03	0.02	0.00	0.83	0.80	0.78	0.79	*ND*

ND = not determined; FM = Fishmeal; M = Methionine

[1] Calculated from NRC 2006.

[2] Calculated from a 60% crude protein Florida pompano muscle (Gothreaux *et al.*, 2010).

Table 13. Water quality parameters during experiment 1 (10 weeks) and experiment 2 (8 weeks).

Parameter	Experiment 1 Mean (± SD)	Experiment 2 Mean (± SD)
Temperature (°C)	27.9 (1.5)	28.8 (6.5)
Salinity g/L	16.5 (2.5)	24.4 (2.8)
Dissolved oxygen (mg/L)	6.8 (1.1)	6.5 (0.9)
pH	7.7 (0.2)	7.7 (0.2)
Total ammonia nitrogen (mg/L)	0.1 (0.1)	0.3 (0.3)

Table 14. Apparent digestibility coefficients for dry matter, crude protein and gross energy of diets with 50% soybean meal, 15% fishmeal (FM15) or 14.7% meat and bone meal blend (FM0), fed to juveniles (2.99 ± 0.28 g mean initial weight) Florida pompano *T. carolinus* L. (Experiment 1).

Variable	FM15	FM0	P - value	PSE[1]
Dry matter (%)	60.8[a]	48.4[b]	0.0017	1.1726
Crude protein (%)	87.6[a]	77.7[b]	0.0022	1.0018
Gross energy (%)	71.8[a]	61.9[b]	0.0048	1.2373

FM = Fishmeal

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different ($P<0.05$).

Table 15. Performance and nutrient retention of juveniles (2.99 ± 0.28 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean meal and graded levels of fishmeal (15 – 0%) and meat and bone meal blend (0 – 14.7%) after 10 weeks of feeding (Experiment 1).

Variable	FM15	FM10	FM05	FM0	P - value	PSE[1]
Final weight (g)	10.78	12.33	12.61	9.53	0.0798	0.7912
Weight gain (%)	293.6	300.2	298.6	220.1	0.0639	20.3349
Average daily gain (g/fish/day)	0.11	0.13	0.13	0.09	0.0718	0.0107
Food conversion ratio	2.32[b]	2.30[b]	2.25[b]	2.96[a]	0.0183	0.1357
Daily protein gain (g/fish/day)	0.01	0.02	0.02	0.01	0.1816	0.0015
Daily energy gain (kcal/fish/day)	0.16	0.17	0.18	0.12	0.1107	0.0155
Survival (%)	97.8[a]	97.8[a]	91.1[ab]	84.5[b]	0.0079	2.2225
Nutrient Retention						
Protein retention efficiency (%)	16.5	16.1	15.6	13.7	0.4378	1.2196
Energy retention efficiency (%)	14.3	13.4	14.2	11.2	0.1778	0.9906

FM = Fishmeal

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

Table 16. Proximate composition of whole body of juveniles (2.99 ± 0.28 g mean initial weight) Florida pompano *T. carolinus* L. fed diets with 50% soybean meal and graded levels of fishmeal (15 – 0%) and meat and bone meal blend (0 – 14.7%) after 10 weeks of feeding (Experiment 1).

Variable	Initial	FM15	FM10	FM05	FM0	P - value	PSE[1]
Dry matter (%)	25.0	25.9	23.7	25.4	24.8	0.5218	1.0406
Crude protein (%)	46.4	48.7	49.6	48.7	49.5	0.7011	0.7155
Gross energy (kcal/100g)	546.9	564.2	541.5	570.1	559.2	0.3471	10.9126

FM = Fishmeal

[1]Pooled standard error of treatment means (n=3).

Table 17. Performance and nutrient retention of juveniles (14.39 ± 0.58 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 41.2% soybean meal, 14% meat and bone meal blend and supplemental methionine after 8 weeks of feeding (Experiment 2).

Performance	M0.6	M0.7	M0.8	M0.9	P - value	PSE[1]
Final weight (g)	70.28	63.55	51.60	52.27	0.0521	4.5420
Weight gain (%)	380.1	327.8	270.8	267.2	0.0531	26.8986
Average daily gain (g/fish/day)	0.99	0.87	0.67	0.68	0.0536	0.0777
Food conversion ratio	2.09	2.35	2.90	2.93	0.0839	0.2319
Daily protein gain (g/fish/day)	0.74	0.76	0.66	0.72	0.4985	0.0471
Daily energy gain (kcal/fish/day)	2.01[a]	1.53[ab]	1.14[ab]	1.07[b]	0.0092	0.1542
Survival (%)	76.0	89.3	80.0	82.7	0.5781	6.6999
Nutrient Retention						
Protein retention efficiency (%)	20.3	18.7	16.9	15.3	0.3127	1.8369
Energy retention efficiency (%)	21.5[a]	16.0[b]	14.5[b]	11.0[b]	0.0085	1.5361

M = Methionine

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

Table 18. Proximate composition of whole body of juveniles (14.39 ± 0.58 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets with 41.2% soybean meal, 14% meat and bone meal blend and supplemental methionine after 8 weeks of feeding (Experiment 2).

Variable	Initial	M0.6	M0.7	M0.8	M0.9	P - value	PSE[1]
Dry matter (%)	28.4	32.4	30.2	29.9	27.2	0.0547	1.0859
Crude protein (%)	40.8	46.1	51.1	51.7	54.2	0.4116	3.2649
Gross energy (kcal/100g)	616.1	620.3	589.8	584.5	554.2	0.0825	15.0638

M = Methionine

[1] Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

CHAPTER IV

EVALUATION OF THE SUPPLEMENTATION OF TAURINE TO FISHMEAL-FREE DIETS FOR FLORIDA POMPANO *Trachinotus carolinus* L.

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is an organic acid that has a variety of functions including constituent of bile, osmoregulation, cell membrane stabilization, antioxidation, and early development of visual, muscular and neural systems (Huxtable 1992). Taurine is not a classical amino acid, since it lacks a carboxyl group and has not been found within any known protein structure but is often classified as an amino acid. It is considered the most abundant free amino acid in animal tissues, accounting for the following percentages of the free amino acid pool: 2.8% in plasma, 25% in liver, 50% in kidney, 53% in muscle and 19% in brain (Brosnan & Brosnan 2006).

Seafood is the primary source of taurine for human nutrition. In crustaceans and molluses, taurine may range from 300-800 mg per 100g of edible portion and overall seafood contains between 0.5 to 1.0% taurine (Zhao *et al.* 1998). Taurine content in aquatic animals is considerably high relative to that of terrestrial animals and plants, which may explain why some fish species appear to have limited abilities to biosynthesize taurine (Yokoyama *et al.* 2001; Goto *et al.* 2003). Limited ability of taurine biosynthesis in fish is presumably due to the low activity of key enzymes on the pathway for taurine synthesis.

In mammals, taurine is biosynthesized from methionine through a metabolic pathway that involves the transformation of methionine to cystathionine by cystathionine synthetase followed by the transformation of cystathionine to cysteine by cystathionase, the oxidation of cysteine to cysteine sulphinate, and the decarboxylation of cysteine sulphinate to hypotaurine (Worden & Stipanuk 1985). The $_L$-cysteinesulphinate decarboxylase (CSD) is a key enzyme in this pathway and its activity has been observed to vary among fish species. Although this physiological ability for taurine biosynthesis from methionine has been observed in rainbow trout, CSD activity has been found to be low in species such as Japanese flounder *Paralichthys olivaceous*, red sea bream *Pagrus major*, yellowtail *Seriola quinqueratiata* and bluefin tuna (Yokoyama *et al.* 2001). In addition, the activity of the cysteamine dioxygenase (CAO), a key enzyme of a secondary pathway for taurine synthesis was found to vary markedly between fish species (Goto *et al.* 2003).

A limited number of taurine requirement studies in fish have been reported and positive responses to supplemental taurine have been observed in Japanese flounder *P. olivaceous* (Park *et al.* 2002; Kim *et al.* 2005a; Kim *et al.* 2005b), yellowtail *S. quinqueratiata* (Matsunari *et al.* 2005; Takagi *et al.* 2006a; Takagi *et al.* 2008), cobia *Rachycentron canadum* (Lunger *et al.* 2007), and red sea bream *P. major* (Takagi *et al.* 2006b), suggesting that taurine may be dietary essential. Notably, the highest positive responses to supplemental taurine were observed in studies in which dietary fishmeal, a source abundant in taurine, was partially or totally replaced by vegetable protein sources.

There is increasing interest on the replacement of fishmeal in aquaculture diets with alternative proteins. Soybean meal has a good amino acid profile with the exception of the low levels of methionine, lysine and threonine, and the absence of taurine. Hence, supplementation of taurine in diets containing low levels of marine ingredients and high levels of plant proteins may be necessary.

Studies conducted with Florida pompano have shown that this species grow well when fed diets in which fishmeal has been partially replaced by plant-based ingredients such as soybean products (Lazo *et al.* 1998; Riche 2009; Riche & Williams 2011). Our previous studies utilizing diets with 50% soybean meal in combination with poultry-by-product meal or a meat and bone meal blend demonstrated that dietary fishmeal could be reduced from 15 to 5%. Nevertheless, to date, attempts to use complete fishmeal-free diets for pompano have impaired fish performance.

The present study was conducted to answer the hypothesis that pompano might respond to supplemental taurine as observed in other marine finfishes particularly when fed diets lacking fishmeal. Therefore, graded levels of taurine was applied to fishmeal-free diets containing soybean meal and poultry by-product meal or meat and bone meal blend as the major dietary protein sources.

MATERIAL AND METHODS

Fish

Juvenile pompano, 2.63 g mean weight, obtained from Harbor Branch Oceanographic Institute, Fort Pierce, FL were transported to the Alabama Department of Conservation and Natural Resources Marine Resource Division, Claude Peteet Mariculture Center (CPMC), located in Gulf Shores, Alabama.

At CPMC, fish were acclimated to the facilities' water parameters by slowly replacing the hauling water with local water (31.59 g/L salinity, 6.60 mg/L dissolved oxygen and 7.51 pH) and subsequently stocked into a 5 m^3 fiberglass recirculating tank equipped with independent biological filter, air lift pumps and supplemental aeration provided by a regenerative air blower and air diffusers. Fish remained in this tank acclimating to local conditions until having grown to adequate size for use in the growth trials. During this period fish were fed to apparent satiation with a 40% crude protein and 9% crude fat commercial diet (EXTR 400, Rangen, Inc).

Diets

Experimental diets were manufactured at Auburn University, Department of Fisheries and Allied Aquacultures, Auburn, Al, USA. They were prepared by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer and grinder (Hobart A200FT, Troy, OH, USA) for 20 minutes. Boiling water was blended into the mixture to promote appropriate consistency for pelleting. Subsequently, the moist mash for each diet was passed through a 3.0 mm die in the grinder. Wet diets were then placed into a forced air drying oven (<45 °C) for approximately 24 h resulting in a moisture content of less than 10%. Dry diets were stored at -20 °C, and prior to use each diet was crumbled and sieved to an appropriate size. All experimental diets were formulated to be isonitrogenous and isolipidic, containing 40% crude protein and 8.0% lipids, using 50% soybean meal as the major protein source.

Experiment 1. Taurine supplementation to fishmeal-free diets based on poultry by-product meal

In experiment 1, in order to evaluate the effects of taurine supplementation to fishmeal-free diets on the nutrition performance and body composition of juvenile pompano, four diets were designed to contain graded levels of taurine (Table 19). Soybean meal (50% of the diet) was the major dietary protein source and poultry by-product meal (P) (14.27% of the diet) was the sole dietary animal protein, contributing with 59.50% and 23.54% of dietary crude protein, respectively.

The basal diet (PT0) was not supplemented with taurine (T) and served as the control. With progressive supplementation, the diets PT25, PT50 and PT75 contained taurine at 0.25, 0.50 and 0.75%, respectively. With exception of taurine, other ingredient levels were maintained constant.

Experiment 2. Taurine supplementation to fishmeal-free diets based on meat and bone meal blend

In experiment 2, poultry by-product meal was replaced with meat and bone meal blend (Table 19). All diets had 50% soybean meal along with 14.16% meat and bone meal blend (M), contributing with 83.04% of dietary crude protein (59.5% and 23.54%, respectively). The same dietary levels of supplemental taurine (T) (0, 0.25, 0.50 and 0.75%) used in experiment 1 were employed in the diets MT0, MT25, MT50 and MT75 in the experiment 2.

Experimental systems and husbandry

The experiments were conducted in semi-recirculating systems, each comprised of 12 circular fiberglass culture tanks, a reservoir tank, biological filter, water pump and supplemental aeration supplied by a regenerative blower and air diffusers. The culture tanks used for experiment 1 and 2 were different volume, with 0.9 m^3 and 0.7m^3, respectively. The experimental systems are enclosed by a greenhouse which provides a natural 14h L/10h D cycle. Ventilation was provided to reduce water temperatures during the summer. Routine systems maintenance consisted of partial water exchanges and of siphoning of solids as necessary.

Water quality parameters were monitored routinely. Dissolved oxygen, temperature, salinity and pH were monitored twice daily using an YSI 556 multi probe meter (Yellow Spring Instruments Co., Yellow Springs, OH, USA). Total ammonia nitrogen was analyzed on a weekly basis using an ion selective electrode (Orion EA 940, Thermo Electron Corporation, Beverly - MA, USA).

Before the commencement of the growth trials, fish were manually graded to reduce size variations. Size sorted fish were stocked to culture tanks of experiment 1(mean weight 6.25 ± 0.22 g) and 2 (mean weight 5.87 ± 0.20 g) at a density of 25 (28 fish/m^3) and 15 (21 fish/m^3) fish per tank, respectively. Both experiments initiated concomitantly. Fish were counted and weighed in groups of 15 and 25, then given a two minutes prophylactic treatment against *Amyloodinium ocellateum*, which consisted on the immersion of fish groups into a chloroquine phosphate (Marex, Aquatronics, Oxnard, CA) solution at 21.1 mg/ L followed by a fresh water bath. This prophylactic treatment was employed every time fish were handled.

Fish in each experimental tank were fed three times daily one of the randomly assigned experimental diets. An initial daily feed input was set at 8% of fish's biomass and was reduced as the biomass in the tanks increased, reaching the 6% of fish's biomass at the end of experimental period. Three replicates were used per treatment. Experimental fish were sampled every two weeks for growth and survival evaluations and for feed input readjustment.

Data acquisition and analytical Procedures

The following parameters were used to evaluate fish performance in each of the experiments:

- Weight gain (%) = [(final body weight – initial body weight)/ initial body weight) × 100]
- Average Daily Gain (g/fish/day) = (weight gain/days of experiment)
- Food conversion ratio; FCR = (as is feed intake/wet weight gain)
- Daily protein gain (g/fish/day) = [(final body weight × final body protein) – (initial body weight × initial crude protein)]/days of the experiment
- Daily energy gain (kcal/fish/day) = [(final body weight × final body energy) – (initial body weight × initial body energy)]/days of the experiment.
- Protein retention efficiency (%); PR = [(final body weight × final body protein) – (initial body weight × initial body protein)]/total protein intake (g)] × 100
- Energy retention efficiency (%); ER = [(final body weight × final body energy) – (initial body weight × initial body energy)]/total energy intake (g) × 100

At the end of experimental period, after final sampling to complete experimental data collection, all experimental fish were returned to their respective experimental tanks and allowed to recover for 5 days under normal water quality and feeding conditions. Subsequently, at day 6, after morning feeding, five fish from each experimental tank were euthanized with 200 mg/ L of tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA) and bled via caudal venipuncture for measurement of postprandial serum taurine. The serum was separated from the other blood components by centrifugation in a 3300 rpm centrifuge (Fisher Scientific Centrific, Model 228) and kept frozen (- 45 °C) until amino acid analyzes.

Other four fish from each experimental tank were euthanized and subsequently homogenized in a food processor and frozen at – 60 °C for proximate analyses. Dry matter was determined by placing representative portions of each sample in an oven at 105 °C until constant weight be obtained. Protein content of whole fish body and experimental diets was determined by the micro-Kjeldahl method (Ma & Zuazaga 1942) and Gross energy was analyzed with a semimicro - bomb calorimeter (Model 1425, Parr Instrument Co. Moline, IL, USA).

Statistical analyses

All data were analyzed using one-way analysis of variance to determine significant ($P<0.05$) differences among treatment means. When significant differences were identified the Student – Neuman Keul's multiple –range test was used to determine significant differences between treatment means. Linear regression analysis was used to evaluate the relation between independent and dependent variables for select data. The Statistical analyses were performed using the SAS® software package (SAS Institute Inc., Cary, NC USA).

RESULTS

Florida pompano adapted well to the experimental conditions and accepted both commercial and experimental diets with no noticeable palatability problems. The prophylactic preventive treatments were effective in preventing disease outbreaks. Water quality parameters for the experiments 1 and 2 (Table 22) were within adequate ranges for pompano (Watanabe 1995; Weirich & Riche 2006) during the experimental period. Dissolved oxygen values were maintained consistently near saturation levels and the salinity was continuously near 30 g/L.

Experiment 1. Taurine supplementation to fishmeal-free diets based on poultry by-product meal

Analyzed crude protein values of experimental diets were very similar among diets ranging from 39.2% to 39.8% and did not differ from the formulated values of 40% crude protein (Table 19). The proximate analysis and amino acid composition of major dietary ingredients are reported in Table 20, and the amino acid composition of experimental diets and the 40% crude protein pompano muscle is presented in Table 21.

Taurine levels were analyzed for all experimental diets and were equivalent to calculated values in the formulations (Table 21). Slightly different values were observed among analyzed amino acid composition of diet 1 (PT0) and calculated values for the other experimental diets (PT25, PT50 and PT75). Compared to pompano muscle concentration, dietary lysine was lowest in the PT0 diet and valine the least abundant in the PT25, PT50 and PT75 diets. Dietary gross energy values ranged from 475.0 to 479.0 kcal/100g and were considered similar among experimental diets.

Taurine additions to the basal diet had a significant impact on pompano performance (Table 23). At the highest levels of taurine supplementation (0.75%, PT75), pompano performance was improved relative to the diet without supplementation. Fish fed the PT75 diet had significant higher final weight (55.44g), weight gain (773.3 %), and average daily gain (0.86 g/fish/day) compared to fish fed the fishmeal-free diet without supplemental taurine (PT0) (final weight = 41.49g, weight gain = 587.7%, and average daily gain = 0.62g/fish/day). Daily energy gain, FCR, PR, ER, and survival did not differ among treatments according to the analysis of variance. However, the linear regression analysis indicated a significant negative effect of supplemental taurine on FCR. The regression analysis also indicated a significant positive effect of supplemental taurine on nutrient retention (protein and energy). Pompano final weight positively responded to increasing levels of taurine but tended to a plateau between the 0.5 and 0.75% of taurine in the diet (Figure 3).

Taurine concentration in the fishmeal-free diets in experiment 1 did not cause significant differences in whole pompano composition (Table 24) and serum osmolality (Table 25). Dry matter ranged from 30.8% to 31.9% while crude protein and gross energy ranged from 46.3 to 49.2% and from 586.9 to 618.4 kcal/100g, respectively. Serum osmolality values ranged from 308.5 to 363.8 mmol/kg and did not differ among treatments.

Experiment 2. Taurine supplementation to fishmeal-free diets based on meat and bone meal blend

Analyzed protein values for the test diets ranged from 38.0% to 38.9% and were consistent but slightly lower than the formulated value of 40% crude protein (Table 19). The calculated amino acid composition of experimental diets and of the 40% pompano muscle are presented in Table 21. As observed in experiment 1, the analyzed taurine values were very similar compared with those calculated in the formulations for all experimental diets. In reference to the amino acid levels in the pompano muscle, methionine was the least abundant amino acid in the MT0 diet and valine in the other experimental diets. Lysine was the second lowest amino acid for all experimental diets.

Pompano performance was significantly affected by the addition of taurine to experimental diets (Table 26). Fish fed diets containing at least 0.25 g of taurine per 100 g of diet (MT25, MT50 and MT75) had similar performance. Fish fed the MT0 diet, with no supplemental taurine, had significantly reduced performance as compared to those offered the highest level. Significant lowest final weight (28.24 g), weight gain (386.7%), average daily gain (0.27 g/fish/day), daily protein gain (0.37 g/fish/day), daily energy gain (0.35 kcal/fish/day), PR (8.9%), ER (7.4%), and survival (37.8%) were observed in fish fed the MT0 diet. The FCR was significant higher in fish fed the MT0 diet compared to fish fed the other experimental diets. Regression analysis indicated a significant positive effect of supplemental taurine on final weight, weigh gain, PR and ER.

The dose response curve presented in Figure 5 demonstrates that pompano final weight increased positively with dietary taurine levels. Fish performance was stronger from the unsupplemented diet (MT0) to the first supplemental taurine level (0.25%, MT25) and lessened when higher supplemental levels were reached [0.5 (MT50) and 0.75% (MT75)].

Overall, the supplementation of taurine to experimental diets did not appear to cause major effects on whole body proximate composition (Table 27). Dry matter values ranged from 24.6 to 28.9% and crude protein ranged from 46.5 to 49.1%, with no significant differences among treatments. However, lower gross energy (538.7 kcal/100 g) was observed in pompano fed the MT0 diet while no differences were observed for gross energy in fish fed the other experimental diets ($P<0.05$). The serum osmolality was not affected by supplemental taurine in the diets (Table 25). Serum osmolality values did not differ among treatments and were fairly similar to those of experiment 1 ranging from

318.2 to 329.3 mmol/kg. However, postprandial serum taurine ranged from nearly zero to approximately 250.0 µg/ ml and was directly related with dietary taurine levels (Figure 4).

DISCUSSION

Pompano as in other marine finfishes may have a limited ability to biosynthesize taurine and thus must rely on dietary sources. Fishmeal is a natural source of taurine, yet, the use of fishmeal in aquatic feeds is expected to progressively decline (Tacon & Metian 2008). Consequently, the supplementation of this amino acid may be necessary when diet ingredients lack taurine.

Previous studies in this lab utilizing poultry by-product meal and a meat and bone meal blend in soybean meal based diets for pompano have successfully reduced dietary fishmeal to as low as 5% (7.85% of dietary crude protein), however, the complete replacement was not possible without impairing fish performance. Subsequent studies indicated that the depressions in performance were not related with limiting levels of dietary methionine and lysine but could be due to taurine limitations.

Fishmeal is considered a taurine rich feed ingredient while terrestrial protein sources such as soybean meal, meat and bone meal products and poultry by-product meal either lack or have very low levels of taurine (NRC 2006). Consequently, as fishmeal is replaced taurine may become a limiting nutrient.

Taurine supplementation to soybean meal based diets with either poultry by-product meal or meat and bone meal blend as the sole animal protein sources did not affect serum osmolality, or dry matter, protein and energy content in pompano. Taurine supplementation to fishmeal-free diets for yellowtail *S. quinqueradiata* increased serum osmolality at levels equivalent to those of a 58% fishmeal diet (Takagi et al., 2008). Conversely, in the present study, dietary taurine did not affect serum osmolality even at the highest supplementation level (0.75%). However, serum taurine levels ranged from nearly zero to approximately 250.0 μg/ml and were directly related to levels of dietary taurine. Similar results were observed when taurine was supplemented to diets for yellowtail *S. quinqueratiata* and cobia *R. canadum* in the studies conducted by Takagi *et al.* (2008) and Lunger *et al.* (2007), respectively.

The supplementation of taurine to fishmeal-free diets in this study resulted in improved pompano performance. Fish weight gain increased from 587.7% in fish fed the diet without taurine (PT0) to 773.3% when fish were fed the PT75 diet containing 0.75% of supplemental taurine in experiment 1. Under the same dietary taurine levels, in experiment 2 pompano weight gain increased from 386.7% to 1198.0% in fish fed the MT0 and MT75 diets, respectively. This improvement in performance was coupled with a considerable decline in FCR, which indicates a higher feed efficiency of fish fed the diets with supplemental taurine. The FCR declined from 3.76 to 2.01 in fish fed the diets MT0 and MT75, respectively, and was negative correlated with supplemental taurine in both experiments.

The lower survival observed in fish fed the MT0 diet in experiment 2 probably resulted from an almost complete absence of taurine in this diet. Significantly lower survival rates of pompano fed fishmeal-free diets replaced with meat and bone meal blend were observed in previous studies in this lab. Low survival rates were also observed in studies with yellowtail *S. quinqueradiata* (Takagi *et al.* 2006a; Takagi *et al.* 2008) when fish were fed non-fishmeal diets unsupplemented with taurine. In experiment

2, chronic pompano mortality was observed in all tanks during the entire experimental period however; mortality was much greater in fish fed the MT0 diet. Since diet formulation was equivalent for both experiments, the higher survival rates obtained in experiment 1 are probably related with the higher taurine content in the PT0 diet containing poultry by-product meal. Low levels of taurine can be found in poultry by-product meal (NRC 2006) but is hardly found in meat and bone meal products.

In general, the performance of fish fed diets supplemented with taurine in this study appears satisfactory relative to that of other studies in which fishmeal was employed in the diets. Riche (2009) observed increases of 675% in weight gain and 0.51g/day in average daily gain of pompano juveniles (6.3 ± 0.50 g mean initial weight) fed a 50% crude protein diet containing fishmeal (35.4%) and soybean meal (23.4%) for 10 weeks. In a seven weeks experiment, Lazo *et al.* (1998) observed a 482% (21.9 g) increase in weight gain of pompano (4.54 g mean initial weight) fed a 40% crude protein diet containing fishmeal and soybean meal at 26.7 and 45%, respectively. Albeit these studies may not be directly compared, it appears that with proper taurine supplementation, pompano can accept and utilize fishmeal-free diets efficiently.

In the fishmeal replacement study conducted by Riche & Williams (2011), pompano growth performance and survival were drastically depressed when dietary fishmeal levels were below 8.98% (>40% fishmeal replacement) and 6.25% (>80% fishmeal replacement), replaced with soy protein isolate and soybean meal, respectively. In general, these negative responses to high dietary levels of soy-based ingredients in pompano studies are attributed to differences in diet palatability, presence of antinutritional factors and/or a deficiency in one or more amino acids. However, in most cases, dietary taurine was either not reported or not taken into consideration as a possible cause for the negative responses to low-fishmeal, high-vegetable based diets.

A limited number of studies on taurine requirement exist, and positive responses to supplemental taurine have been observed in Japanese flounder *P. olivaceous* (Park *et al.* 2002; Kim *et al.* 2005a; Kim *et al.* 2005b), yellowtail *S.quinqueratiata* (Matsunari *et al.* 2005; Takagi *et al.* 2006a; Takagi *et al.* 2008), cobia *R. canadum* (Lunger *et al.* 2007), and red sea bream *P. major* (Takagi *et al.* 2006b).

Park *et al.* (2002) suggested that 1.4% of taurine was required for optimum growth of juvenile Japanese flounder. Accordingly, the supplementation of taurine at 1.0% to diets containing over 60% fishmeal improved growth and feed efficiency of Japanese flounder and 1.5% dietary taurine was recommended (Kim *et al.* 2005a). In a subsequent study Kim *et al.* (2008) observed a significant improvement in the performance of Japanese flounder when taurine comprised 1.6% of the diet (1.5% from supplemental taurine).

Matsunari *et al.* (2005) observed an improvement in growth of yellowtail when dietary taurine level was above 1.0% compared to the lowest dietary level (0.39%). When fishmeal-free diets were employed for this species, a positive response was observed when dietary taurine levels were beyond 3.0% (Takagi *et al.* 2006a) and in a further study, these authors recommended 4.5% of supplemental taurine to fishmeal-free diets based on soy protein concentrate for optimum growth of yellowtail (Takagi *et al.* 2008). The utilization of 0.5% of dietary taurine in low fishmeal diets for cobia resulted in improved growth and feed efficiency compared to the unsupplemented diets (Lunger *et al.* 2007), and no more than 0.5% supplemental taurine was required for optimum growth of red sea bream fed fishmeal-free diets based on soy protein concentrate (Takagi *et al.* 2006b). In this study, improvement in pompano performance fed diets in which poultry by-product meal and meat and bone meal blend were used to replace fishmeal was observed with dietary taurine level as low as 0.25%.

The variation in taurine requirement among different species was found to be related with different abilities of taurine biosynthesis. Accordingly, the activity of the L-cysteinesulphinate decarboxylase (CSD), a key enzyme in the major pathway for taurine synthesis, was analyzed for most of the aforementioned species and was observed to vary considerably (Yokoyama *et al.* 2001). The main pathway for taurine synthesis involves the oxygenation of cysteine to cysteinesulphinate followed by the decarboxylation (mediated by CSD) to hypotaurine and then to taurine (Griffith 1987). A secondary pathway for taurine synthesis involves cysteamine dioxygenase; however, the activity of this enzyme in fish also varies considerably (Goto *et al.* 2003). Therefore, the positive responses to dietary taurine in some fishes appear to be strongly linked to differences in activity of key enzymes.

Results from this study indicated that Florida pompano have a requirement for taurine. Although no significant differences in performance were found when dietary taurine level was 0.25% or higher, it appeared that pompano growth was optimized at higher taurine levels (0.5 and 0.75%). Therefore, further investigation on biosynthesis, dietary requirement and physiological roles of taurine in Florida pompano is necessary.

REFERENCES

Brosnan, J.T. & Brosnan, M.E. (2006) The sulfur-containing amino acids: An overview. *J. Nutr.*, 136, 1636S-1640S.

Gothreaux, C.T., Reigh, R.C., Williams, M.B. & Chesney, E.J. (2010) Amino Acid Availability of Soybean Meal for Florida Pompano. *N. Am. J. Aquacult.,* 72, 309-313.

Goto, T., Matsumoto, T., Murakami, S., Takagi, S. & Hasumi, F. (2003) Conversion of cysteate into taurine in liver of fish. *Fish. Sci.,* 69, 216-218.

Griffith, O.W. (1987) Mammalian sulfur amino-acid metabolism - an overview. *Methods in Enzymology,* 143, 366-376.

Huxtable, R.J. (1992) Physiological actions of taurine. *Physiol. Rev.,* 72, 101-163.

Kim, S.K., Matsunari, H., Takeuchi, T., Yokoyama, M., Furuita, H., Murata, Y. & Goto, T. (2008) Comparison of taurine biosynthesis ability between juveniles of Japanese flounder and common carp. *Amino Acids,* 35, 161-168.

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

Kim, S.K., Takeuchi, T., Yokoyama, M., Murata, Y., Kaneniwa, M. & Sakakura, Y. (2005b) Effect of dietary taurine levels on growth and feeding behavior of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquaculture,* 250, 765-774.

Lazo, J.P., Davis, D.A. & Arnold, C.R. (1998) The effects of dietary protein level on growth, feed efficiency and survival of juvenile Florida pompano (*Trachinotus carolinus*). *Aquaculture,* 169, 225-232.

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.

Ma, T. & Zuazaga, G. (1942) Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. *Industrial & Engineering Chemistry Analytical Edition,* 14, 280-282.

Matsunari, H., Takeuchi, T., Takahashi, M. & Mushiake, K. (2005) Effect of dietary taurine supplementation on growth performance of yellowtail juveniles *Seriola quinqueradiata*. *Fish. Sci.,* 71, 1131-1135.

NRC (Natural Research Council). (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, DC., USA, 114 p.

NRC (Natural Research Council). (2006) *Nutrient requirements of dogs and cat*. National Academy Press, Washington, DC., USA, 398 p.

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

Riche, M. & Williams, T. (2011) Fish meal replacement with solvent extracted soybean meal or soy protein isolate in a practical diet formulation for Florida pompano (*Trachinotus carolinus*, L.) reared in low salinity. *Aquacult. Nutr.*, 17: no. doi: 10.1111/j.1365-2095.2010.00808.x ...2011.

Riche, M. (2009) Evaluation of Digestible Energy and Protein for Growth and Nitrogen Retention in Juvenile Florida Pompano, *Trachinotus carolinus*. *J. World Aquac. Soc.*, 45-57.

Tacon, A. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture,* 285, 146-158.

Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H. & Ukawa, M. (2008) Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. *Aquaculture,* 280, 198-205.

Takagi, S., Murata, H., Goto, T., Hayashi, M., Hatate, H., Endo, M., Yamashita, H. & Ukawa, M. (2006a) Hemolytic suppression roles of taurine in yellowtail *Seriola quinqueradiata* fed non-fishmeal diet based on soybean protein. *Fish. Sci.,* 72, 546-555.

Takagi, S., Murata, H., Goto, T., Ichiki, T., Endo, M., Hatate, H., Yoshida, T., Sakai, T., Yamashita, H. & Ukawa, M. (2006b) Efficacy of taurine supplementation for preventing green liver syndrome and improving growth performance in yearling red sea bream *Pagrus major* fed low-fishmeal diet. *Fish. Sci.,* 72, 1191-1199.

Watanabe, W.O. (1995) Aquaculture of the Florida pompano and other jacks (Family Carangidae) in the western Atlantic, Gulf of Mexico and Caribbean basin: status and potential. In: *Culture of High-Value Marine Fishes* (Main, K.L. & Rosenfeld, C. eds), pp. 185-205. Oceanic Institute, Honolulu, HI, USA.

Weirich, C. & Riche, M. (2006) Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia and nitrite at various salinities. *Aquacult. Res*., 855-861.

Williams, T. (2008) An Assessment of Alternative Feed Ingredients in Practical Diets for Florida Pompano (*Trachinotus carolinus*) Held in Low Salinity Recirculating Systems. Master's thesis.The University of Maine Orono.

Worden, J.A. & Stipanuk, M.H. (1985) Acomparison by species, age and sex of cysteinesulfinate decarboxylase activity and taurine concentration in liver and brain of animals. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.,* 82, 233-239.

Yokoyama, M., Takeuchi, T., Park, G. & Nakazoe, J. (2001) Hepatic cysteinesulphinate decarboxylase activity in fish. *Aquacult. Res*., 32, 216-220.

Zhao, X.H., Jia, J.B. & Lin, Y. (1998) Taurine content in Chinese food and daily taurine intake of Chinese men In *Taurine 3 - Cellular and Regulatory Mechanisms* (Schaffer, S., *et al.* eds.), Vol. 442, pp. 501-505.

TABLES

Table 19. Formulation of experimental diets (g/100 g as is basis) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (8 weeks) and experiment 2 (12 weeks).

	Experiment 1				Experiment 2			
Ingredients	PT0	PT25	PT50	PT75	MT0	MT25	MT50	MT75
Poultry by product meal[1]	14.27	14.27	14.27	14.27	-	-	-	-
Meat and bone meal blend[2]	-	-	-	-	14.16	14.16	14.16	14.16
Soybean meal[3]	50.0	50.0	50.00	50.00	50.00	50.00	50.00	50.00
Menhaden fish oil[1]	5.20	5.20	5.20	5.15	5.30	5.30	5.30	5.30
Corn starch[4]	4.02	4.57	4.66	5.06	4.00	4.95	4.80	4.84
Whole wheat[4]	16.00	15.50	15.50	15.00	16.00	15.00	15.00	15.00
Corn gluten meal[5]	6.80	6.50	6.15	6.00	6.90	6.70	6.60	6.30
Lecithin (soy refined)[4]	1.00	1.00	1.00	1.00	1.50	1.50	1.50	1.50
ASA mineral premix[6]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA vitamin premix[7]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride[4]	0.20	0.2	0.2	0.2	0.20	0.20	0.20	0.20
Stay C 250 mg/kg[8]	0.10	0.1	0.1	0.1	0.10	0.10	0.10	0.10
$CaPO_4$[4]	1.60	1.60	1.60	1.60	1.00	1.00	1.00	1.00
DL-methionine[9]	0.06	0.06	0.07	0.07	0.09	0.09	0.09	0.10
Taurine[9]	0.00	0.25	0.50	0.75	0.00	0.25	0.50	0.75
Total	100	100	100	100	100	100	100	100
Crude protein[10]	40.1	40.1	40.1	40.2	40.2	40.1	40.3	40.4
(Crude protein)[11]	(39.2)	(39.4)	(39.8)	(39.7)	(38.7)	(38.0)	(38.5)	(38.9)
Lipid[10]	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Gross energy (kcal/100g)[11]	479.0	475.8	475.0	475.3	497.1	496.0	494.9	489.7

P = Poultry by-product meal; M = Meat and bone meal blend; T = Taurine

[1] Griffin Industries, Inc., Mobile, Alabama, USA.

[2] Meat and bone meal with blood, 65 RDB, Mid-South Milling Company, Inc., Memphis, Tennessee, USA.

[3] De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Alabama, USA.

[4] MP Biochemicals Inc., Solon, Ohio, USA.

[5] Grain Processing Corporation, Muscatine, IA, USA.

[6] ASA Premix (g $100g^{-1}$ premix) : cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250, ferrous sulfate heptahydrate, 4.0, manganous sulfate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193, and α cellulose 81.826.

[7] ASA Premix (g/kg Premix): thiamin HCL, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU g^{-1}), 2.40; vitamin D_3 (400,000 IU g^{-1}), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.

[8] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[9] Aldrich-Sigma, St. Louis, MO.

[10] Calculated.

[11] Analyzed.

Table 20. Proximate analysis and amino acid composition of major dietary ingredients (fishmeal, FM; meat and bone meal blend, MBM; soybean meal, SBM; and whole wheat, WW) used in the diets (g/100g as is basis).

Ingredients	FM[1]	MBM[1]	SBM[1]	WW[2]
Proximate analysis				
Dry matter	90.34	94.63	86.58	88.50
Crude protein	62.80	66.50	47.60	15.80
Crude fat	9.47	7.85	1.62	1.59
Fiber	0.31	0.88	2.70	2.78
Ash	20.19	18.85	5.94	1.53
Amino acids				
Methionine	1.75	0.97	0.82	0.21
Cystine	0.54	0.75	0.88	0.27
Lysine	4.91	4.50	2.81	0.36
Phenylalanine	2.38	2.88	2.16	0.63
Leucine	4.32	5.38	3.36	0.89
Isoleucine	2.36	1.36	1.86	0.51
Threonine	2.26	2.23	1.56	0.37
Valine	2.71	3.19	1.78	0.59
Histidine	1.32	2.11	1.16	0.30
Arginine	3.87	3.88	3.26	0.64
Glycine	5.07	7.78	1.89	-
Aspartic acid	6.63	5.76	6.55	-
Serine	1.94	2.47	1.95	-
Glutamic acid	8.78	7.42	9.64	-
Proline	3.32	4.58	2.36	-
Hydroxyproline	0.89	2.12	0.02	-
Alanine	3.78	4.74	1.72	-
Tyrosine	2.08	1.83	1.49	0.43
Taurine[3]	0.32	-	-	-
Total essential amino acids[4]	*25.88*	*26.50*	*18.77*	*4.50*

[1] New Jersey Feed Laboratory, Inc. 1686 Fifth Street Trenton, NJ 08638.
[2] NRC 1993.
[3] NRC 2006.
[4] Except Tryptophan

Table 21. Amino acid composition of experimental diets (40% crude protein) fed to juveniles Florida pompano *T. carolinus* L. in experiment 1 (8 weeks) and experiment 2 (12 weeks), and of the 40% crude protein pompano muscle (g/100g dry matter basis).

	Experiment 1				Experiment 2				*Fish*
Amino acid	PT0[1]	PT25[2]	PT50[2]	PT75[2]	MT0[1]	MT25[2]	MT50[2]	MT75[2]	*Muscle*[3]
Methionine	0.73	0.83	0.83	0.82	0.70	0.82	0.81	0.82	*0.85*
Cystine	0.69	0.69	0.68	0.68	0.71	0.69	0.71	0.69	*0.78*
Lysine	2.08	2.24	2.22	2.21	2.15	2.30	2.26	2.24	*2.57*
Phenylalanine	1.98	1.87	1.85	1.83	1.97	1.92	1.90	1.90	*0.96*
Leucine	3.28	3.34	3.28	3.26	3.30	3.39	3.36	3.33	*2.51*
Isoleucine	1.63	1.59	1.57	1.56	1.43	1.42	1.41	1.41	*1.61*
Threonine	1.66	1.36	1.35	1.34	1.58	1.37	1.34	1.33	*1.37*
Valine	1.71	1.65	1.63	1.62	1.73	1.70	1.69	1.68	*1.99*
Histidine	0.95	0.95	0.94	0.94	1.07	1.05	1.04	1.04	*0.65*
Arginine	2.56	2.62	2.60	2.59	2.76	2.51	2.49	2.49	*1.41*
Tyrosine	1.41	1.36	1.35	1.34	1.39	1.34	1.33	1.32	*0.49*
Taurine[1]	0.07	0.35	0.64	0.85	0.01	0.25	0.49	0.82	*ND*
Taurine	0.05	0.31	0.57	0.83	0.00	0.26	0.52	0.78	*ND*

ND = not determined; P = Poultry by-product meal; M = Meat and bone meal blend; T = Taurine.

[1] Analyzed.

[2] Calculated from NRC 2006.

[3] Calculated from 60% crude protein Florida pompano muscle (Gothreaux *et al.*, 2010).

Table 22. Water quality parameters during experiment 1 (8 weeks) and experiment 2 (12 weeks).

Parameter	Experiment 1 Mean (± SD)	Experiment 2 Mean (± SD)
Temperature (°C)	28.7 (1.2)	28.7 (1.5)
Salinity g/L	29.2 (2.2)	28.5 (2.1)
Dissolved oxygen (mg/L)	6.1 (1.0)	6.5 (0.9)
pH	7.6 (0.2)	7.7 (0.2)
Total ammonia nitrogen (mg/L)	0.3 (0.2)	0.3 (0.3)

Table 23. Performance of juveniles (6.25 ± 0.22 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.27% poultry by-product meal and graded levels of supplemental taurine after 8 weeks of feeding (Experiment 1).

Variable	PT0	PT25	PT50	PT75	P - value	PSE[1]
Final weight (g)	41.49[b]	49.56[ab]	49.91[ab]	55.44[a]	0.0223	2.4124
Weight gain (%)	587.7[b]	674.2[ab]	703.0[ab]	773.3[a]	0.0336	35.0105
Average daily gain (g/fish/day)	0.62[b]	0.76[ab]	0.77[ab]	0.86[a]	0.0246	0.0417
Food conversion ratio	1.86	1.72	1.70	1.59	0.0670	0.0603
Daily protein gain (g/fish/day)	0.46[b]	0.51[a]	0.53[a]	0.56[a]	0.0038	0.01270
Daily energy gain (kcal/fish/day)	1.19	1.39	1.49	1.60	0.1154	0.1060
Survival (%)	92.0	96.0	96.0	92.0	0.8018	3.9999
Nutrient Retention						
Protein retention efficiency (%)	20.2	22.3	22.5	24.1	0.0900	0.8903
Energy retention efficiency (%)	21.2	24.2	22.0	24.0	0.3542	1.3194

P = Poultry by-product meal; T = Taurine.

[1] Pooled standard error of treatment means (n=3)

Mean values across a row with different superscripts are statistically different (P<0.05)

Table 24. Proximate composition (dry matter basis) of whole body of juveniles (6.25 ± 0.22 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.27% poultry by-product meal and graded levels of supplemental taurine after 8 weeks of feeding (Experiment 1).

Variable	Initial	PT0	PT25	PT50	PT75	P - value	PSE[1]
Dry matter (%)	28.4	31.5	31.9	30.8	31.0	0.7047	0.7074
Crude protein (%)	40.8	46.6	46.3	49.2	49.1	0.3374	1.2971
Gross energy (kcal/100g)	616.1	608.7	618.4	586.9	599.6	0.2050	9.6761

P = Poultry by-product meal; T = Taurine.

[1] Pooled standard error of treatment means (n=3).

Table 25. Serum osmolality of juveniles Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.27% poultry by-product meal or 14.16% meat and bone meal blend and graded levels of supplemental taurine in experiment 1 (8 weeks, PT diets) and experiment 2 (12 weeks, MT diets).

		Supplemental Taurine (g/100g)					
Experiments	Water	*Zero*	0.25	0.50	0.75	P - value	PSE[1]
			Osmolality (mmol/kg)				
Experiment 1	826.0	308.5	335.6	334.4	363.8	0.1756	15.5166
Experiment 2	802.0	329.3	322.4	328.9	318.2	0.9814	22.0152

[1] Pooled standard error of treatment means (n=3).

Table 26. Performance of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.16% meat and bone meal blend and graded levels of supplemental taurine after 12 weeks of feeding (Experiment 2).

Variable	MT0	MT25	MT50	MT75	P - value	PSE[1]
Final weight (g)	28.24[b]	57.41[a]	74.48[a]	75.62[a]	0.0085	7.8015
Weight gain (%)	386.7[b]	899.0[a]	1126.7[a]	1198.0[a]	0.0111	135.6767
Average daily gain (g/fish/day)	0.27[b]	0.62[a]	0.82[a]	0.84[a]	0.0086	0.0938
Food conversion ratio	3.76[a]	2.46[b]	2.13[b]	2.01[b]	0.0002	0.1542
Daily protein gain (g/fish/day)	0.37[b]	0.54[a]	0.61[a]	0.60[a]	0.0168	0.0458
Daily energy gain (kcal/fish/day)	0.35[b]	1.08[a]	1.43[a]	1.38[a]	0.0205	0.2065
Survival (%)	37.8[b]	86.7[a]	75.6[a]	75.6[a]	0.0154	8.3894
Nutrient Retention						
Protein retention efficiency (%)	8.9[b]	15.7[a]	18.5[a]	19.3[a]	0.0029	1.3935
Energy retention efficiency (%)	7.4[b]	15.2[a]	17.5[a]	18.1[a]	0.0097	1.7881

M = Meat and bone meal blend; T = Taurine.

[1]Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05).

Table 27. Proximate composition (dry matter basis) of whole body of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed fishmeal-free diets containing 50% soybean meal, 14.16% meat and bone meal blend and graded levels of supplemental taurine after 12 weeks of feeding (Experiment 2).

Variable	Initial	MT0	MT25	MT50	MT75	P - value	PSE[1]
Dry matter (%)	28.4	24.6	28.9	28.7	27.6	0.0591	1.0213
Crude protein (%)	40.8	47.1	46.5	48.1	49.1	0.6183	1.4597
Gross energy (kcal/100g)	616.1	538.7[b]	598.8[a]	606.8[a]	590.0[a]	0.0048	8.8979

M = Meat and bone meal blend; T = Taurine.

[1]Pooled standard error of treatment means (n=3).

Mean values across a row with different superscripts are statistically different (P<0.05)

FIGURES

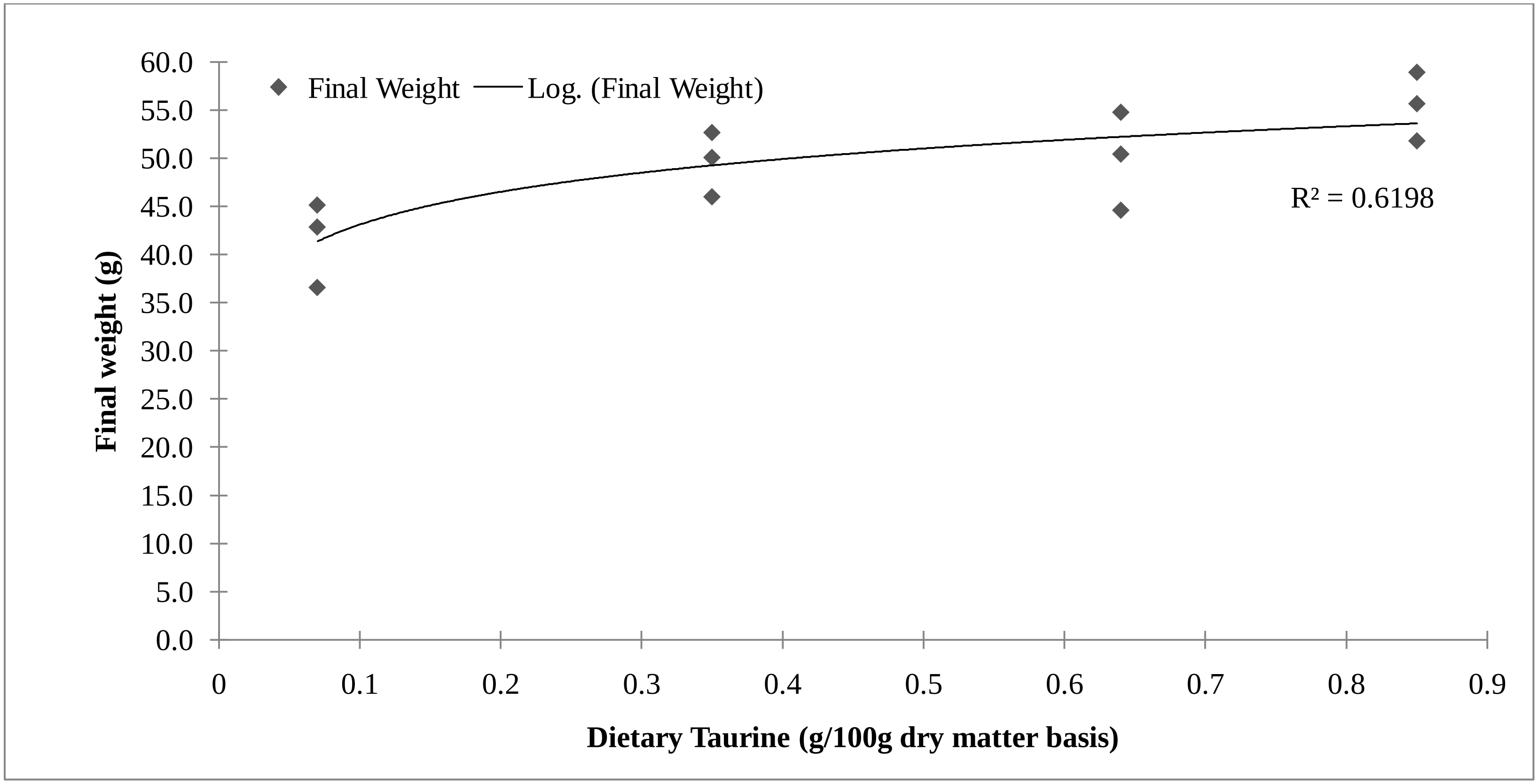

Figure 3. Growth response of juveniles (6.25 ± 0.22 g mean initial weight) Florida pompano *T. carolinus* L. fed poultry by-product meal based diets with supplemental taurine after 8 weeks of feeding (Experiment 1).

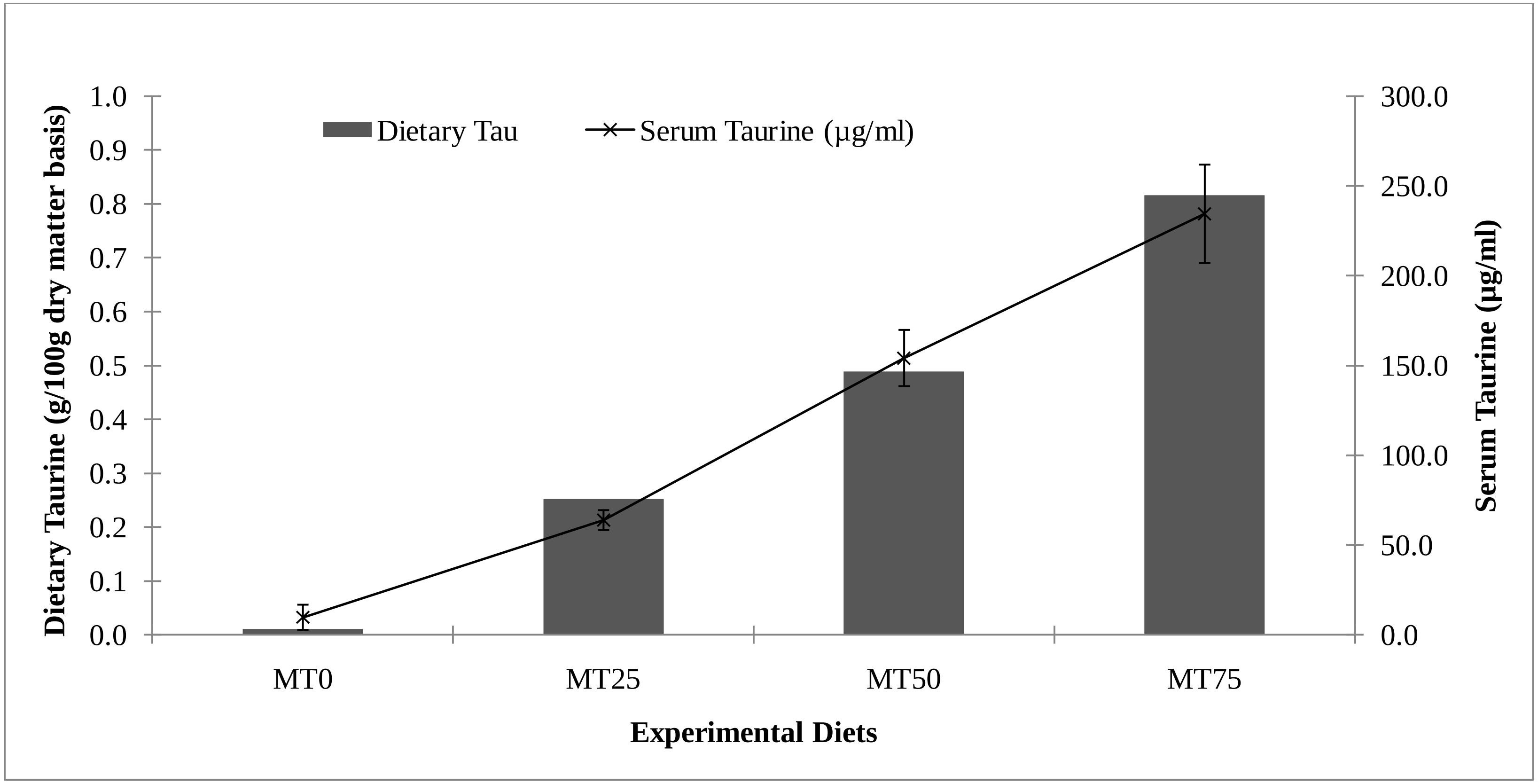

Figure 4. Dietary taurine and postprandial serum taurine levels of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed meat and bone meal blend based diets during 12 weeks of experiment 2. Vertical bars represent the standard error of the mean serum taurine (SEM, n = 3).

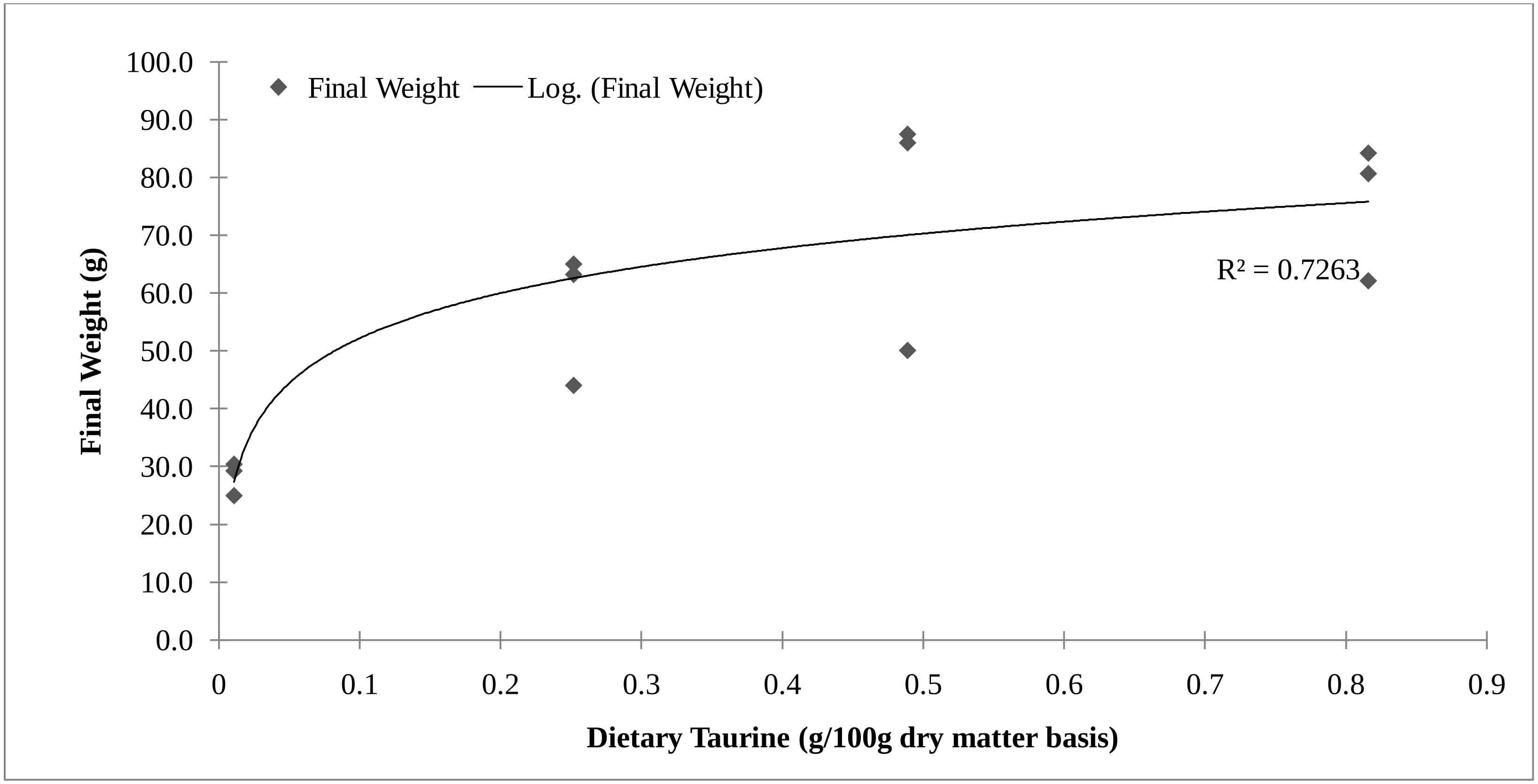

Figure 5. Growth response of juveniles (5.87 ± 0.20 g mean initial weight) Florida pompano *T. carolinus* L. fed meat and bone meal blend based diets with supplemental taurine after 12 weeks of feeding (Experiment 2).

CHAPTER V

SUMMARY AND CONCLUSIONS

This study will contribute to the development of the pompano industry in the United States and has add to our undergoing of the replacement of fishmeal in practical diets for the Florida pompano. The reduction or the complete replacement of fishmeal in aquatic feeds constitutes a major step towards a more sustainable industry. Globally, the utilization marine ingredients in aquatic feeds must become progressively more restricted due to their natural scarcity in the light of an expanding aquaculture industry.

The dietary level of fishmeal used in the control diets of the first and second studies was 15%. The reason for this “typically” low fishmeal level is because it represents the lowest level of fishmeal that has been shown to not depress Florida pompano performance by several researches. The effects of a further fishmeal replacement were evaluated with the use of poultry by-product meal and a meat and bone meal blend as alternative substitutes and the results demonstrated the technical applicability of these by-products in Florida pompano diets.

In the first study, the isonitrogenous replacement of fishmeal by poultry by-product meal resulted in significant depressions in pompano performance when less than 5% fishmeal was present in the diet. When meat and bone meal blend was used to replace fishmeal depression in performance was seen when dietary fishmeal levels were less than 5%. In both studies the lower digestibility of the fishmeal-free diets seemed to be a contributing cause of poorer pompano performance. The fishmeal-free diets were also evaluated for potential limitations in methionine, lysine and taurine. Methionine or lysine supplementation in the fishmeal-free diets containing poultry by-product meal or meat and bone meal blend did not improve fish production.

Taurine supplementation to the fishmeal-free diets based in poultry by-product meal or meat and bone meal blend had a positive impact on pompano performance. Diets containing 0.25% taurine promoted higher pompano performance compared to diets without supplemental taurine. Albeit the optimal requirement for taurine was not physiologically elucidated, shifts in growth, feed conversion and serum taurine levels indicate taurine is assimilated and that a dietary taurine requirement exists. Further studies are necessary to determine the optimum dietary taurine requirement in pompano.

LITERATURE CITED

Ahmed, I., Khan, M.A. & Jafri, A. (2003) Dietary methionine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquacult. Int.*, 11, 449-462.

Ai, Q.H., Mai, K.S., Tan, B.P., Xu, W., Duan, Q.Y., Ma, H.M. & Zhang, L. (2006) Replacement of fish meal by meat and bone meal in diets for large yellow croaker, *Pseudosciaena crocea*. *Aquaculture,* 260, 255-263.

Austreng, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of gastrointestinal-tract. *Aquaculture,* 13, 265-272.

Barrows, F.T., Bellis, D., Krogdahl, A. *et al*. (2008) Report of the plant products in aquafeed strategic planning workshop: An integrated, interdisciplinary research roadmap for increasing utilization of plant feedstuffs in diets for carnivorous fish. *Rev Fish Sci.* 2008;16:449–455.

Bellinger, J.W. & AVAULT JR, J.W. (1971) Food habits of juvenile pompano, *Trachinotus carolinus,* in Louisiana. *Transactions of the American Fisheries Society,* 100, 486-494.

Berry, F. & Iversen, E. (1967) Pompano: biology, fisheries and farming potential. Gulf Caribb. Fish. Inst. *Proc. 19th Annu. Sess.(1966),* 116-128.

Borlongan, I.G. & Coloso, R.M. (1993) Requirements of juvenile milkfish (*Chanos-chanos forsskal*) for essential amino-acids (vol 123, pg 125, 1993). *J. Nutr.*, 123, 972-972.

Brosnan, J.T. & Brosnan, M.E. (2006) The sulfur-containing amino acids: An overview. *J. Nutr.,* 136, 1636S-1640S.

Coyle, S.D., Tidwell, J.H. & Webster, C.D. (2000) Response of largemouth bass *Micropterus salmoides* to dietary supplementation of lysine, methionine, and highly unsaturated fatty acids. *J. World Aquac. Soc.,* 31, 89-95.

Davis, D. A., & Arnold, C. R. (2004) Red Drum, *Sciaenops ocellatus* Production Diets: Replacement of Fish Meal with Soybean Meal. *J. Appl. Aquacult.*, 15, 173-181.

Davis, D. A., Rhodes, M. & Quintero, H.E. (2009) Update on nutrition research with florida pompano *(Trachinotus carolinus)*. *Abstracts of the 25th Annual Meeting of the Alabama Fisheries Association.*

Dong, F.M., Hardy, R.W., Haard, N.F., Barrows, F.T., Rasco, B.A., Fairgrieve, W.T. & Forster, I.P. (1993) Chemical-composition and protein digestibility of poultry by-product meals for salmonid diets. A*quaculture,* 116, 149-158.

FAO, 2010. The state of World fisheries and aquaculture 2010. FAO Fisheries and aquaculture department. Rome, 2010.

Fields, H.M. (1962) Pompanos *(Trachinotus spp.)* of south Atlantic coast of the United States, US Fish and Wildlife Service; for sale by the Supt. of Docs., US Govt. Print. Off.

Finucane, J. (1969a) Pompano mariculture in Florida. *H. W Jr., Youngken Food-drugs from the sea proceedings,* 135–143.

Finucane, J.H. (1969b) Ecology of pompano (*Trachinotus carolinus*) and permit (*T. falcatus*) in florida. *Transactions of the American Fisheries Society,* 98, 478-&.

Fowler, L.G. (1991) Poultry by-product meal as a dietary-protein source in fall chinook salmon diets. *Aquaculture,* 99, 309-321.

Gatlin, D.M., Barrows, F.T., Brown, P. *et al.* (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res.,* 38, 551-579.

Gaylord, T.G. & Gatlin, D.M. (1996) Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture,* 139, 303-314.

Gaylord, T.G. & Rawles, S.D. (2005) The modification of poultry by-product meal for use in hybrid striped bass *Morone chrysops X M-saxatilis* diets. *J. World Aquac. Soc.,* 36, 363-374.

Gilbert, C.R. (1986) Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (South Florida): Southern, gulf, and summer flounders.[*Paralichthys lethostigma*; *Paralichthys albigutta*; *Paralichthys dentatus*]. Florida State Museum, Gainesville (USA).

Goda, A.M., El-Haroun, E.R. & Chowdhury, M.A.K. (2007) Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. *Aquac. Res.,* 38, 279-287.

González-Félix, M.L., Davis, D.A., Rossi Jr, W. & Perez-Velazquez, M. (2010) Evaluation of apparent digestibility coefficient of energy of various vegetable feed ingredients in Florida pompano, *Trachinotus carolinus*. *Aquaculture,* 310, 240-243.

Gothreaux, C. (2008) Measurement of Nutrient Availability in Feedstuffs for Florida Pompano and Development of Formulated Diets for Pompano Aquaculture. Master's thesis. Louisiana State University, Baton Rouge, Louisiana, USA.

Gothreaux, C.T., Reigh, R.C., Williams, M.B. & Chesney, E.J. (2010) Amino Acid Availability of Soybean Meal for Florida Pompano. *N. Am. J. Aquacult.,* 72, 309-313.

Goto, T., Matsumoto, T., Murakami, S., Takagi, S. & Hasumi, F. (2003) Conversion of cysteate into taurine in liver of fish. *Fish. Sci.,* 69, 216-218.

Goto, T., Takagi, S., Ichiki, T., Sakai, T., Endo, M., Yoshida, T., Ukawa, M. & Murata, H. (2001) Studies on the green liver in cultured red sea bream fed low level and non-fish meal diets: Relationship between hepatic taurine and biliverdin levels. *Fish. Sci.,* 67, 58-63.

Griffin, M.E., White, R.W. & Brown, P.B. (1994) Total sulfur amino-acid requirement and cysteine replacement value for juvenile hybrid striped bass (*Morone saxatilis* x *Morone chrysops*). *Comp. Biochem. Physiol., A-Physiology,* 108, 423-429.

Griffith, O.W. (1987) Mammalian sulfur amino-acid metabolism - an overview. *Methods in Enzymology,* 143, 366-376.

Gunter, G. & Hall, G.E. (1963) *Biological Investigations of the St. Lucie Estuary (Florida) in Connection with Lake Okeechobee Discharges Through St. Lucie Canal*, Gulf Coast Research Laboratory.

Hardy, R.W. & Barrows, F.T. (2002) Diet formulation and manufacture. In: *Fish Nutrition* (Halver, J.E. ed.), 3nd edn, Vol. 1, pp. 506-596. Academic Press Inc., San Diego, CA, USA.

Hedrick, R.L., Popma, T.J. & Davis, D.A. (2005) Pond production and fatty acid profiles of the fillets of channel catfish reared on diets with different protein sources. *N. Am. J. Aquacult.,* 67, 304-311.

Heilman, M.J. & Spieler, R.E. (1999) The daily feeding rhythm to demand feeders and the effects of timed meal-feeding on the growth of juvenile Florida pompano, Trachinotus carolinus. *Aquaculture,* 180, 53-64.

Huxtable, R.J. (1992) Physiological actions of taurine. *Physiol. Rev.,* 72, 101-163.

Jackson, A.J. & Capper, B.S. (1982) Investigations into the requirements of the tilapia sarotherodon-mossambicus for dietary methionine, lysine and arginine in semi-synthetic diets. *Aquaculture,* 29, 289-297.

Jordan, D.S. & Evermann, B.W. (1898) The fishes of North and Middle America: a descriptive catalogue of the species of fish-like vertebrates found in the waters of North America, north of the Isthmus of Panama, Govt. print. off.

Jory, D., Iversen, E. & Lewis, R. (1985) Culture of fishes of the genus *Trachinotus* (Carangidae) in the western Atlantic: prospects and problems. *Journal of the World Mariculture Society,* 16, 87-94.

Kikuchi, K., Sato, T., Furuta, T., Sakaguchi, I. & Deguchi, Y. (1997) Use of meat and bone meal as a protein source in the diet of juvenile Japanese flounder. *Fish. Sci.,* 63, 29-32.

Kim, S.K., Matsunari, H., Takeuchi, T., Yokoyama, M., Furuita, H., Murata, Y. & Goto, T. (2008) Comparison of taurine biosynthesis ability between juveniles of Japanese flounder and common carp. *Amino Acids,* 35, 161-168.

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

Kim, S.K., Takeuchi, T., Yokoyama, M., Murata, Y., Kaneniwa, M. & Sakakura, Y. (2005b) Effect of dietary taurine levels on growth and feeding behavior of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquaculture,* 250, 765-774.

Kureshy, N., Davis, D.A. & Arnold, C.R. (2000) Partial replacement of fish meal with meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. *N. Am. J. Aquacult.,* 62, 266-272.

Lazo, J.P., Davis, D.A. & Arnold, C.R. (1998) The effects of dietary protein level on growth, feed efficiency and survival of juvenile Florida pompano (*Trachinotus carolinus*). *Aquaculture,* 169, 225-232.

Li, M.G.H., Manning, B.B. & Robinson, E.H. (2002) Comparison of various animal protein sources for growth, feed efficiency, and body composition of juvenile channel catfish *Ictalurus punctatus*. *J. World Aquac. Soc.*, 33, 489-493.

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.

Ma, T. & Zuazaga, G. (1942) Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. *Industrial & Engineering Chemistry Analytical Edition,* 14, 280-282.

Mai, K., Wan, J., Ai, Q., Xu, W., Liufu, Z., Zhang, L., Zhang, C. & Li, H. (2006) Dietary methionine requirement of large yellow croaker, *Pseudosciaena crocea* R. *Aquaculture,* 253, 564-572.

Mainard, L.A.W. & Loosli, J.K. (1969) Animal Nutrition (6th Edn.), pp. 613. McGraw-Hill, New York, NY.

Matsunari, H., Takeuchi, T., Takahashi, M. & Mushiake, K. (2005) Effect of dietary taurine supplementation on growth performance of yellowtail juveniles *Seriola quinqueradiata*. *Fish. Sci.,* 71, 1131-1135.

McGinnis, A. & Kasting, R. (1964) Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. *J. Agric. Food Chem.*, 12, 259-262.

Moe Jr, M., Lewis, R. & Ingle, R. (1968) Pompano mariculture: Preliminary data and basic considerations. Fla. Board Conserv. *Mar. Res. Lab. Tech. Ser*, 55, 65.

Murthy, H. & Varghese, T. (1998) Total sulphur amino acid requirement of the Indian major carp, *Labeo rohita* (Hamilton). *Aquacult. Nutr.*, 4, 61-65.

Nengas, I., Alexis, M., Davies, S. & Petichakis, G. (1995) Investigation to determine digestibility coefficients of various raw materials in diets for gilthead sea bream, *Sparus auratus* L. *Aquacult. Res.*, 26, 185-194.

Nengas, I., Alexis, M.N. & Davies, S.J. (1999) High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream *Sparus aurata* L. *Aquaculture*, 179, 13-23.

Nguyen, T.N., Davis, D.A. & Saoud, I.P. (2009) Evaluation of Alternative Protein Sources to Replace Fish Meal in Practical Diets for Juvenile Tilapia, *Oreochromis spp*. *J. World Aquac. Soc.*, 40, 113-121.

NRC (Natural Research Council). (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, DC., USA, 114 p.

NRC (Natural Research Council). (2006) *Nutrient requirements of dogs and cat*. National Academy Press, Washington, DC., USA, 398 p.

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

Patro, B., Reigh, R.C., Williams, M.B. (2011) Dietary methionine requirement of Florida pompano. *Aquacultre America 2011 – Meeting Abstract 344.*

Perret, W.S., Latapie, W.R., Pollard, J.F., Mock, W.R., Adkins, G.B., Gaidry, W.J. & White, J. (1971) Fishes and invertebrates collected in trawl and seine samples in Louisiana estuaries. *Section I*, 39-105.

Rawles, S.D., Gaylord, T.G. & Gatlin, D.M. (2006a) Digestibility of gross nutrients by sunshine bass in animal by products and commercially blended products used as fish meal replacements. *N. Am. J. Aquacult.*, 68, 74-80.

Rawles, S.D., Riche, M., Gaylord, T.G., Webb, J., Freeman, D.W. & Davis, M. (2006b) Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* female x *M. saxatilis* male) in recirculated tank production. *Aquaculture*, 259, 377-389.

Riche, M. & Williams, T. (2011) Fish meal replacement with solvent extracted soybean meal or soy protein isolate in a practical diet formulation for Florida pompano (*Trachinotus carolinus*, L.) reared in low salinity. *Aquacult. Nutr.*, 17: no. doi: 10.1111/j.1365-2095.2010.00808.x ...2011.

Riche, M. & Williams, T.N. (2010) Apparent digestible protein, energy and amino acid availability of three plant proteins in Florida pompano, *Trachinotus carolinus* L. in seawater and low-salinity water. *Aquacult. Nutr*, 16, 223-230.

Riche, M. (2009) Evaluation of Digestible Energy and Protein for Growth and Nitrogen Retention in Juvenile Florida Pompano, *Trachinotus carolinus*. *J. World Aquac. Soc.*, 45-57.

Steffens, W. (1994) Replacing fish-meal with poultry by-product meal in diets for rainbow-trout, *Oncorhynchus mykiss*. *Aquaculture*, 124, 27-34.

Swisher, K. (2006) The global market for rendered products. In: *Essential rendering: all about the animal by-products industry* (Meeker, D.L., ed.), pp. 213–228. Kirby Lithographic Company, Inc., Arlington, Virginia.

Tacon, A. & Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, 285, 146-158.

Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H. & Ukawa, M. (2008) Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. *Aquaculture*, 280, 198-205.

Takagi, S., Murata, H., Goto, T., Hayashi, M., Hatate, H., Endo, M., Yamashita, H. & Ukawa, M. (2006a) Hemolytic suppression roles of taurine in yellowtail *Seriola quinqueradiata* fed non-fishmeal diet based on soybean protein. *Fish. Sci.,* 72, 546-555.

Takagi, S., Murata, H., Goto, T., Ichiki, T., Endo, M., Hatate, H., Yoshida, T., Sakai, T., Yamashita, H. & Ukawa, M. (2006b) Efficacy of taurine supplementation for preventing green liver syndrome and improving growth performance in yearling red sea bream *Pagrus major* fed low-fishmeal diet. *Fish. Sci.,* 72, 1191-1199.

Takagi, S., Shimeno, S., Hosokawa, H. & Ukawa, M. (2001) Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream *Pagrus major*. *Fish. Sci.,* 67, 1088-1096.

Turker, A., Yigit, M., Ergun, S., Karaali, B. & Erteken, A. (2005) Potential of poultry by-product meal as a substitute for fishmeal in diets for Black Sea turbot *Scophthalmus maeoticus*: Growth and nutrient utilization in winter. *Isr. J. Aquacult./Bamidgeh*., 57, 49-61.

Wang, Y., Guo, J.L., Bureau, D.P. & Cui, Z.H. (2006) Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibea miichthioides*). *Aquaculture,* 252, 476-483.

Wang, Y., Kong, L., Li, C. & Bureau, D.P. (2010) The potential of land animal protein ingredients to replace fish meal in diets for cuneate drum, *Nibea miichthioides,* is affected by dietary protein level. *Aquacult. Nutr,* 16, 37-43.

Watanabe, W.O. (1995) Aquaculture of the Florida pompano and other jacks (Family Carangidae) in the western Atlantic, Gulf of Mexico and Caribbean basin: status and potential. In: *Culture of High-Value Marine Fishes* (Main, K.L. & Rosenfeld, C. eds), pp. 185-205. Oceanic Institute, Honolulu, HI, USA.

Webster, C.D., Thompson, K.R., Morgan, A.M., Grisby, E.J. & Gannam, A.L. (2000) Use of hempseed meal, poultry by-product meal, and canola meal in practical diets without fish meal for sunshine bass (*Morone chrysops* X M. *saxatilis*). *Aquaculture,* 188, 299-309.

Webster, C.D., Tiu, L.G., Morgan, A.M. & Gannam, A. (1999) Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone chrysops x M. saxatilis* fed practical diets. *J. World Aquac. Soc.*, 30, 443-453.

Weirich, C. & Riche, M. (2006) Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia and nitrite at various salinities. *Aquacult. Res.*, 855-861.

Weirich, C.R. & Riley, K.L. (2007) Volitional spawning of Florida pompano, Trachinotus carolinus, induced via administration of gonadotropin releasing hormore analogue (GnRHa).

Weirich, C.R. (2011) An overview of U.S. pompano research and culture efforts to date. *Aquacultre America 2011 – Meeting Abstract 344.*

Weirich, C.R., Groat, D.R., Reigh, R.C., Chesney, E.J. & Malone, R.F. (2006) Effect of feeding strategies on production characteristics and body composition of Florida pompano reared in marine recirculating systems. *North American journal of aquaculture,* 68, 330-338.

Weirich, C.R., Wills, P.S., Baptiste, R.M., Woodward, P.N. & Riche, M.A. (2009) Production Characteristics and Body Composition of Florida Pompano Reared to Market Size at Two Different Densities in Low-Salinity Recirculating Aquaculture Systems. *North American Journal of Aquaculture*, 71, 165-173.

Williams, S., Lovell, R.T. & Hawke, J.P. (1985) Value of menhaden oil in diets of florida pompano. *Prog. Fish-Cult.,* 47, 159-165.

Williams, T. (2008) An Assessment of Alternative Feed Ingredients in Practical Diets for Florida Pompano (*Trachinotus carolinus*) Held in Low Salinity Recirculating Systems. Master's thesis.The University of Maine Orono.

Wilson R.P. (1989) Protein and amino acid requirements of fishes. In: Progress in Fish Nutrition (Shiau, S.ed.) pp.51 – 76. National Taiwan Ocean University, Keelung, Taiwan.

Worden, J.A. & Stipanuk, M.H. (1985) Acomparison by species, age and sex of cysteinesulfinate decarboxylase activity and taurine concentration in liver and brain of animals. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.,* **82,** 233-239.

Yigit, M., Erdem, M., Koshio, S., Ergun, S., Turker, A. & Karaali, B. (2006) Substituting fish meal with poultry by-product meal in diets for black Sea turbot *Psetta maeotica*. *Aquacult. Nutr*., 12, 340-347.

Yokoyama, M., Takeuchi, T., Park, G. & Nakazoe, J. (2001) Hepatic cysteinesulphinate decarboxylase activity in fish. *Aquacult. Res.,* **32,** 216-220.

Zhang, S., Xie, S., Zhu, X., Lei, W., Yang, Y. & Zhao, M. (2006) Meat and bone meal replacement in diets for juvenile gibel carp (*Carassius auratus gibelio*): effects on growth performance, phosphorus and nitrogen loading. *Aquacult. Nutr.,* **12,** 353-362.

Zhao, X.H., Jia, J.B. & Lin, Y. (1998) Taurine content in Chinese food and daily taurine intake of Chinese men In *Taurine 3 - Cellular and Regulatory Mechanisms* (Schaffer, S., *et al.* eds.), Vol. 442, pp. 501-505.