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Evaluation of Gross Protein/Energy Ratios for Adult Florida Pompano, Trachinotus carolinus, Fed Practical Diets, in Relation to Growth, Feed Utilization, and Nitrogen Retention

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By

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Evaluation of Gross Protein/ Energy Ratios for Adult Florida Pompano, *Trachinotus carolinus,* Fed Practical Diets, in Relation to Growth, Feed Utilization, and Nitrogen Retention (December 2013)

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Florida pompano (*Trachinotus carolinus*) is a strong candidate for aquaculture production growth in the United States. Demonstrated through research, the species meets many of the criteria for species selection. The species is very hardy, can be spawned in captivity, has superior growth rates, easily accept various diets, and has maintained a high market price. However, research is needed to complete our knowledge of the nutritional requirements of Florida Pompano at different ages in order to develop cost-effective and environmentally-friendly diets. To date, only the nutritional requirements for juvenile Florida pompano weighing up to 45 g has been examined. Our goal was to examine the nutritional requirements in terms of protein and energy for Florida Pompano close to its typical marketable weight, between 400 and 600 g.

Six diets were formulated to be isoenergetic (*Gross energy GE*= 21 kJ/g) but contain different Gross Protein/Gross Energy (*GP/GE*) ratios. The formulated *GP/GE* ratios were 18, 19, 21, 22, 23, and 25 g/MJ. Fish meal, soy bean meal and corn gluten meal were used as protein source, with fish oil used to increase the lipid content of diets. Fish average weight was 260.8±21g at the start of the experiment. The pompano were randomly distributed into a flow-through system consisting of 18, 1,000-l conical tanks, at a stocking biomass of 3,000±80 g per tank (3.0 kg/ m^3). Fish were hand-fed the

experimental diets (three tanks per diet) once daily, for 10 minutes or until apparent satiation for 88 days. Tanks were harvested at 30, 60, and 88 days and at this time weights and lengths of individual fish were recorded.

The high survival (100%) and good overall health condition of the fish during the entire trial demonstrates the absence of any nutrient deficiency. Results indicated that while Florida pompano increased in size and age the optimal GP/GE decreased, from 21-22 g/MJ at 382g (first harvest), to 19-21 g/MJ at 486g (second harvest), ending at 19 g/MJ at 577g (third harvest); following a similar trend as reported in literature. Nitrogen retention also decreased with an increasing ratio, with 25 g/MJ displaying the lowest retention. There were no significant differences in either visceralsomatic (VSI) or hepatosomatic indices (HSI) with p-values were 0.32 and 0.29 respectively.

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Chapter 1 INTRODUCTION

1.1 Statement of Problem:

Aquaculture is the fastest growing food production sector globally and will soon surpass capture fisheries in the production of seafood. Aquaculture production has increased from 47.3 to 63.6 million tons from 2006 to 2011, while capture fisheries have stayed stagnant, from 90 to 90.4 million tons during that time period (FAO, 2012). As the most popular source of protein around the world and with nearly 85% of world marine stocks being overfished or fully exploited, seafood will need to be produced via aquaculture at an increasing rate as the population continues to rise (WWF, 2013).

Florida pompano, *Trachinotus carolinus,* is a member of the Carangidae family, which encompasses all jacks (FWC, 2013). The species usually inhabits shore lines to water at a depth of 120ft; ranging from as far north as Massachusetts, to as far south as Southeast Brazil (Smith, 2004). Yet, they are absent from clear water coastal areas like that of the Bahamas (Robins and Ray, 1986). The species is a popular game fish commonly caught surf fishing, however, it is also important for commercial fisheries (FWC, 2013). According to the National Marine Fisheries Service's (2013) land query results, the commercial fishery harvested 102.4 metric tons worth a total market value of US$ 913,536. Overall, the numbers have decreased over the past few years, from a peak of 223.4 metric tons in 2005. Dockside prices have remained high over the past two decades with the most recent published dockside prices of $3.65 per pound whole, with an average market price for that year of $8.00 per pound and a general market size for pompano between 1.0 to 1.5 pounds (Main et al. 2007). Florida pompano has been known to grow to a maximum weight of 8 lbs. (Robins and Ray, 1999). The species is a strong candidate for potential growth in aquaculture production in the United States. The history of the culture of Florida pompano dates back over thirty years (Moe et al., 1968; Hoff et al., 1972; Tatum, 1972; Smith, 1973; Wagstaff, 1975). One of the first studies to show the potential of the species was Jory et al., (1985) who demonstrated that pompano are hardy, have the ability to maintain a high market price, and can be spawned in captivity. Since then many studies have been conducted with Florida pompano, which have demonstrated easier modes of reproduction in captivity, the ability to be raised in marine and low-salinity environments, having tolerance for low dissolved oxygen, being able to be handled with relative ease, can accept balanced diets, and have superior growth rates in captivity (Cuevas, 1978; Riche, 2009; Riche and Williams, 2010). The larval rearing can also be accomplished with relative ease, as the larvae are hardy and easily weaned from rotifers, to artemia, and subsequently onto a dry pellet diet (Main et al., 2007). Conversely, research and development efforts have pointed to several findings of concern with regards to pompano nutrition. The primary concern is pompano's low feed conversion ratio compared to other common cultured marine species. This can be attributed to the species high metabolic rate and poor digestibility (Tatum, 1973; McMaster, 1988; Lazo et al., 1998). This is thought to be due to the short digestive tract that leads to a shorter transit time, which in turn decreases the amount of nutrients absorbed, causing a poor feed conversion rate (Tatum 1973, Williams et al., 1985; Lazo et al., 1998; Wierich et al., 2006; Richie 2009). Another explanation is that pompano are known to eat large amounts of food until satiation is achieved, and this coupled with their short digestive system can possibly lead to a reduced digestion rate (William et al., 1985).

1.2 Background/Literature Review:

Protein is one of the most important ingredients in fish diets due to its responsibility for growth and metabolism. Though fish can synthesize many amino acids, they cannot do this for all and must acquire them through consumption of feed (NRC 2011). Protein is so essential that fish generally require nearly half of their diet to be protein as fish tissue is made up of approximately 65-75% protein on a dry-weight basis, fish tissue is made up of approximately 65-75% protein (Wilson and Halver, 1986). A correct protein level in the diet is important for several reasons. First, a diet that is high in protein and energy can lead to a decrease in feed consumption and growth (Page and Andrews, 1973). Second, a diet too high in protein will cause the fish to convert this excess protein into energy (Wilson and Halver, 1986). This waste of protein for energy, instead of the more desirable result of growth is of particular concern due to the high cost of protein sources in fish feed which reached an all-time high of US$ 1,300/MT in 2012 (Pauly and Froese, 2012)

Research into protein and energy requirements of fish dates back to the late 1950's to early 1960's; these first studies were accomplished on the Chinook salmon (Wilson and Halver, 1986). Several sources report that most carnivorous marine fish have a protein requirement that ranges from 40-55% protein requirement (NRC 1983, Steffens 1989, Wilson 1989, Jobling 1994). Chou et al., (2001) found the cobia requires 44.5% protein in their diets, while spotted sea bass require 45%. Serrano et al., (1992) found red drum require 40%, and it is reported that juvenile flounder need 45-50% protein (Kim et al., 2004; Kim, 2005). Wang et al., (2013) found that golden pompano require a level of 46-49% protein to achieve maximum growth. In Florida pompano Lazo et al., (1998) presented that fish fed a diet containing 45% protein had the highest growth rates, feed conversion efficiency, and feed intake rates, though this was the highest level of crude protein tested. Wantanabe's 1995 findings reported that the species requires 40% protein however, this was from trout feed diets that showed good growth but poor feed efficiency.

Another look at past research on the finfish nutrition will show most of the work accomplished on the early life stages of fish. This trend also occurs in the current research that has been accomplished with Florida Pompano: Lazo et al. 2009, 4.5g; Riche, 2009, 6.3±0.50 g; Riche and Williams, 2010, 75g; Riche and Williams, 2011, 2.5-14.7g; and González-Félix, 2010, 116.9g. These studies make up the limited research that has been reported on the species and with no research accomplished on larger fish, closer to market size. The reason that this difference in age and size is important is due to recent studies presenting that protein, as a factor of weight in diet, generally decreases with an increase in fish size (NRC, 2011). Some examples of results that follow this trend are: Tilapia less than 1g require 35-50% protein, while at 5-25g fish require a lower 30-40% protein level (Balarin and Haller, 1982). Channel catfish require 35% at 14 to 100g, but only 25% at 114 to 500g (Page and Andrews, 1973). Salmonids go from 45-50% to 40% to 35% from young to juvenile to yearlings (Hilton and Slinger, 1981; NRC, 1981). The common carp from fry to fingerlings and sub adults to adults, varies 43-47% to 37-42%, to 28-32% respectively (NRC, 1977). Even though larger fish require lower levels of dietary protein, larger fish have shown lower rates of retention of protein than their smaller counterparts (NRC, 2011). Nutrition studies are carried out through a variety of methods; the most common way can be observed in Lazo's study on dietary protein requirements of Florida pompano. In this study fish were initially weighed, fed diets that ranged in protein levels over a period of time, while feed intake tracked along with growth. At completion, feed conversion, feed efficiency, and other parameters were calculated and statistics were run to determine significant difference. These differences, if any, will show which level of protein is more efficiently used by the fish.

Research into the effect of the gross protein to gross energy ratio (*GP/GE)* have begun to gain in popularity among research initiatives. This ratio is one of the most important and least considered aspect of fish nutrition in aquaculture. Recent studies and research have shown that *GP/GE* is a more rational and precise way of conveying crude protein requirement in fish feed (NRC, 2011). This ratio is important to aquaculture, in particular to the culture of food fish because a correct ratio will allow for an efficient conversion of dietary protein in to body mass maximizing growth and minimizing feed consumption (Lee and Putman, 1973; Riche, 2009). An unbalanced ratio can have several negative effects, such as an excess of dietary energy that can cause a number of liver diseases (Riche, 2009) and buildup of adipose tissue covering the intestines (Cho and Kaushik, 1985, 1990; Craig et al., 1999; Peres and Oliva-Teles, 1999; Martino et al., 2002; Du et al., 2005). On the other side of the ratio an excess amount of protein in the diet can also have several negative effects of which, includes having excess protein in a diet used in catabolic reactions instead of anabolic. With protein sources being the most expensive ingredient in fish feed compared to other more desirable energy sources, (starch, lipids) this use of protein as an energy source can be considered a waste (Wantanabe, 2002; NRC, 2011). Another negative effect of an excess of protein is the increase of nitrogenous wastes and decrease in water quality of the culture system

(Tibbetts, 2005).

As stated, the *GP/GE* ratio is of great importance when formulating a diet for resulting in several studies on various fish species. Wantanabe et al., (2001) found a ratio at 27.5-29.5 MJ kg^{-1} for mutton snapper (*Lutjanus analis*) maximized growth, while in Asian sea bass (*Lates calcarifer*) a ratio of 30 mg/kJ was considered optimal (Catacutan and Coloso, 1995). Japanese sea bass (*Lateolabrax japonicas*) fed a ratio of 25.9 MJ kg^{-1} and had the highest efficiencies (Ai et al., 2004). For Florida pompano the major publication states that a ratio between 23.8 to 25.1 MJ kg^{-1} had the highest efficiency (Riche, 2009). This author believes that previous research on the species may have been inaccurate due to lack of digestible energy to support the highly active nature, high metabolism, and large growth demands.

1.3 Purpose of Study:

The objective of this study is to increase our knowledge of the nutritional requirements of Florida pompano, in particular adult pompano, close to market size. This study addresses gross protein to gross energy ratio in diets to find the most suitable level for the species to maximize growth, survival, and nitrogen retention while keeping feed conversions ratios low. Since sources of protein in fish feed are the most expensive ingredient (Bassompierre et al., 1997), this data is a necessary step closer to creating a more cost-effective and environmentally friendly diet for adult Florida pompano.

Chapter 2. Materials and Methods

2.1 Feed Formulation Strategy:

Six isoenergetic diets were formulated with different gross protein/gross energy (*GP/GE*) ratios based on current data on juvenile Florida pompano, *Trachinotus carolinus,* (Riche, 2009; Lazo et al., 1998). The formulated *GP/GE* ratios were 18, 19, 21, 22, 23 and 25 g MJ^{-1}; using fish meal, soybean meal and corn gluten meal for sources of protein, included at various levels to create diets with increasing *GP/GE* ratios (Table 1). Fish oil was used to increase the lipid content of diets. All diets were supplemented with minerals and vitamin mix. The feeds company Naltech Nutritional Technologies SAC, Lima, Peru was chosen to produce the six diets.

Table 1. Formulation and proximate composition of the experimental diets (*g/100 g* of dry diet) fed to adults, Florida Pompano *Trachinotus carolinus*.

Experimental diets	**Protein/Energy (GP/GE) ratios**					
	Diet 1 18 g/MJ	Diet 2 19 g/MJ	Diet 3 21 g/MJ	Diet 4 22 g/MJ	Diet 5 23 g/MJ	Diet 6 25 g/MJ
Anchovy meal [1]	24.91	25.14	31.82	32.53	41.00	39.95
Soybean meal [2]	17.00	15.00	22.00	19.00	28.00	24.00
Corn gluten meal [3]	10.00	10.00	10.00	10.00	6.19	10.00
Wheat flour [4]	34.48	40.28	22.92	29.21	12.68	14.87
Anchovy oil [5]	10.48	6.46	10.27	6.29	9.92	6.27
Mineral-vitamin premix [6]	0.50	0.50	0.50	0.50	0.50	0.50
Luctamold	0.20	0.20	0.20	0.20	0.20	0.20
Antox	0.05	0.05	0.05	0.05	0.05	0.05
Others	2.39	2.37	2.24	2.22	1.46	4.16
Dietary composition						
Crude protein (g/100 g as feed)	35.2	35.3	40.2	40.3	45.1	45.3
Crude lipid (g/100 g as feed)	14.1	10.2	14.5	10.5	14.8	11.1
Carbohydrates (g/100 g as feed)	30.5	33.9	24.0	27.5	17.8	19.1
Energy (MJ/kg as feed)	19.0	18.0	19.0	18.0	20.0	18.0
GP/GE (g/MJ)	18.0	19.0	21.0	22.0	23.0	25.0

[1] Anchovy meal (Naltech., Lima, Peru.)
[2]Soybean meal (Naltech., Lima, Peru.)
[3] Corn gluten meal (Naltech., Lima, Peru.)
[4]Wheat flour (Naltech., Lima, Peru.)
[5]Menhaden oil (Naltech., Lima, Peru.)
[6]Mineral Premix composition (g/kg): $Ca(H_2PO_4)_2 \cdot H_2O$, 136.00; $Ca(C_6H_{10}O_6) \cdot 5H_2O$, 348.553; FeSO4 $\cdot 7H_2O$, 5.00; $MgSO_4 \cdot 7H_2O$, 132.00; K_2HPO_4, 240.00; $NaH_2PO_4 \cdot H_2O$, 88.00; NaCl, 45.00; $AlCl_3 \cdot 6H_2O$, 0.084; KI, 0.15; $CuSO_4 \cdot 5H_2O$, 0.50; $MnSO_4 \cdot H_2O$, 0.70; $CoCl_2 \cdot 6H_2O$, 1.00; $ZnSO_4 \cdot 7H_2O$, 3.00; $NaSeO_3$, 0.0127. Vitamin Premix composition (g/kg): Ascorbic acid, 50; dl-calcium pantothenate, 5.0; Choline chloride, 36.2; Inositol, 5.0; Menadione sodium bisulfite, 2.0; Niacin, 5.0; Pyridoxine HCl, 1.0; Riboflavin, 3.0; Thiamine mononitrate, 0.5; dl-alpha-tocopherol acetate (250 IU/g), 8.0; Vitamin A palmitate (500,000 IU/g), 0.2; Vitamin micro-mix[c], 10.0; Cellulose, 874.1 Vitamin Micro-mix composition (g/100g): Biotin, 0.50; Folic acid, 1.8; Vitamin B12, 0.02; Cholecalciferol (40 IU/ug), 0.02; Cellulose, 97.66

2.2 Analytical Procedure:

Samples of the six diets and body samples (minced and lyophilized) were sent for a full protein, lipid, energy, and amino acid profile by the Department of Wildlife and Fisheries Sciences, at Texas A&M University.

2.3 Experimental Conditions:

The experimental unit used in this study consisted of eighteen conical tanks, each with a volume of 1,000 liters, on flow-through (Figure 1). The tanks contained a central standpipe, with water levels controlled by an external tree, and an oxygen stone. Well water was pumped from a settling tank by a 2hp centrifugal pump (Hayward™, Hayward industries, (http://www.haywardnet.com/aboveground/products/pumps/) and then mechanically filtered through a sand filter containing crushed coral media (Triton II, Pentair Pool Ltd., Minneapolis, MN, USA) before entering the tanks at a flow rate of 1000% daily exchange. Throughout the experiment, tanks were siphoned as needed and the system was backwashed every other day to ensure consistent water flow. Water parameters were measured using a YSI Professional Plus Meter (YSI, Inc., 1700/1725

Brannum Lane, Yellow Springs, Ohio, 45387). Water temperature, dissolved oxygen, pH, and salinity were recorded daily.

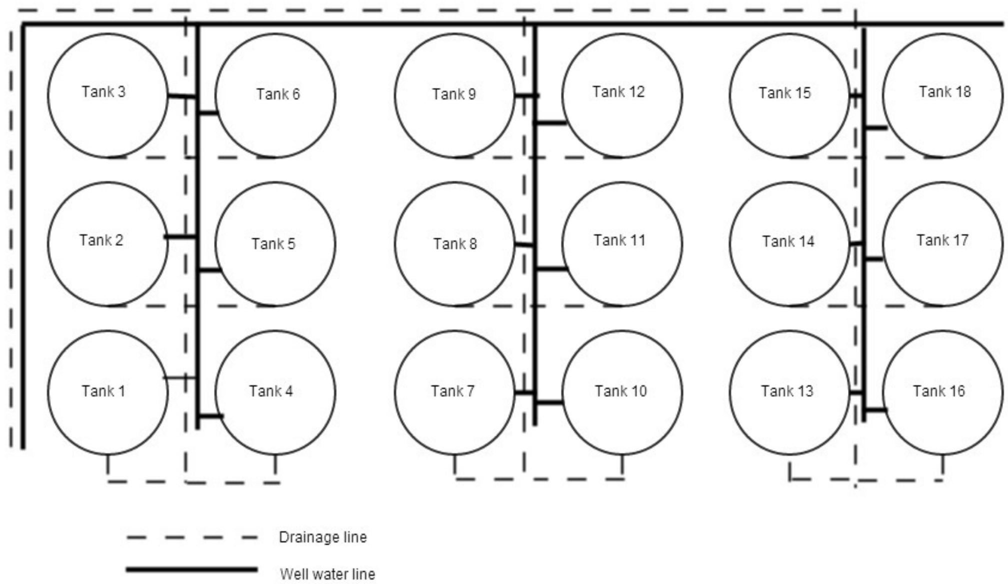

Figure 1 System Overview

The Florida pompano juveniles used in the experiment were obtained from fish spawned and raised at the University of Miami experimental fish hatchery (UMEH). Fish weight and length were recorded and fish were randomly distributed at an initial stocking density of 3kg/cm3. The six practical diets were randomly assigned to tanks, each having three replicates. Fish were fed by hand once per day at 0900hr for 88 days, to apparent satiation or up to 10 minutes. The total amount of feed consumed per day was recorded daily.

2.4 Harvest, Sample Collection and Production Performance:

Before the start of the experiment, during the stocking of the tanks, three fish were randomly selected and euthanized using tricane methansulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA). The fish were minced in a meat grinder (Hobart model 4812, 701 S. Ridge Ave.Troy, OH 45374) and samples were pooled. Three samples were taken and stored at -80°C for two days and then lyophilized. Samples were weighed, vacuumed sealed, and sent for protein, lipid, and ash analysis.

Each of the three harvested fish were anesthetized with 15 ppm of 100% clove bud oil (Spice USA, Inc., Hialeah, FL, USA) and individual weight and length were recorded to measure growth performances. After all fish were removed from the tanks, each tank was disinfected using Virkon S (Farnam Companies, Inc. 301 West Osborn Road Phoeniz, AZ. 85013). Before fish returned to their designated tanks, at stocking and after the second harvest, fish were given a two to five minute freshwater bath as prophylactic treatment to ensure optimal health. Harvests were performed at 30, 60, and 88 days from the start of the trial.

Upon the completion of the final harvest, three fish from each tank were euthanized using 40ppm of tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA). Each fish was dissected and the visceral mass extracted. The entire mass and individual liver were weighed to obtain the visceral-somatic index (VSI) and hypo-somatic index (HIS). The fish from each tank were minced in a meat grinder and homogenized for whole-body proximate analysis. A sample for each tank was taken (18 samples, three replicates per diet) and placed in -80°C for two days. Samples were lyophilized, weighed, and sent for analysis.

Growth performance was determined using the following variables:

ADG = average daily gain; SRG = specific growth rate; MDI = mean daily intake; GEI = gross energy intake; GPI = gross protein intake; FE = feed efficiency; PER = protein efficiency ratio; FCR = feed conversion ratio; NRE= nitrogen retention efficiency; VSI= visceral-somatic index; HSI= hepato-somatic index

1. ADG = (g/d)

2. SGR = 100 (ln average final weight- ln average initial weight)/numbers of days

3. MDI = (g/fish/day)

4. FE = (weigh final-weight initial)/total feed intake

5. PER = weight gain in g/ protein intake

6. FCR = feed intake/ wet weight gain

7. NRE (%) = [[(FBW x N content$_{final}$) – (IBW x N content$_{initial}$)]/DNI] x 100
   (Azevedo et al., 2004)
   (FBW=final body weight; IBW=initial body weight; DNI=digestible N intake)

8. VSI= VSI weight * 100/body weight

9. HSI= HSI weight * 100/body weight (AOAC, 2000)

2.5 Statistical Methods:

The data from the feeding trials was analyzed using one-way analysis of variance (ANOVA) with the diets as the treatments and the tanks as experimental units. Duncan's multiple range test was then used to rank significant difference among means. Statistical analyses were performed using Statistical Analysis System (SAS) version 9.2 (SAS Institute Inc., Cary, N.C.). A P-value equal to or less than 0.05 was used to indicate statistical significance.

Chapter 3: Results

Over the 88 day experimental trials, water quality of all tanks in the system were stable and normal for well water in the region. The average and ranges of water temperature (24.3± 0.5 °C), salinity (33.1± 0.05 ppt), dissolved oxygen (8.68± 1.7 mg/L), and pH (7.2-8.0) were considered optimal for Florida pompano at an adult size.

3.1 Experiment 1 (30 days)

The first experiment was harvested at 30 days, with 100% survival. During this trial the fish averaged an initial biomass of 3 kg/m^3 at the start of the experiment, with a final biomass average of 4.4 kg/m^3. As displayed in table 2 there was no significant differences amongst the growth parameters except for protein efficiency ratio (PER), where that highest ratio, 25 g MJ^{-1}, was significantly higher than the rest. The largest growth based on mean weight gain in grams and average daily growth (ADG), were observed in the 21 and 22 g MJ^{-1} diets where mean weight gain averaged 128 and 129g; ADG of 4.2 and 4.2 respectively. Diet 4, 22 g MJ^{-1}, recorded the highest SGR at 1.35. The lowest growth levels were detected in the diets 1 (18 g MJ^{-1}) and 6 (25 g MJ^{-1}). The feed conversion ratio (FCR) were all high, above 2, the lowest being 2.1 at 22 g MJ^{-}

Table 2. Experiment 1. Final biomass 4.4 kg/m^3: Mean gain, intake, and selected efficiency parameters in Pompano, *Trachinotus carolinus* (means of three replicates, means±std).

Diet *Growth after 30 days*	Diet 1 18 g MJ^{-1}	Diet 2 19 g MJ^{-1}	Diet 3 21 g MJ^{-1}	Diet 4 22 g MJ^{-1}	Diet 5 23 g MJ^{-1}	Diet 6 25 g MJ^{-1}
Initial biomass (g fish tank $^{-1}$)	2916±46^a	3147±229^a	3042±139^a	2977±38^a	2975±11^a	2958±18^a
Final biomass (g fish tank -1)	4188±191^a	4502±638^a	4492±210^a	4474±231^a	4453±383^a	4299±220^a
Initial mean wt (g)	259±25	288±38	269±21	255±10	247±1	247±22
Final mean wt (g)	371±26^a	412±70^a	396±26^a	384±30^a	371±32^a	359±34^a
Weight gain wt (g)	112±6.0^a	124±39^a	128±7.5^a	129±25^a	123±33^a	112±19^a
ADG [1]	3.7±0.2^a	4.1±1.3^a	4.2±0.25^a	4.2±0.8^a	4.1±1.0^a	3.7±0.6^a
SGR [2]	1.20±0.1^a	1.17±0.2^a	1.29±0.1^a	1.35±0.2^a	1.33±0.3^a	1.24±0.2^a
Intake						
MDI [3]	9.4±0.9^a	10±2.3^a	10±0.5^a	9.0±1.0^a	9.3±1.1^a	8.9±1.2^a
Efficiency						
FE [4]	0.39±0.03^a	0.40±0.04^a	0.42±0.02^a	0.47±0.04^a	0.45±0.05^a	0.42±0.02^a
PER[5]	0.86±0.07^a	0.81±0.08^a	0.78±0.04^a	0.92±0.08^a	0.85±0.11^a	0.93±0.06^b
FCR [6]	2.5±0.22^a	2.5±0.23^a	2.4±0.14^a	2.1±0.20^a	2.4±0.33^a	2.4±0.16^a
Survival (%)	100	100	100	100	100	100

Values with different superscripts within the same column were significantly different ($P<0.05$).
[1]ADG = average daily gain; [2] SRG = specific growth rate; [3] MDI = mean daily intake; [4] FE = feed efficiency; [5]PER = protein efficiency ratio; [6]FCR = feed conversion ratio.

3.2 Experiment 2 (60 days)

Fish in experiment 2 were harvested at 30 days from the previous experiment ending and over this time period survival was 100%. At harvest the average biomass was 5.6 kg/m^3. As shown in table 3, there were several parameters that displayed significant differences from other diets. The growth parameters ADG and mean weight gain were significantly lower in diets 5 (23 g MJ^{-1}) and 6 (25 g MJ^{-1}). The efficiency parameters of diet 6 performed significantly worse than the other diets at feed efficiency (FE) and FCR.

The largest growth over the experiment was observed at the ratios of 19 g MJ^{-1}and 21 g MJ^{-1} with 119 and 118 g in mean weight gain respectively. These two diets, 2 and 3 also showed the highest levels of ADG and SGR as well. The lowest performing diets based on growth were diets 5 and 6, the highest ratio diets, with a mean average weight of 91±10 g and 91±11g respectively. The FCRs for all diets during this trial were once again all above to the lowest at 19 g MJ^{-1} (2.3±0.06) and the highest at 25 g MJ^{-1} (3.1±0.17).

Table 3. Experiment 2. Final biomass 5.6 kg/m^3: Mean gain, intake, and selected efficiency parameters in Pompano, *Trachinotus carolinus* (means of three replicates, means±std).

Diet *Growth after 30 days*	Diet 1 18 g MJ^{-1}	Diet 2 19 g MJ^{-1}	Diet 3 21 g MJ^{-1}	Diet 4 22 g MJ^{-1}	Diet 5 23 g MJ^{-1}	Diet 6 25 g MJ^{-1}
Initial biomass (g fish tank $^{-1}$)	4188±191^a	4502±638^a	4492±210^a	4474±231^a	4453±383^a	4299±220^a
Final biomass (g fish tank -1)	5404±160^a	5803±669^a	5845±451^a	5700±197^a	5545±341^a	5388±310^a
Initial mean wt (g)	371±26^a	412±70^a	396±26^a	384±30^a	371±32^a	359±34^a
Final mean wt (g)	477±90	527±110	516±72	489±74	462±90	449±74
Weight gain wt (g)	108±18^a	119±6.9^a	118±20^a	104±4.5^a	91±10^b	91±11^b
ADG [1]	3.6±0.6^a	3.9±0.2^a	4.0±0.7^a	3.5±0.1^a	3.0±0.3^b	3.0±0.4^b
SGR [2]	0.8±0.07^a	0.8±0.09^a	0.9±0.1^a	0.8±0.08^a	0.7±0.1^a	0.7±0.02^a
Intake						
MDI [3]	9.8±1.3^a	9.8±1.2^a	10.2±0.6^a	9.7±0.6^a	9.2±0.5^a	9.8±0.3^a
Efficiency						
FE [4]	0.39±0.01^a	0.43±0.04^a	0.41±0.03^a	0.38±0.04^a	0.35±0.03^a	0.32±0.04^{bc}
PER[5]	0.85±0.03^a	0.86±0.09^a	0.76±0.07^a	0.76±0.09^a	0.71±0.07^a	0.72±0.09^a
FCR [6]	2.5±0.01^a	2.3±0.06^a	2.4±0.05^a	2.6±0.11^a	2.8±0.01^a	3.1±0.17^{bc}
Survival (%)	100	100	100	100	100	100

Values with different superscripts within the same column were significantly different ($P<0.05$).
[1]ADG = average daily gain; [2] SRG = specific growth rate; [3] MDI = mean daily intake; [4] FE = feed efficiency; [5]PER = protein efficiency ratio; [6]FCR = feed conversion ratio.

3.3 Experiment 3 (88 days)

The harvest of the fish in experiment 3 occurred at 28 days from the previous harvest. Survival was 100% during this trial. As shown in table 4, the biomass average 6.6 kg/m^3 per tank at harvest. There was no significant differences in growth and efficiency parameters. However, diet 6 had the highest FCR at 4.8 with a large standard deviation of ±1.99.

Diet 2 (19 g MJ^{-1}) exhibited the highest mean weight gain and ADG of 104.8±29g and 37±1.0 while diet 5 displayed the largest SGR of 6.8±0.15. The lowest performing diet based on growth was diet 6, in mean weight gain, ADG, and SGR.

Nitrogen retention efficiency (NRE) was calculated using the formula shown in the material and methods of this paper. Nitrogen content in the carcass was found using the Kjeldahl Method with a conversion factor of 6.25 (AOAC, 2000). NR showed a general trend of decreasing retention with increasing level of protein/energy ratio. Diet 6 was observed to be significantly lower than the rest of diets at 20.35±6.26%, except from diet 5 and 3, while the highest percentage, diet 1, was 43.38±8.68%. This trend can be observed in Figure 1.

Table 4. Experiment 3. Final biomass 6.6 kg/m^3: Mean gain, intake, and selected efficiency parameters in Pompano, *Trachinotus carolinus* (means of three replicates, means±std).

Diet *Growth after 28 days*	Diet 1 18 g MJ^{-1}	Diet 2 19 g MJ^{-1}	Diet 3 21 g MJ^{-1}	Diet 4 22 g MJ^{-1}	Diet 5 23 g MJ^{-1}	Diet 6 25 g MJ^{-1}
Initial biomass (g fish tank $^{-1}$)	5405±160^a	5803±670^a	5845±451^a	5701±197^a	5546±341^a	5388±310^a
Final biomass (g fish tank -1)	6406±311^a	6746±605^a	6877±275^a	6774±158^a	6719±276^a	6226±463^a
Initial mean wt (g)	477±90^a	527±110^a	516±72^a	489±74^a	462±90^a	449±74^a
Final mean wt (g)	565±121	634±98	607±96	580±96	560±95	519±81
Weight gain wt (g)	89.8±26^a	104.8±29^a	91±18^a	92±24^a	98±19^a	69±18.5^a
ADG [1]	3.2±0.9^a	3.7±1.0^a	3.3±0.65^a	3.3±0.8^a	3.5±0.68^a	2.45±0.66^a
SGR [2]	0.6±0.12^a	0.65±0.21^a	0.58±0.13^a	0.61±0.17^a	0.68±0.15^a	0.51±0.16^a
Intake						
MDI [3]	11.9±1.9^a	11.8±0.7^a	11.6±0.4^a	11.3±0.2^a	11.5±0.19^a	10.9±1.2^a
Efficiency						
FE [4]	0.26±0.04^a	0.31±0.09^a	0.28±0.05^a	0.28±0.06^a	0.30±0.06^a	0.23±0.08^a
PER[5]	0.57±0.09^a	0.63±0.18^a	0.52±0.1^a	0.56±0.13^a	0.60±0.12^a	0.51±0.18^a
FCR [6]	3.8±0.7^a	3.4±1.2^a	3.6±0.7^a	3.5±0.8^a	3.4±0.7^a	4.8±1.99^a
Body Analysis						
NRE[7]	43.38±8.68^a	39.51±6.55^a	31.91±5.32^{ab}	41.76±1.46^a	29.14±0.82^{ab}	20.35±6.26^b
Survival (%)	100	100	100	100	100	100

Values with different superscripts within the same column were significantly different (P<0.05).
[1]ADG = average daily gain; [2] SRG = specific growth rate; [3] MDI = mean daily intake; [4] FE = feed efficiency; [5]PER = protein efficiency ratio; [6]FCR = feed conversion ratio, [7]NR= nitrogen retention efficiency,

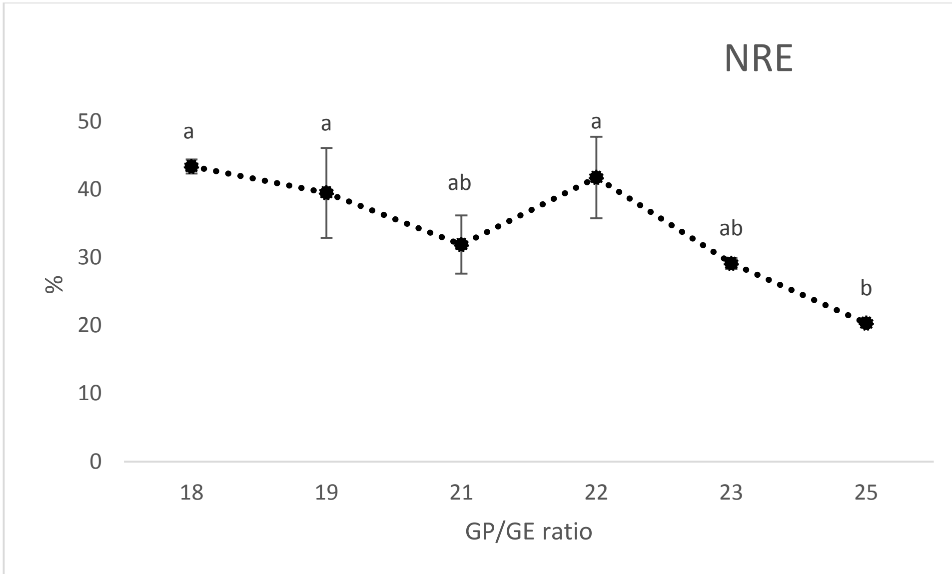

Figure 2 Nitrogen retention (NR) of experiment 3 fed diets of varying gross protein/gross energy ratio. Significant differences between dietary treatments within each group are displayed by different letters. Each point represents the mean and standard deviation (n=3).

3.4 Biological Indices

As displayed in table 5 there was no significant differences in the visceralsomatic or hepatosomatic indices among the six dietary treatments. The p-values were 0.32 and 0.29 respectively.

Table 5. Biological indices of commercial-size pompano at the end of feeding trial*

DIETARY TREATMENT	VSI[1]	HSI[2]
1	5.16	1.22
2	5.83	1.24
3	4.47	1.09
4	5.62	1.14
5	4.93	1.44
6	4.94	1.21

*Values in a column that do not have the same superscript letters are significantly different according to Duncan's multiple range test ($P<0.05$)
[1]VSI: Visceral-somatic index = VSI weight * 100/body weight
[2]HSI: Hepato-somatic index = HIS weight * 100/ boy weight

3.5 Overall Combined Results

Figure 2 displays the specific growth rates over the 88 day of the trial. Throughout the study no significant differences among the diet was visible, nor can a generally trend be created. However a decreasing trend can be observed when comparing progressive performance from experiment 1 to experiment 3. As the fish reach a larger size class there is a decrease in SGR.

Figure 3 presents the feed efficiencies over each experiment. A trend can be observed between experiments 1 and 2, where there is a shift from higher ratios being optimal to a higher performance in lower ratios in experiment 2. This trend does not continue in experiment 3, however there is a general decreasing trend among all diets compared to experiments 1 and 2.

Figure 4 displays the protein efficiency ratio over the three experiments. This ratio shows similar trends to that of figure 3, FE. Experiments 1 and 2 appear to be the opposite trends, with higher ratios performing more optimal in experiment 1 and less optimal in experiment 2. Also as shown in FE and SGR, there is a general trend as the age class of the fish increases the PER decreases.

Figure 5 shows the feed conversion ratios during the three experiments. The trends present are different than the other parameters measured. Over the first experiment FCRs were all nearly equal, with 22 g MJ^{-1} performing the best. This trend changes over the second experiment where the higher ratios performed worse than the lower ratios, with 25 g MJ^{-1} being significantly higher than the other diets. During this final trial, all FCRs were higher than those of experiments 1 and 2 at each ratio. There was a significant observed increase in the standard deviations particularly at 25 g MJ^{-1} which ranged from over 7 to 2.5.

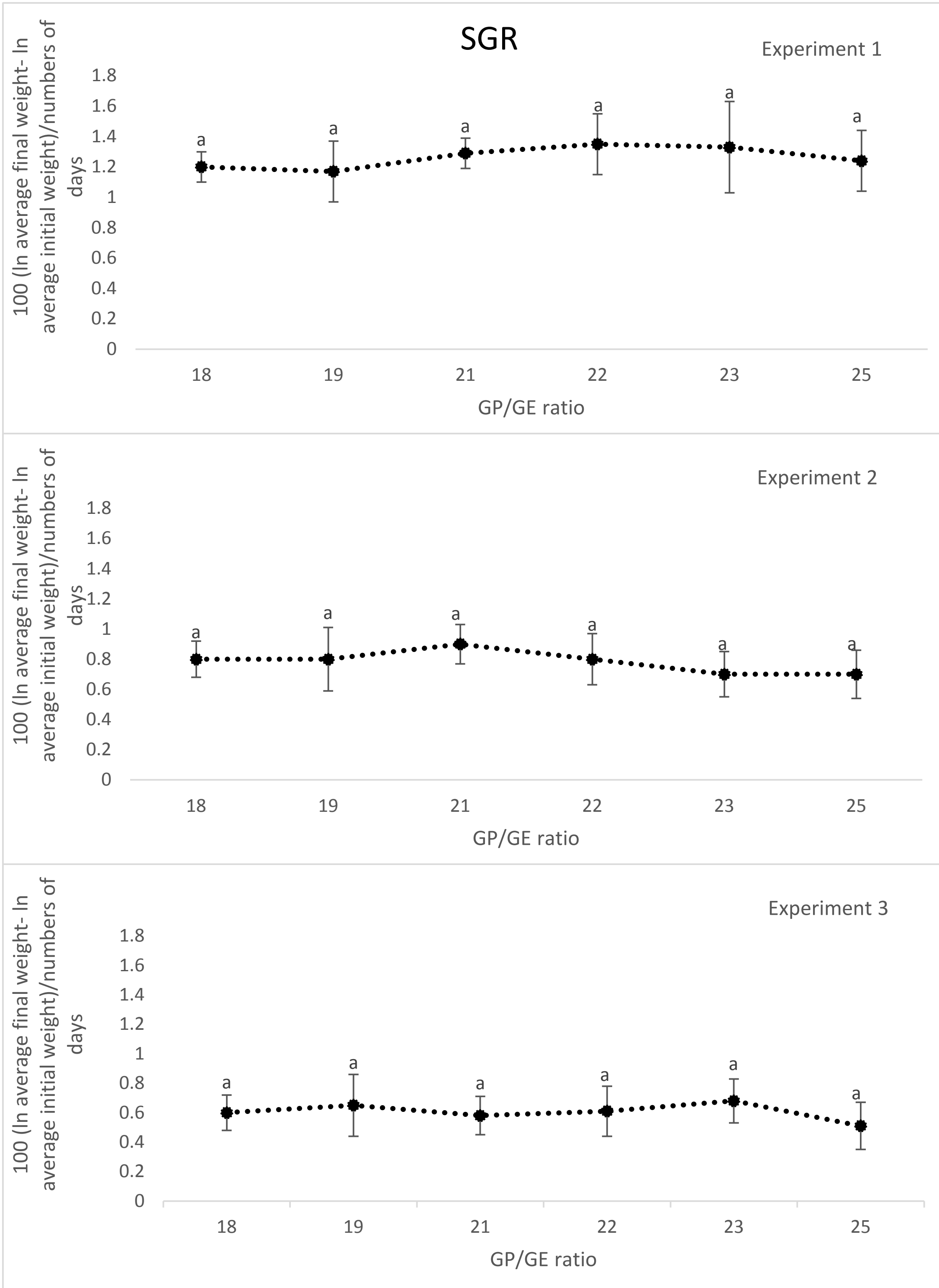

Figure 3 Specific growth rates (SGR) of 3 experiments fed diets of varying gross protein/gross energy ratio. Significant differences between dietary treatments within each group are displayed by different letters. Each point represents the mean and standard deviation (n=3).

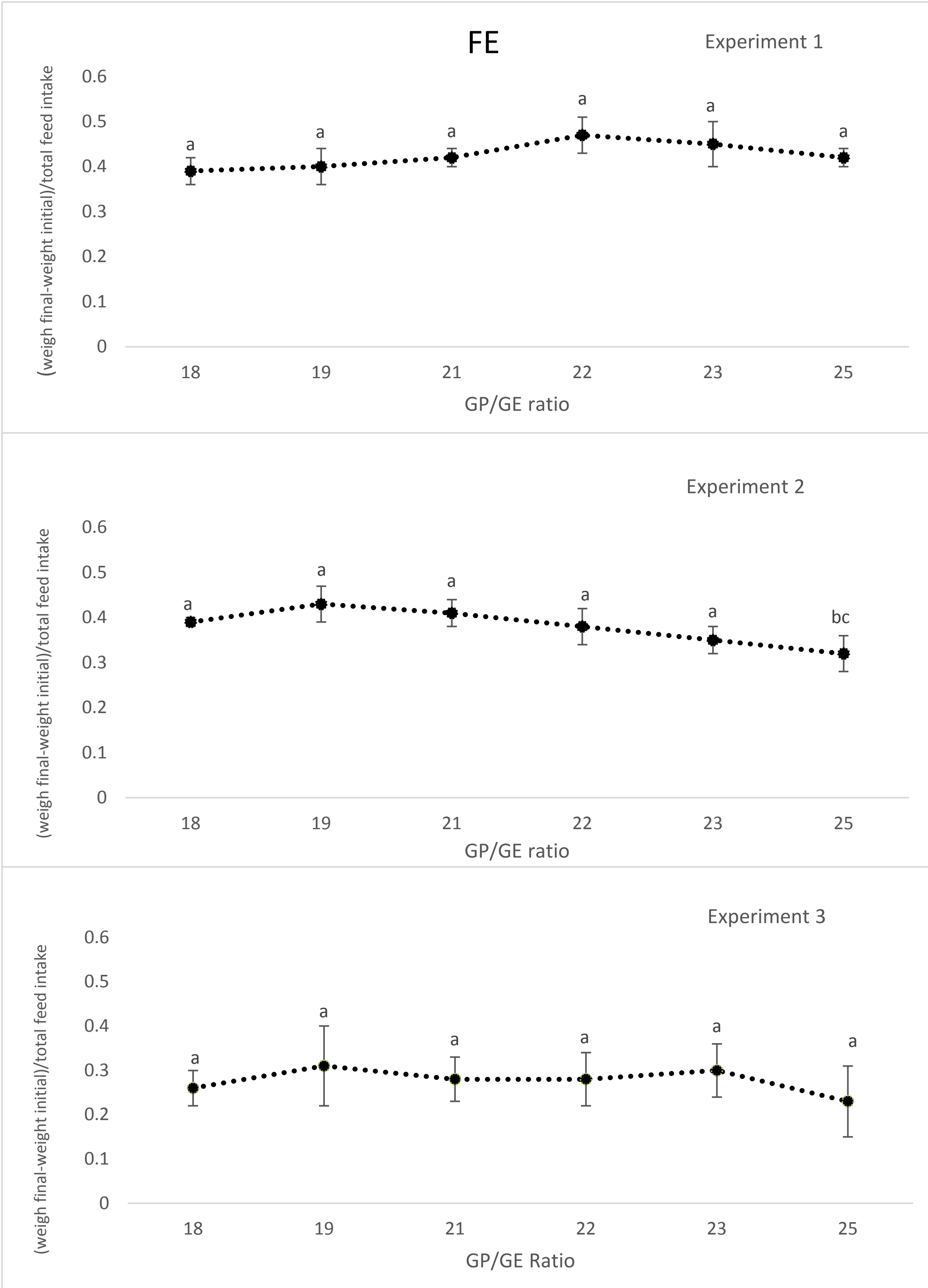

Figure 4 Feed efficiency (FE) of 3 experiments fed diets of varying gross protein/gross energy ratio. Significant differences between dietary treatments within each group are displayed by different letters. Each point represents the mean and standard deviation (n=3).

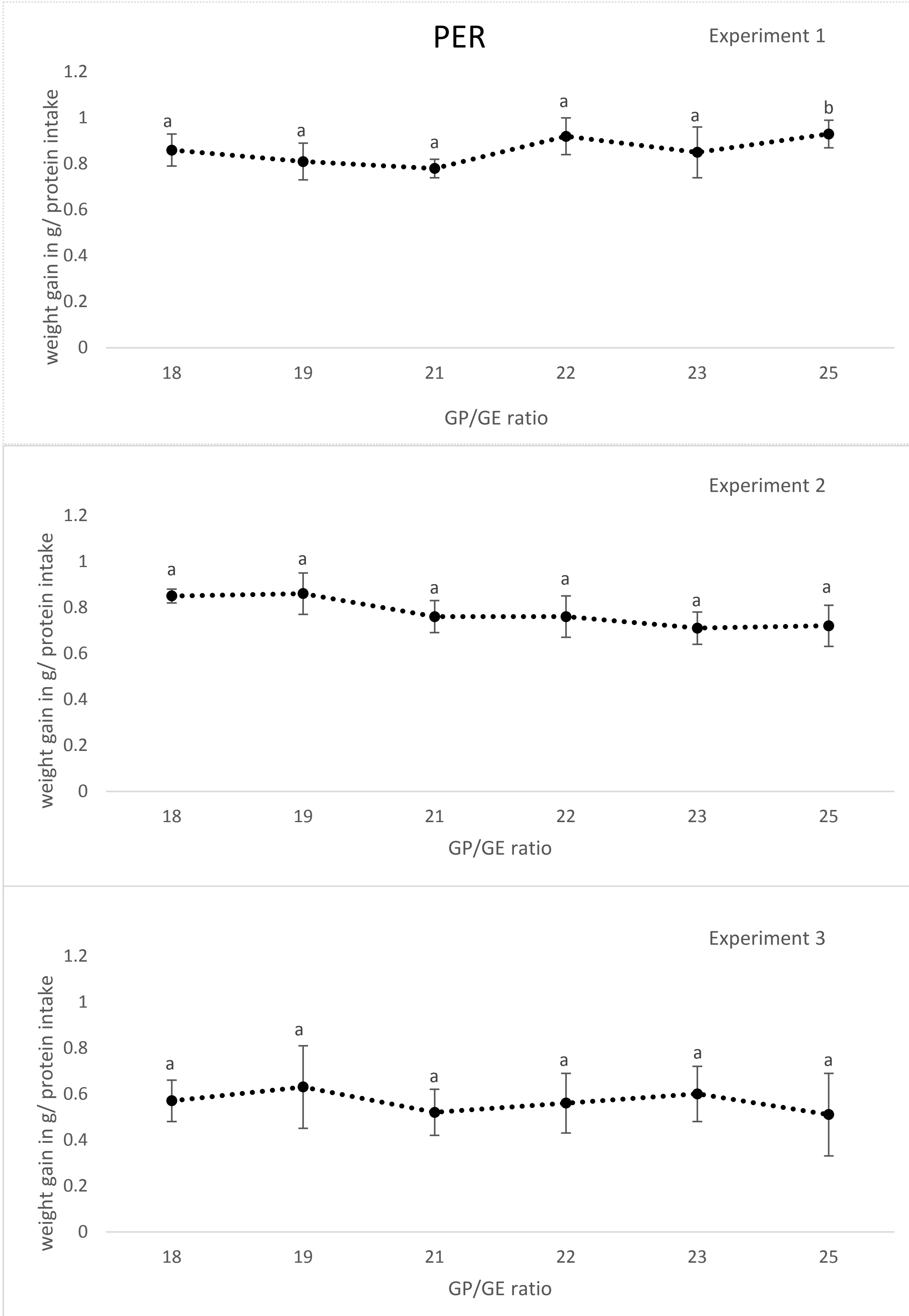

Figure 5 Protein efficiency ratio (PER) of 3 experiments fed diets of varying gross protein/gross energy ratio. Significant differences between dietary treatments within each group are displayed by different letters. Each point represents the mean and standard deviation (n=3)

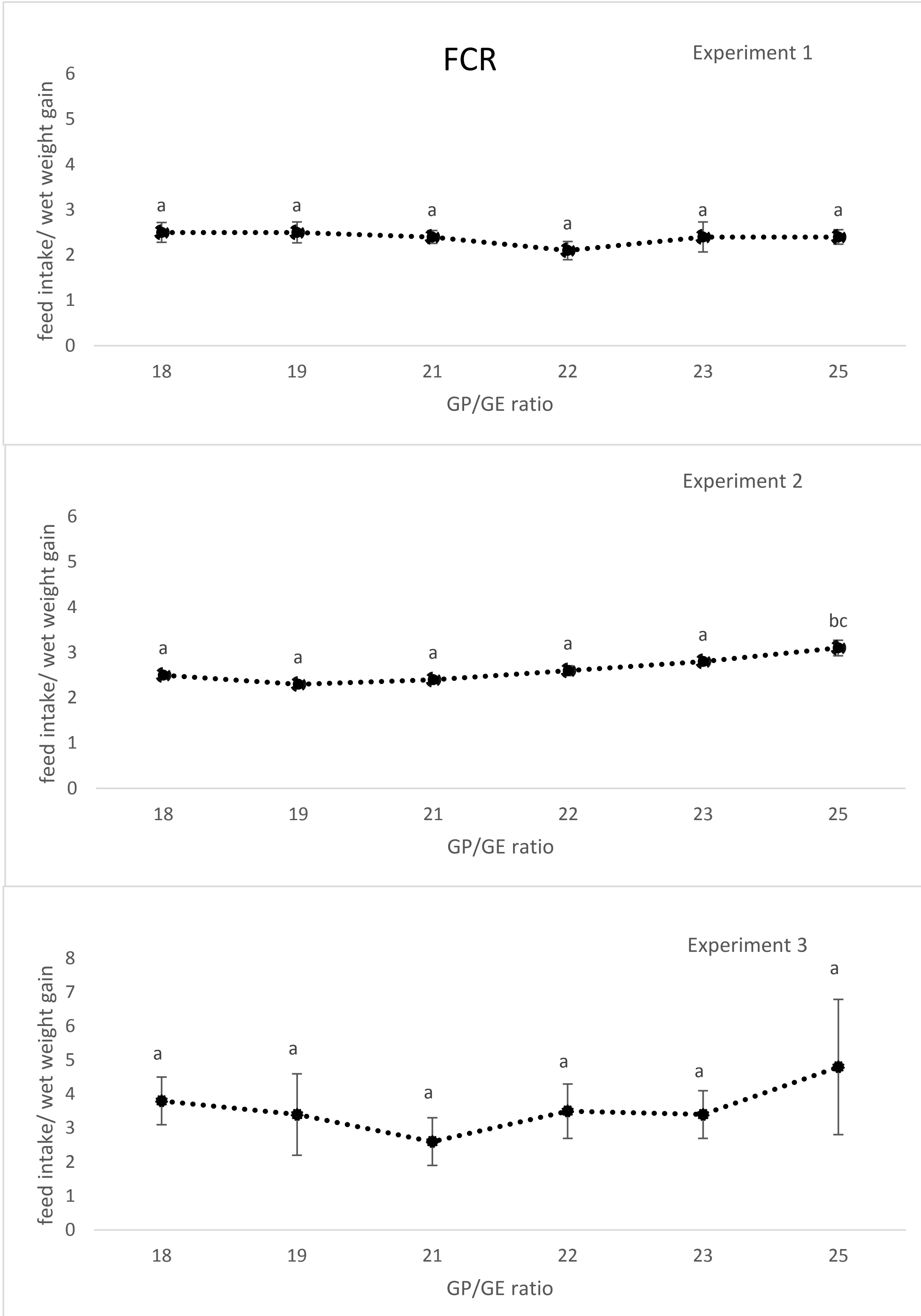

Figure 1 Feed conversion ratio (FCR) of 3 experiments fed diets of varying gross protein/gross energy ratio. Significant differences between dietary treatments within each group are displayed by different letters. Each point represents the mean and standard deviation (n=3)

Chapter 4: Discussion

4.1 Growth Study

Over the course of the three harvests and 88 days of the three ranges of Florida pompano sizes during the experiment, the high survival (100%) and good overall health condition of the fish indicates the absence of any nutrient deficiency. There was a visible trend from harvest to harvest for several of the parameters. Through the first experiment, diets of 22 and 21 g MJ^{-1} was optimal for growth and several efficiencies while after the second experiment the best diets based on GP/GE decreased to 19 and 21 g MJ^{-1}. Both of these are lower than that of previous studies done on Florida pompano by Riche (2009) where it was reported a ratio of 23.8 to 25.1 g MJ^{-1} provided the best performance. In comparable studies that were also based on efficiency and growth optimization of protein/energy ratios were found: Japanese sea bass, 25.9 mg/KJ (Ai et al., 2004), Asian sea bass 30.6 mg KJ^{-1} (Catacutan and Coloso, 1995) and 23 MJ kg^{-1} (Lee et al., 1994), and mutton snapper 27.5-29.5 g MJ^{-1} (Watanabe et al., 2001). However all of these studies were accomplished on juvenile fish ranging from 1.34 to 12.2g. Results of this thesis however, follow that of Atlantic salmon where a ratio of 18.8 g MJ^{-1} provided optimal growth for small fish, while a lower ratio of 16.4 g MJ^{-1} was optimal for larger fish (Eien and Roem, 1997); and that of gilthead seabream where the protein/energy ratio required decreased from 28.35 to 19.5 g MJ^{-1} as the fish grew larger (Lupatsch et al., 2001).

The performance of adult Florida pompano at the higher levels of protein in their diets during experiments two and three when fish were closer to harvest weight was significantly lower. During these experiments diets with 35-40% were shown to be more optimal for growth and efficiency. This finding is not in agreement to what was reported by Lazo et al. (1998) where it was concluded that the protein requirement for juvenile pompano is no less than 45%, from golden pompano (*Trachinotus auratus)* where fish fed 46-49% protein displayed the highest weight gains and lowest FCRs (Wang et al., 2013), and from juvenile flounder where 45-50% was optimal (Kim et al., 2005). Due to there being no significant difference in PER during these two experiments it can be inferred that the energy level present in the diets did not limit the efficiency of protein use (Lazo et al., 1998). However, this does support other findings that with increase size and age in fish the protein requirement decreases.

The final harvest displayed lower growth and efficiencies than that of the other two experiments at all levels of GP/GE ratios. With this result it is reasonable to conclude that Florida pompano at this size go through the onset of sexual maturity or another physiological change that causes the feed efficiencies to decrease so drastically. It can be inferred that the species in an aquaculture setting should be harvested at a size closer to 500g before these efficiencies change, which is similar to what was reported by Main et al., (2007) in the SRAC species profile, of 463 to 680.4 g.

As presented in Table 4 and Figure 1, the nitrogen retention efficiencies (NRE) for Florida pompano appeared to decrease with an increase of GP/GE ratio, and as these diets were isoenergetic, this is an increase in protein in the diets. These results is comparable to that Lupatsch et al. (2001) whom reported that when an adequate level of energy is present in the diet, lower levels of protein are more efficient at NRE. The energy levels within this study (18-20 MJ kg^{-1} as feed) were similar to research on golden pompano, where energy levels in diets ranged from 19.5 to 21 MJ kg^{-1} (Wang et al.

2013). However, these were higher than that tested in other studies for Florida pompano, Riche (2009) used a maximum of 15.9. In this study it was reported that at increasing energy, NR was also increasing. Therefore an appropriate level cannot be determined from this study. Lastly, similar to the trend displayed in this thesis, it has been reported that larger fish commonly display a lower protein retention than their smaller counterparts (NRC, 2011).

4.2 Body Caracas Analysis

As there was no significant differences (P-value= 0.32) in the visceralsomatic index it can be concluded that there was no dietary effect on the liver or fat deposition. As described in several studies, mesenteric fat is generally a sign of surplus dietary energy (Craig et al., 1999; Peres and Oliva-Teles, 1999; Martino et al., 2002; Du et al., 2005; Riche, 2009). This result is similar to what was reported by Wang et al., (2013) for golden pompano, where there was also no significant difference in VSI among different dietary protein and lipid levels.

The hepatosomatic index along all treatments displayed no significant differences (P-value= 0.29), even with a difference in dietary lipid levels (10 and 14 g/100g as feed). This is unlike from other species such as red drum, a difference of 3 to 10 g/100g as feed, where a higher level of lipids displayed a higher HIS (Serrano et al., 1992), and golden pompano which exhibited a decrease in HIS with an increase dietary protein and lipids (Wang, 2013).

4.3 Conclusion

The results from this study provide a starting point for further research into the nutritional requirements of harvestable size Florida pompano. Using these results there are several studies that can accomplished to provide greater insight in to these requirements. The first study is to look at way to improve FCRs in the nutrition of Florida pompano. As demonstrated in this thesis and review of the literature the species has always displayed a poor FCR usually above two, and as feeds is the most expensive piece of aquaculture production, a decrease in the efficiency parameter will allow for Florida pompano to be a more desirable option for future increase in production. One possibility for this would be to test a variety of feeding methods. As stated in the introduction, pompano have a short digestive tract, a sign of carnivory, and thus feed has a short transit time to be digested. This coupled with the species being down to eat large amounts of food until satiation is achieved could be what is leading to these high FCRs (Tatum, 1973; Williams et al. 1985; Lazo et al. 1998; Wierich et al. 2006, Richie 2009), even though feed intake is regulated by energy density of the feed. A study such as this has been accomplished on sockeye salmon, showing by feeding continuously throughout the day instead of satiation three times per day growth can increase (Shelbourne et al. 1973).

A second study which is currently being accomplished at the University of Miami is to look at the replacement of fish meal in the diets of Florida pompano with the use of soybean meal, Navita®, Hamlet protein, and Selecta. All of these products are regularly used in literature as fish meal replacement in other species.

In conclusion, Florida pompano appears that it will have a great future in the field of aquaculture. As established in the literature and this thesis the species meets many of the criteria that are required to establish a successful business model, with fast growth rates, ability to spawn and be raised in captivity, can withstand low levels of dissolved oxygen and low salinity, and have maintained a high market price. These criteria coupled with a limited commercial market and a need for growth in fish production will lead to the choice for this species, while future research is still necessary in the nutrition of Florida pompano. With work on adult fish in this thesis along with the work done on juveniles gives a great starting point for future endeavors.

After completion of writing this thesis results from the analysis of diets and feces have been obtained. After further calculations with these results, which will be presented in a future publication, some conclusions have changed. One diet (diet 2) was removed from the study due to inconsistencies in analysis amongst labs. Table 6 displays the growth parameter using the current data. The optimal level of DP/DE using the calculated values were 26.2-23.3 g MJ^{-1}, with the lowest ratio of 21.7 g MJ^{-1} displaying inferior growth and efficiencies compared to the other diets. This result is similar to previous findings using Florida pompano. Riche (2009), found that a ratio 23.8and 25.1 mg kJ $^{-1}$ to be optimal for the species.

Table 6: Growth, feed efficiency and selected efficiency parameters in Pompano, *Trachinotus carolinus* fed diets of varying DP/DE ratios for 88 days (means of three replicates, means±std).

Diet *Growth after 88 days*	Diet 1: 26.2g/MJ	Diet 2: 25.4g/MJ	Diet 3: 24.3g/MJ	Diet 4: 23.3g/MJ	Diet 5: 21.7g/MJ
Final biomass (g fish tank -1)	6877±275 [a]	6746±605[a]	6774±158 [a]	6719±276 [a]	6226±463 [a]
Initial mean wt (g)	269±21	288±38	255±10	247±1	247±22
Final mean wt (g)	607±96	634±98	580±96	560±95	519±81
Weight gain wt (g)	338.2±10 [a]	347.2±26 [a]	325.7±10 [a]	312±23.5 [ab]	272.2±32 [b]
SGR[1]	0.92±0.05 [a]	0.90±0.04 [a]	0.93±0.01 [a]	0.92±0.05 [a]	0.84±0.09 [a]
MDI[2]	10.4±0.4 [a]	10.3±1.4 [a]	9.8±0.5 [a]	9.8±0.6[a]	9.6±0.7[a]
FE[3]	0.37±0.005 [a]	0.38±0.02[a]	0.38±0.02 [a]	0.36±0.02[ab]	0.32±0.03 [b]
PER[4]	0.68±0.01 [a]	0.76±0.05[a]	0.74±0.04 [a]	0.72±0.04[a]	0.71±0.07 [a]
FCR[5]	2.7±0.03 [a]	2.6±0.17 [a]	2.6±0.15[a]	2.7±0.16[a]	3.14±0.33 [b]
NRE[6]	29.17±1.032[a]	33.48±6.60[a]	26.03±4.28[a]	23.42±5.99[a]	23.45±0.78[a]
Survival (%)	100	100	100	100	100

Values with different superscripts within the same column were significantly different (P<0.05). [1]SGR=Specific Growth Rate, [2]MDI= Mean Daily Intake, [3]FE=Feed Efficiency, [4]PER=Protein Efficiency Ratio, [5]FCR= Feed Conversion Ratio.

The results for body carcass analysis remained the same as the thesis above, no significant difference were found in either VSI or HIS over the course of the study

(Table 7). Also included in the recently received data was digestibility results for the experimental diets. Yttrium oxide (Y_2O_3) was added to the diets at 0.05% in order to determine the digestibility of dry matter (DM), crude protein (CP), gross energy (GE) and amino acids (Table 8). Results from this section concluded with no significant differences present (P-value > 0.05) in DM, CP, or GE. However, for amino acids four (isoleucine, leucine, phenylalanine, lysine) displayed significant differences between diets 1 (26.2 g MJ^{-1}) and 3 (24.3 g MJ^{-1}). The results for ADC protein and energy were similar to previous studies accomplished using Florida Pompano (William et al., 1985; Riche, 2009). However, the results were considerably lower than was has been reported for other marine species (Bureau et al., 1999; Peres and Olive-Teles, 1999; Santinha et al., 1999; Zhou et al., 2004)

Table 7. Biological indices of commercial-size pompano at the end of feeding trial (Calculated Values)*

DIETARY TREATMENT	VSI[1]	HSI[2]
1	4.47	1.09
2	5.83	1.24
3	5.62	1.14
4	4.93	1.44
5	4.94	1.21

*Values in a column that do not have the same superscript letters are significantly different according to Duncan's multiple range test (*P<0.05*)
[1]VSI: Visceral-somatic index = VSI weight * 100/body weight
[2]HSI: Hepato-somatic index = HIS weight * 100/ boy weight

Table 8 Apparent digestibility coefficients (ADC) of DM, CP, GE and of Amino Acids for Florida pompano fed diets of varying DP/DE ratios for 88 days.

	Experimental diets				
	1 26.2 g MJ^{-1}	2 25.4 g MJ^{-1}	3 24.3 g MJ^{-1}	4 23.3 g MJ^{-1}	5 21.7 g MJ^{-1}
DM	49.88	53.63	61.41	52.37	50.76
CP	65.08	66.30	74.04	67.20	66.06
GE	65.36	64.31	73.96	66.40	65.25
Essential					
Arginine	77.72	83.21	86.77	81.75	84.53
Histidine	71.89	71.37	80.11	76.19	77.78
Isoleucine	66.40	73.48	80.14	71.31	77.25
Leucine	69.26	74.65	82.15	74.72	79.53
Lysine	76.52	90.33	86.90	81.35	81.92
Methionine	71.70	73.64	83.35	77.72	81.30
Phenylalanine	70.20	76.79	81.49	74.37	79.67
Threonine	60.20	64.24	75.05	67.32	71.18
Valine	65.75	71.77	79.22	70.95	75.92
Nonessential					
Alanine	68.27	72.99	81.07	73.58	77.68
Aspartic Acid[a]	56.19	62.09	72.28	62.86	68.66
Cysteine	49.71	44.81	68.38	64.91	59.26
Glutamic Acid[b]	66.05	71.80	79.88	73.71	77.15
Glycine	58.23	64.57	70.46	63.10	66.69
Proline	61.67	66.46	75.75	69.72	73.21
Serine	62.94	64.07	75.98	69.33	73.63

*Values in a row that do not have the same superscript letters are significantly different according to Duncan's multiple range test ($P<0.05$). [a] Aspartic acid + asparagine [b] Glutamic acid + glutamine

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