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The effect of protein to lipid ratios on growth, digestibility, and feed utilization of striped bass (*Morone saxatilis*) raised in seawater at 21 °C

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Abstract

A 12-week feeding trial assessed the impact of protein-to-lipid ratios (P:L) on the growth, feed and protein utilization, digestive enzyme activity, and fillet composition of juvenile striped bass (*Morone saxatilis*) raised at summer local seawater temperature conditions (21 °C). A 3×2 factorial design, using three protein levels (40, 44, and 48%) and two lipid levels (12 and 16%) resulting in six diets (40:12, 44:12, 48:12, 40:16, 44:16, and 48:16) was used. Hatchery-reared fish with an initial weight of 11.4 ± 0.2 g were used. Laboratory-formulated experimental diets were randomly assigned to triplicate groups of 22 fish in 500-L tanks connected to a recirculating seawater (35.4 ± 3 ppt) system, and fish were fed manually to apparent satiation four times a day. Survival rates were higher than 96% across all treatments, remaining unaffected by dietary treatments. Fish fed the 44:12 and 48:12 diets resulted in significantly higher weight gain (WG) (274.1 ± 18.4 and 306.8 ± 19.8, respectively), final body weight (FBW) (42.4 ± 1.2 g and 47.1 ± 2.4 g, respectively), and specific growth rate (SGR) (1.6 ± 0.06 and 1.7 ± 0.06, respectively). Diets with 12% lipid resulted in feed conversion rate (FCR) values closer to 1, and the 48:16 (1 ± 0.03) treatment resulted in no significant differences compared to the 12% lipid dietary treatments. Similar trends were observed for protein efficiency ratio (PER), with comparable values for the 40:12 (2.0 ± 0.1), 44:12 (2.2 ± 0.1), 48:12 (2.0 ± 0.1), and 48:16 (2.1 ± 0.1) treatments. A strong relationship between dietary P:E ratios and SGR, PER, and FCR was observed among treatments, which helped explain the interactions between protein and lipid obtained. An estimated dietary P:E ratio between 11 and 12 is suggested for efficient protein and feed utilization for this species under our culture conditions. The apparent digestibility coefficient (ADC %) was significantly higher in the 12% lipid diets ($65.01 \pm 3.06\%$), influenced solely by lipid content. Enzyme activity for trypsin and chymotrypsin in the pyloric caeca (PC) and intestines (I) were higher in the 48:12 treatment (893.5 ± 18.1, 1087.3 ± 55.5, 12.2 ± 0.3, and 8.3 ± 0.2 Units/g organ, respectively). L-aminopeptidase activity (PC) was higher in the 48:12 treatment (1007.7 ± 101.8 Units/g organ), while L-aminopeptidase (I) had higher values in the 48:16 treatment (2217.2 ± 161.3 Units/g organ). In the case of lipase (PC), activity was higher in the 12% lipid treatments, while in the intestine, the highest activity was recorded in the 44:12 treatment (1084.4 ± 87.0). The activity of proteases,

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except for L-aminopeptidase (PC), directly correlated with improvements in SGR, PER, feed intake (FI), and FCR which helps explain the observed results. The Hepatosomatic index (HSI) was significantly higher in the 40:12 treatment while the liposomatic index (LSI) and viscerosomatic index (VSI) indices showed no significant differences between the 40:12, 44:12, 48:12, and 48:16 treatments, respectively. Importantly, the lipid content in the fillet did not significantly decrease with reduced lipid content in the diet, an important issue for market acceptance. Results from the present study suggest an optimal P:L ratio of 48:12 for juvenile striped bass raised in seawater under local summer conditions (21 °C).

Keywords Striped bass · Temperature · Protein · Lipids · E:P ratios · Feed utilization

Introduction

The energy generated by the catabolism of certain dietary nutrients is essential for an organism's normal development and growth (NRC 2011). Providing nutrients in adequate quantities is essential to optimize the biological performance and feeding efficiency in fish under culture conditions. One of the most crucial nutrients in the formulation of feeds for marine fish is protein. Proteins supply organisms with amino acids, which are used to build new proteins, replace existing proteins, or serve as a source of energy (Watanabe 1982; Wilson 2002). Therefore, a protein-deficient diet will result in weight loss. On the other hand, an excess of proteins beyond the organism's requirements will result in higher production costs, a reduction in water quality, and consequently, a less environmentally friendly aquaculture (Wilson 2002; Timmons and Ebeling 2010).

Improving the efficiency of protein utilization can be achieved by ensuring appropriate proportions of other dietary components that provide energy, such as lipids or carbohydrates, in quantities and ratios to reduce the catabolism of amino acids (NRC 2011). However, in fish, especially carnivorous species, there is a preference for lipids over carbohydrates as an energy source. Moreover, lipids are the most efficient nutrients modulating feed intake and can regulate energy use and storage (Bureau et al. 2002; NRC 2011; Tocher and Glencross 2015). However, a diet with excess energy can lead to a reduction in feed intake concomitant with other nutrients such as proteins (NRC 2011; Rahimnejad et al. 2015; Liu et al. 2020). In addition, excess lipids in the diet can result in mortalities associated with fatty livers and other lipotoxic disorders (Roberts and Elis 2011). In this context, finding the appropriate quantity and ratios of lipids to protein in food formulation will help use protein in the diet more efficiently.

Striped bass (*Morone saxatilis*), a member of the Moronidae family, is an anadromous fish with great potential for aquaculture. For example, striped bass exhibits rapid growth and excellent feed conversion efficiency under captive conditions, making it a highly profitable species. Additionally, its market position is solid due to its high demand and commercial value, positioning it as a species of high economic and aquacultural potential (Schultz 2003; Schultz 2010; Kenter et al. 2018; Andersen et al. 2021). Despite a wealth of literature supporting the feasibility of striped bass farming (Andersen et al. 2021), much of the available information focuses on its hybrid species (striped bass × white bass, *Morone chrysops*) cultured in freshwater. Assessing protein and lipid requirements, studies have reported protein needs of 55% crude protein and 16% lipids (Millikin 1982). Subsequently, experiments evaluating the interaction of

protein and lipid of small (1.4 g) striped bass raised in brackish water, a 12% lipid level with dietary protein levels of 37%, 47%, and 57%, did not show significant differences in growth among them but were lower when lipid was increased (17%) or decreased (7%) (Millikin 1983). It is worth noting that these studies were conducted on organisms with an average initial weight of 1.4 g, highlighting the need to determine the optimal protein-lipid ratio for later growth stages and specific climatic conditions in the cultured site.

Currently, striped bass is cultivated in sea cages in Ensenada, Baja California, Mexico, by Pacifico Aquaculture with a yearly production of 3 MT but a goal of 25 MT in the next 2 years (Billund Aquaculture 2023). The average seawater temperatures range from 15 °C in winter to 20 °C in summer, which are below their optimal grow-out temperatures of 24 °C (Andersen et al. 2021; Del Rio-Zaragoza et al. 2020). The present study is the first of two studies conducted to investigate the effect of protein to lipids ratios for stripers cultured under temperature conditions similar to those found in the cultured area during the winter and summer seasons in Ensenada, B.C., Mexico. The main objective of these studies is to optimize the use of feeds when raising striped bass in seawater and improve the rearing practices of this species under commercial conditions. Determining the effect of protein to lipid ratios is crucial to ensure an adequate supply of nutrients for the healthy growth and development of fish. These studies will allow producers to adjust their feeding practices to ensure sustainable and profitable production in the Ensenada region.

Material and methods

Experimental diets

A (3×2) factorial design was utilized to evaluate the effect of three dietary protein and two lipid levels using formulated diets to meet marine fish nutritional requirements (NRC 2011). For this, six diets were prepared to contain three crude protein (CP) levels (40%, 44%, and 48%) and two lipid levels (12% and 16%) named accordingly 40:12, 44:12, 48:12, 40:16, 44:16, and 48:16. Sardine fish meal (68% CP, Procesadora mar de Ensenada S de R L de C.V.) and Poultry meal (65% CP, Scoular de México, S.A.) at a constant ratio (3.8) were used as the main protein sources and sardine fish oil (Proteínas Marinas y Agropecuarias SA de CV, Guadalajara, Jalisco, México) as the main lipid source. Additionally, 6 g/100 g of gelatin was used as a binder (Table 1). Ingredients were weighted and mixed using a laboratory mixer (Robot-Coupe, model R10, USA) and pelleted to 5 mm using a commercial meat grinder (Tor-Rey, Model M32-5, Mexico). Pellets were air-dried at 60 °C for 24 h in a 10 cubic feet forced-air oven (VWR 1600 HAFO series, USA) and stored refrigerated at 4 °C throughout the study until used for feeding. Protein and lipid levels in the diets for this study were selected based on previous studies conducted with striped bass and striped bass hybrids (Millikin 1982, 1983; Nematipour et al. 1992). The proximate composition of the diets was determined as described in the "Chemical analyses" section, and the values are presented in Table 1. The gross energy (GE) in the diets was calculated using physiological values of 9.44, 4.11, and 5.64 kcal/g for lipids, carbohydrates, and proteins, respectively (NRC 2011).

Table 1 Feed formulations for juvenile of striped bass with different P:L ratios and proximate composition (g/100 g on a dry matter basis)

Ingredients (g/100 g)	Dietary protein and lipid level					
	40:12	44:12	48:12	40:16	44:16	48:16
Fish meal (68% CP)[1]	39.6	44.5	49.4	39.6	44.5	49.4
Poultry meal (65% CP)[2]	10.4	11.7	13.0	10.4	11.7	13.0
Corn meal (3% CP)[3]	19.4	13.7	8.0	15.4	9.6	3.9
Fish oil[4]	7.0	6.5	6.0	11.0	10.6	10.1
Corn starch (pregelatinized)[5]	14.0	14.0	14.0	14.0	14.0	14.0
Gelatin	6.0	6.0	6.0	6.0	6.0	6.0
Rovimix for carnivorous fish[6]	3.0	3.0	3.0	3.0	3.0	3.0
Methionine	0.1	0.1	0.1	0.1	0.1	0.1
Taurine	0.5	0.5	0.5	0.5	0.5	0.5
Total (g)	100	100	100	100	100	100
	Proximate composition (%)					
Crude protein	40.8 ± 0.5	43.7 ± 1.0	48.2 ± 2.6	39.7 ± 0.2	43.5 ± 0.4	47.4 ± 1.2
Crude lipids	12.1 ± 0.3	12.3 ± 0.7	12.4 ± 0.6	16.7 ± 0.5	16.3 ± 0.7	16.7 ± 0.5
Dry matter	95.0 ± 0.3	94.4 ± 0.3	94.6 ± 0.1	95.8 ± 0.1	95.5 ± 0.1	95.8 ± 0.1
Ash	10.3 ± 0.1	11.1 ± 1.3	14.5 ± 0.2	10.7 ± 2.1	12.5 ± 1.1	14.6 ± 1.1
NFE	36.8 ± 2.1	32.9 ± 0.3	24.9 ± 2.5	32.9 ± 2.0	27.7 ± 1.1	21.3 ± 2.2
GE kcal/ g	4.08	4.08	4.17	4.32	4.32	4.40
P: E ratio	10.0	10.7	11.6	9.2	10.1	10.8

[1]Procesadora mar de Ensenada S de R L de C.V

[2]Scoular de México

[3]Nixtamalized corn meal MASECA ®

[4]Proteínas Marinas y Agropecuarias SA de CV, Guadalajara, Jalisco, México

[5]Maizena® cornstarch

[6]Rovimix, Stay-C whit vitamins and minerals DMS, Guadalajara, México

Fish source and feeding trial

Striped bass juveniles were produced and donated by a commercial hatchery in the area, Pacífico Aquaculture S.A.P.I. de C.V. The juveniles, with an initial weight of 6.2 ± 0.9 g, were brought to the Fish Nutrition Laboratory at the Centro de Investigación Científica y de Estudios Superiores de Ensenada (CICESE, Ensenada, B.C., México). Fish were acclimated for 2 weeks in a 9000-L tank equipped with a bubble bead biofilter (DF-6) and a Titan HP-2 heat pump. During the acclimation period, fish were fed a commercial diet at 4% of their biomass using a 2 mm commercial diet (currently used by Pacífico Aquaculture S.A.P.I. de C.V.) from EWOS, Canada, with 47% CP and 16% lipid. After 2 weeks of acclimatization at 21 °C, a total of 396 fish with an average weight of 11.4 ± 0.2 g were randomly distributed in a recirculating system composed of 18–500 L circular tanks (22 fish per tank), a 500 L compensating tank, a Titan HP-2 heat pump, a Polygeyser biofilter (Model DF-6, Patent #5.770.080 y 6.517.724), and a 1 Hp water pump (Century 1 HP). Each dietary treatment was evaluated in triplicate ($n=3$). During the bioassay, tanks were siphoned daily, and the walls and bottom were cleaned weekly. Photoperiod was set to 12L:12D using fluorescent lighting. Fish were fed manually to apparent satiation (based on visual and behavioral observations of the fish)

four times a day (8:00, 11:00, 14:00, and 18:00) for 12 weeks. Special care was taken to avoid over-feeding, but all unconsumed feed was removed from the tanks, dried, and weighed. Feed intake was recorded as the feed offered minus the dry unconsumed feed. Water quality parameters were monitored daily using a YSI Pro-2030 multiparameter (YSI Inc., USA) and were maintained at temperature (20.9 ± 0.3 °C), dissolved oxygen (6.6 ± 0.2 mg L^{-1}), and salinity (35.4 ± 3 ppt) throughout the study. Ammonia levels were measured with an APA® aquarium kit three times a week and maintained below 0.5 ± 0.3 mg/L.

Fish sampling

This research meets the ARRIVE, European Union Council (2010/63/EU), and Mexican Government (NOM-062—ZOO-1999) guidelines for the production, care, and use of experimental animals (Fuentes-Quesada et al. 2020). All the fish were individually weighed (grams) and measured (centimeters) every 2 weeks throughout the experiment. Three fish from each tank at the end of the experiment were humanely euthanized with an overdose of clove oil and stored at -20 °C for subsequent muscle composition analysis. Another three fish from each tank were dissected to remove the intestine and pyloric ceca for digestive enzyme activity analysis. The weight of the liver, visceral fat, and viscera were weighed to calculate the hepatosomatic (HSI), liposomatic (LSI), and visceral (VSI) indices. Indices for fish growth and feed utilization were calculated as follows:

$$\text{Weight Gain (WG\%)} = [(\text{final weight} - \text{initial weight})/ \text{initial weight}] \times 100$$
$$\text{Final body weight (FBW)} = \text{final weight} - \text{initial weight}$$
$$\text{Feed Conversion Ratio (FCR)} = \text{total feed consumed} / \text{wet weight gained}$$
$$\text{Protein Efficiency Ratio (PER)} = \text{weight gain} / \text{protein intake}$$
$$\text{Feed Intake (FI)} = (\text{total feed consumed} / \text{fish per tank})/\text{day}$$
$$\text{FI\_ABW} = (\text{total feed consumed} /((\text{initial body weight} + \text{final body weight})/2))/\text{day}$$
$$\text{Hepatosomatic Index (HSI)} = (\text{liver weight} / \text{body weight}) \times 100$$
$$\text{Liposomatic index (LSI)} = (\text{visceral fat weight}/ \text{body weight}) \times 100$$
$$\text{Viscerosomatic Index (VSI)} = (\text{viscera weight} / \text{body weight}) \times 100$$
$$\text{Survival} = (\text{final number of fish} / \text{initial number of fish}) \times 100$$

Chemical analyses

The proximate composition of experimental diets and fish muscle samples was determined following standardized procedures (AOAC 2005) and reported on a dry matter basis. All samples were analyzed in triplicate. Briefly, a muffle furnace was used to ash samples at 550 °C for 2 h to determine total ash content. The moisture content was determined after drying the samples at 135 °C for 2 h or until a constant weight was obtained. Crude protein (N×6.25) was estimated using the micro-Kjeldahl method, and crude lipids using Soxhlet extraction with petroleum ether. Soluble and insoluble carbohydrates were included in the nitrogen-free extract (NFE), and their quantity was calculated by the difference:

$$\text{NFE(\%)} = 100 - (\% \text{ crude protein} + \% \text{ crude lipid} + \% \text{ ash})$$

Apparent digestibility

Feces were collected using a glass tube as a siphon (with care not to generate stress in the fish) after the first hour feeding and dried at 60 °C for 24 h until a constant weight was obtained (Madrid et al. 2019). Insoluble ash content was used as an indigestible marker and determined using the well-established acid-insoluble ash method, as described for marine fish by Fuentes-Quezada et al. (2018), and all diets contained more than 10% ash. Briefly, 50 mg of ash was boiled in 5 mL of 2N HCl using 50 mL beakers covered with a watch glass to prevent evaporation. After 5 min, the solution was filtered with previously inciner-ated Whatman TM filter paper (microfiber glass filter, diameter 47 mm), and the residue was rinsed with distilled water. The residue was then incinerated again in a muffle furnace at 450 °C for 8 h, cooled, and the insoluble ash was evaluated by the difference in weight.

The acid insoluble ash (AIA) was calculated as follows:

$$AIA\,(\%) = [(\text{Weight of the filter plus ashes}) - (\text{Initial weight of the filter}) / \text{sample weight}] \times 100$$

and the apparent digestibility coefficient (ADC) of the diets was calculated as follows:

$$ADC(\%) = 100 - (100 \times AIA\,(\%)\text{ in the feed} / AIA\,(\%)\text{ in the feces})$$

Digestive enzyme activity

The digestive enzyme extracts were obtained following the method described by Fuentes-Quesada et al. (2020). Briefly, the entire digestive system was homogenized in 10 mL of distilled water at 4 °C for 2 min using a tissue grinder (POLYTRON® PT 1200, Kine-matica AG, Switzerland), and then centrifuged at 18,500 g for 45 min at 4 °C (5810R, Eppendorf, USA). The resulting supernatant was collected and stored at -80 °C in 1 mL aliquots until use. The spectrophotometric measurements were performed using a Varios-kan Flash plate reader (Thermo Scientific). Negative and positive controls were prepared using distilled water and commercial enzymes (at a concentration of 0 and 1 mg mL^{-1}), respectively.

The estimation of trypsin activity was performed according to the method described by Erlanger et al. (1961). Briefly, the substrate used was BAPNA (Nα-benzoyl-DL-arginine-p-nitroanilide hydrochloride) from Sigma B-4875 at a concentration of 1 mM in 500 μL of DMSO. The reaction conditions consisted of 175 μL of TRIS buffer of 50 mM and CaCl2 of 20 mM, a pH of 7, a temperature of 37 °C, and 25 μL of homogenate. After 30 min of incubation, the reaction was stopped with 30% acetic acid and the absorbance was meas-ured at 410 nm after 10 min of stabilization.

Chymotrypsin activity was determined using the method of Hummel (1959) as modified by Applebaum et al. (2001). The substrate used was BTEE (N-Benzoyl-L-tyrosine ethyl ester) from Sigma 13,110-F at a concentration of 0.56 mM in a Tris–HCl buffer of 100 mM and CaCl2 of 25 mM, with a pH of 7.8 and the addition of methanol at 2.5% (v/v) at 37 °C. The reaction was recorded every minute for 30 min at a wavelength of 256 nm in a 96-well quartz plate.

The method of Apple (1974) was used to determine the activity of leucine aminopepti-dase (LAP). The reaction was performed using L-leucine-P-nitroanilide as substrate, and the absorbance was measured at 405 nm after the addition of 30% acetic acid to stop the reaction. The reaction was performed in HCl-Tris buffer 50 mM at pH 8.0 and a tempera-ture of 37 °C for 30 min.

Lipase activity was estimated using the method described by Gjellesvik et al. ([1992]) with 0.56 mM 4-Nitrophenyl myristate (Sigma 70,124) as substrate dissolved in 0.5 mL DMSO. The reaction was performed in 150 mM Tris–HCl buffer, 15 mM sodium tauro-cholate, pH 8.5 at 37 °C, and the enzymatic activity was recorded every minute for 30 min at 405 nm.

Statistical analyses

A two-way ANOVA was conducted to analyze the somatic index, fish growth, feed utilization, proximate composition, fatty acid profile, apparent digestibility, and digestive enzyme activity. If the interactions between both factors (proteins and lipids) were statistically significant, differences were determined using Duncan's multiple range test (Rawles and Gatlin III [1998]). Prior to the analysis, normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene tests, respectively. The statistical analysis was performed using IBM SPSS 21 software, and a significance level of $p < 0.05$ was used for all tests.

Results

Biological performance and survival of striped bass

Protein and lipid content in the diets resulted in significant effects in fish growth and feed utilization, where fish fed the low lipid diets resulted in significantly higher growth and fish fed the low protein diets resulted in lower growth (Table [2]). However, a significant interaction was observed between both factors. The interaction between both factors indicated that although fish fed the 44% or 48% protein and 12% lipid in their diet resulted in higher growth, fish fed the 48% and 16% lipid diet resulted in similar fed and protein efficiency compared to the latter diets and higher growth compared to the other two protein levels at this lipid level. In general, fish fed diets with a 48:12 and 44:12 ratio resulted in higher WG values ($306.8 \pm 19.8\%$ and $274.1 \pm 18.4\%$, respectively), FBW (47.1 ± 1.2 g and 42.4 ± 1.2 g, respectively), SGR ($1.7 \pm 0.06\%$ and $1.6 \pm 0.06\%$, respectively), and FI (13.4 ± 1.5 g/kg ABW/day and 12.7 ± 0.8 g/kg ABW/day, respectively). Additionally, fish fed the diets 48:12, 44:12, and 48:16 resulted in FCR values close to 1. Both the level of lipids and protein in the diet, as well as their interaction, had a significant effect on FCR. The interaction between both factors indicated that fish fed the diet with 48% protein and 16% lipid diets, resulted in better FCR when compared to the other 16% lipid diets, with values closer to 1 and similar to the 12% lipid diets. PER values were influenced by both factors as well as their interaction, where fish fed the 48% protein and 16% lipids improved protein utilization (2.1 ± 0.1), resulting in similar values compared to all dietary treatments containing 12% lipids. No significant effects of either protein or lipid levels were observed on the survival rate at the end of the feeding trial for fish fed the experimental diets. Fish fed the 12% diets had significantly higher FI compared to fish fed the higher lipid (e.g., 16%) diets. In addition, fish fed higher protein content diets had higher FI values at each lipid level (Table [2]). If values are expressed as FI-ABW (g/kg/day) in general, higher FI values were observed with the lower lipid level diets, but only significantly higher than the 44:16 treatment resulting in a significant interaction.

Table 2 Biological performance and survival of juveniles of striped bass fed the experimental diets for 12 weeks*

Levels of P:L	Survival (%)	WG (%)[1]	FBW (g)[2]	SGR[3]	FCR[4]	PER[5]	FI (g/fish/day)[6]	FI-ABW (g kg^{-1} day^{-1})[7]	ADC (%)[8]
40:12	100±0	186.0±2.9^c	33.0±0.8^b	1.3±0.01^c	1.2±0.02^{c,b}	2.0±0.1^a	0.27±0.01	12.6±0.2^{ab}	64.4±2.0
44:12	98.3±3	274.1±18.4^a	42.4±1.2^a	1.6±0.06^a	1.0±0.05^c	2.2±0.1^a	0.34±0.03	12.7±0.8^{ab}	69.7±11.7
48:12	100±0	306.8±19.8^a	47.1±2.4^a	1.7±0.06^a	1.0±0.07^c	2.0±0.1^a	0.39±0.04	13.4±0.8^a	61.0±9.1
40:16	100±0	62.4±7.8^e	18.4±0.7^d	0.6±0.06^e	1.8±0.17^a	1.4±0.1^c	0.13±0.01	9.3±0.5^c	57.5±16.3
44:16	100±0	134.9±8.2^d	26.5±1.1^c	1.0±0.04^d	1.4±0.04^b	1.7±0.1^b	0.22±0.01	11.8±0.2^{ab}	52.5±1.7
48:16	96.7±6	226.8±16.7^b	37.4±2.9^b	1.4±0.06^b	1±0.03^c	2.1±0.1^a	0.27±0.03	11.1±0.9^b	51.3±2.9
p values**									
Protein (%)	0.58	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01 (40<44<48)	<0.01	0.55
Lipids (%)	0.67	<0.01	<0.01	<0.01	<0.01	0.02	0.00 (16<12)	0.02	0.02 (12˃16)
PxL	0.28	<0.01	0.02	<0.01	<0.01	<0.01	0.63	0.02	0.61

* If an interaction between both factors is observed, differences were assessed using Duncan's multiple range test

** If a significant main effect occurred, the *p* value is reported, and within parentheses, the differences between levels of the factor are indicated

[1] Weigh gain

[2] Final body weight

[3] Specific growth rate

[4] Feed conversion rate

[5] Protein efficiency ratio

[6] Feed intake (g/fish/day)

[7] Feed intake (g/kg ABW/day)

[8] Apparent digestibility coefficient

Apparent digestibility coefficient (%)

ADC for the different dietary treatments resulted in significant effects for lipid but not for protein content or their interaction. Fish fed diets containing 12% lipids had significantly higher ADC values (mean 65.01 ± 8.4%) compared to those fed 16% lipid diets (mean 53.8 ± 8.8%) (Table 2).

Body condition indices

HSI values were significantly affected by dietary protein and lipid levels and their interaction. HSI values for fish fed the 12% lipid diets tended to decrease as protein content increased and at the 16% lipid level, HSI values tended to increase as protein content increased (Table 3). In contrast to what was expected, LSI and VSI values for treatments fed the 12% lipid diets tended to be higher than those for the 16% lipid diets. However, a significant interaction was found for fish fed the 16% lipid and 48% protein, potentiating and increasing both index values (3.7 ± 0.3 and 4.6 ± 0.4, respectively), resulting in similar values to treatments 40:12 (3.9 ± 0.2 and 3.9 ± 0.6, respectively), 44:12 (3.8 ± 0.2 and 4.7 ± 0.3, respectively), and 48:12 (3.5 ± 0.3 and 4.4 ± 0.4, respectively) (Table 3).

No significant effects of any factor (protein or lipid) or evidence of interaction between them were observed for the lipid content in the muscle of juvenile striped bass at the end of the feeding trial with values ranging from 15 to 19% (Table 5). Particularly interesting is that lipid content in the muscle was not significantly affected by the lipid level in the diets.

Enzyme activity

Trypsin activity in the pyloric caeca was not significantly affected by the levels of protein in the diet but was significantly influenced by lipid level and their interaction. The highest activity was observed in fish fed the 48:12 diet (893.5 ± 18.1 U/g organ), with no significant differences among the treatments 44:12, 40:12, and 44:16 (768.9 ± 61.7, 800.4 ± 97.1, 797.9 ± 213.4 U/g organ, respectively). On the other hand, trypsin activity in the intestine was significantly influenced by the inclusion levels of protein and lipid in the diet, with higher activity in fish fed 48% protein and 12% lipid. The interaction between both factors resulted in higher values for the treatments 40:12, 44:12, 48:12, and 48:16 (1113.7 ± 33.7, 1062.9 ± 39.5, 1087.3 ± 55.5, 1223.9 ± 16.2 U/g organ, respectively) (Table 4).

Chymotrypsin activity in the pyloric caeca of fish was significantly affected by protein and lipid levels in the diet. Significantly higher values were observed in fish fed the 12% lipid diets. The interaction between lipid and protein levels resulted in a significant increase in the activity for fish fed the 44:16 (8.7 ± 0.2 U/g organ) diets compared to the other protein levels at this lipid level. Similarly, in the pyloric caeca, chymotrypsin activity in the intestine was significantly influenced by the level of protein and lipid inclusion in the diet. In general, chymotrypsin activity was higher for fish fed the 12% lipid level except for the 48:16 treatment resulting in a significant interaction (Table 4).

The activity of L-aminopeptidase in the pyloric caeca showed significant differences with dietary protein and lipid levels. The highest activity was observed in fish fed the 48:12 (1007.7 ± 101.8 U/g organ) and the lowest activity in fish fed the 48:16 (469.2 ± 92.7 U/g organ) diet resulting in a significant interaction. In contrast, the

Table 3 Digestive enzyme activity of juveniles of striped bass feed the experimental diets (Units/g organ) after of 12 weeks of feeding*

Levels P:L	Trypsin (PC)	Trypsin (I)	Chymotrypsin (PC)	Chymotrypsin (I)	L-aminopep (PC)	L-aminopep (I)	Lipase (PC)	Lipase (I)
40:12	800.4 ± 97.1^{ab}	1113.7 ± 33.7^{ab}	10.3 ± 0.3^{b}	6.9 ± 0.6^{ab}	900.2 ± 55.9^{ab}	1450.1 ± 12.7^{c}	411.1 ± 10.8	897.2 ± 240.6^{ab}
44:12	768.9 ± 61.7^{ab}	1062.9 ± 39.5^{ab}	11.9 ± 0.2^{a}	6.2 ± 0.6^{b}	628.3 ± 38.1^{cd}	1745.8 ± 88.5^{b}	414.9 ± 45.4	1084.4 ± 87.0^{a}
48:12	893.5 ± 18.1^{a}	1087.3 ± 55.5^{ab}	12.2 ± 0.3^{a}	8.3 ± 0.2^{a}	1007.7 ± 101.8^{a}	1520.4 ± 85.9^{bc}	493.1 ± 83.2	979.4 ± 27.7^{ab}
40:16	572.3 ± 55.4^{b}	855.4 ± 62.9^{c}	3.1 ± 0.6^{e}	2.6 ± 0.6^{d}	691.3 ± 69.52^{bc}	1126.5 ± 95.2^{d}	175.3 ± 3.4	233.1 ± 39.1^{c}
44:16	797.9 ± 213^{ab}	624.9 ± 22.6^{d}	8.7 ± 0.2^{c}	4.3 ± 0.6^{c}	888.1 ± 101.7^{ab}	1001.1 ± 58.2^{d}	199.0 ± 15.6	189.5 ± 21.3^{c}
48:16	540.2 ± 58.1^{b}	1223.9 ± 16.2^{a}	4.2 ± 0.6^{d}	6.4 ± 0.5^{b}	469.2 ± 92.7^{d}	2217.2 ± 161.3^{a}	187.9 ± 15.6	727.6 ± 127.6^{b}
p values**								
Protein (%)	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	0.211	<0.01
Lipids (%)	0.00	<0.01	<0.01	<0.01	<0.01	0.01	<0.01 (16<12)	<0.01
P*L	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.5	<0.01

*If an interaction between both factors is observed, differences were assessed using Duncan's multiple range test

**If a significant main effect occurred, the *p* value is reported, and within parentheses, the differences between levels of the factor are indicated

Table 4 Indices juveniles of juveniles of striped bass fed the experimental diets for 12 weeks*

Levels of P:L	HSI[1]	LSI[2]	VSI[3]
40:12	3.6 ± 0.1^a	3.9 ± 0.2^a	3.9 ± 0.6^a
44:12	3.2 ± 0.3^b	3.8 ± 0.2^a	4.7 ± 0.3^a
48:12	2.4 ± 0.1^c	3.5 ± 0.3^a	4.4 ± 0.4^a
40:16	1.8 ± 0.1^d	2.4 ± 0.2^b	1.9 ± 0.1^b
44:16	$2.0 \pm 0.1^{c,d}$	2.7 ± 0.1^b	2.6 ± 0.1^b
48:16	2.3 ± 0.2^c	3.7 ± 0.3^a	4.6 ± 0.4^a
		p values**	
Protein	0.01	0.02	< 0.01
Lipid	< 0.01	< 0.01	< 0.01
P*L	< 0.01	< 0.01	< 0.01

*If an interaction between both factors is observed, differences were assessed using Duncan's multiple range test

**If a significant main effect occurred, the *p* value is reported, and within parentheses, the differences between levels of the factor are indicated

[1]Hepatosomatic index

[2]Liposomatic index

[3]Viscerosomatic index

highest L-aminopeptidase activity in the intestine was observed for fish fed the 48:16 (2217.2 ± 161.3 U/g organ) diet and the lowest values for fish fed the 40:16 (1126.5 ± 95.2 U/g organ) and 44:16 (1001.1 ± 58.2 U/g organ) diets resulting in a significant interaction (Table 4).

Lastly, lipase activity in the pyloric caeca was significantly influenced by lipid inclusion levels with higher activity levels observed for fish fed the 12% lipid diets (439.7 ± 18.42 U/g organ). However, lipase activity in the intestine was significantly affected by both lipid and protein levels and their interaction. The highest activity levels were observed in fish fed the 12% lipid diets with the exception of the 48:16 (727.6 ± 127.6 U/g organ), which showed similar activity levels to those of 40:12 (897 ± 240.6 U/g organ) and 48:12 (979.4 ± 27.7 U/g organ) treatments (Table 4).

Discussion

Finding the appropriate ratios among the nutrient components of fish feed is of utmost importance to achieve feed optimization, especially for vital components such as lipids and proteins in marine fish feeds (Halver and Hardy 2002; NRC 2011). Information with respect to lipid and protein requirements of striped bass is relatively scarce, with only a few available studies (Millikin 1982, 1983; Berger and Halver 1987). In the present study, juvenile striped bass fed diets containing 44% and 48% protein, with 12% lipids, exhibited higher WG (274.1 ± 18.4 and 306.8 ± 19.8), FBW (42.4 ± 1.2 and 47.1 ± 2.4), and SGR (1.6 ± 0.06 and 1.7 ± 0.06), respectively. The results obtained in this study are consistent with the existing literature, which generally establishes that protein requirement for carnivorous fish is relatively high ranging from 40 to 55% (Oliva-Teles et al. 2015). With respect to lipid level requirements, Millikin (1983) reported a 12% lipid level as adequate for the growth of 1.4 g striped bass reared in brackish water (7 ppt) resulting in similar values

when fed diets containing 37%, 47%, and 57% protein at this lipid level. These findings support the results obtained in our study, with the exception of the 37:12 diet, since the 40:12 in our study did not perform as well as the higher protein diets at this lipid level. The better performance with lower protein content (37%) in the former study was probably related to the fish being cultured closer to their isotonic point and the amount of energy needed for osmoregulation (Shi et al. 2010; Hassan et al. 2013; Wu et al. 2021). Thus, it appears that diets with protein concentrations of 44–48% and 12% lipid promote adequate growth for juvenile striped bass cultured in full-strength seawater at 21 °C.

FCR of fish fed the 12% lipid diets showed values closer to 1 with better conversion compared to diets containing 16% lipid, except for the fish fed diet 48:16 (1 ± 0.03). A similar trend was observed for PER, where fish fed diets containing 12% lipids utilized the protein more efficiently compared to those fed with 16% lipids, except in the case of fish fed diet 48:16 (2.1 ± 0.1) resulting in a significant interaction in both measurements of feed efficiency. This effect could be attributed to a phenomenon called protein-sparing effect, which involves the use of non-protein energy to meet the organism's energy requirements, resulting in a reduction in amino acid catabolism and improvement in indices such as FCR and PER (Bureau et al. 2002; NRC 2011; Gro-Ingunn and Dong-Fang 2015). It is interesting to note that a strong relationship between P:E ratios of the diets and SGR, PER, and FCR was observed (Fig. 1) which helps explain the interaction between protein and lipid in the present study which strongly suggests that more than protein and lipid levels an adequate P:E ratio in the diet is desired for improving feed utilization and growth. Using a second-order polynomial regression between P:E and PER, an optimal P:E ratio of
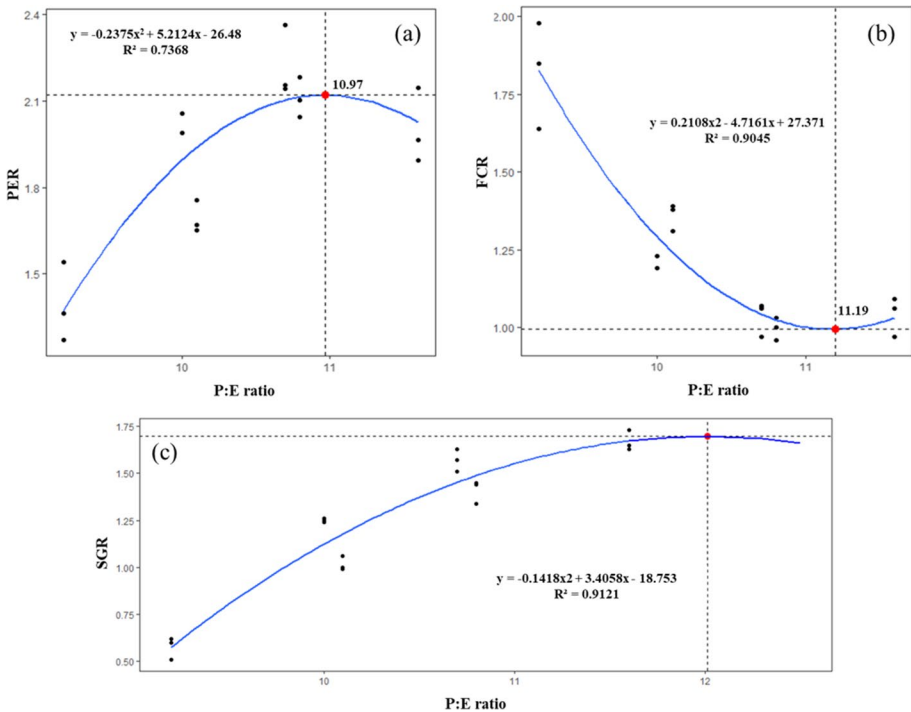

Fig. 1 Second-order polynomial regression between P:E and PER (**a**), P:E and FCR (**b**), P:E and SGR (**c**)

10.97 or higher was estimated, while P:E and FCR, and optimal P:E ratio of 11.19 was estimated, and if SGR is used, an optimal P:E of 12 is desired (Forster 2000). Thus, a P:E ratio between 11 and 12 is recommended, which can be achieved with formulations with a protein content between 44–48% and 12% lipid or the 48:16 diet. Nonetheless, the relationship between the P:L ratio in the diets as used in our experimental design was adequate to evaluate protein sparing and fish performance. We propose that P:L ratios might help explain many differences in reported results among studies in the literature, including incongruities and, in particular, many of the interactions among nutrients in our dietary treatments as previously discussed.

When expressing FI in (g/fish/day), fish fed diets containing 12% lipids consumed more feed (between 0.27 ± 0.01 and 0.39 ± 0.04 g/fish/day) compared to those fed with diets containing 16% lipids (between 0.13 ± 0.01 and 0.27 ± 0.03 g/fish/day). This trend has been reported in species such as cobia (*Rachycentron canadum*) (Wang et al. 2005), ussuri catfish (*Pseudobagrus ussuriensis*) (Wang et al. 2012), giant croaker (*Nibea japonica*) (Han et al. 2014), largemouth bass (*Micropterus salmoides*) (Li et al. 2020a, b), and northern whiting (*Sillago sihana*) (Liu et al. 2020). The amount of energy contained in the diet is one of the most important regulatory factors for feed consumption in fish (Hemre et al. 1995; De la Higuera 2001). Generally, when fish are fed high-energy diets, they exhibit lower feed intake. Additionally, in most species, feed consumption and weight gain are linearly and positively correlated in fish (Silverstein 2006). In other words, the low feed intake in organisms fed the high-energy diets results in lower WG, FBW, and SGR indices.

There are different methodologies to estimate digestibility coefficients of feds and ingredients, as well as the methods to collect feces (Tacón and Rodrigues 1984; Vandenberg and De La Noüe 2008). In the present study, the indirect method with AIA (acid-insoluble ash) was used as an inert marker, which has been widely used in previous studies with good results as long as sufficient ash (i.e. > 10%) is present in the feed samples for the analysis (Fuentes-Quesada et al. 2018; Madrid et al. 2022; Garnica-Gómez et al., 2023). The highest values of ADC of the diet ($65.01 \pm 8.4\%$) were observed for the 12% dietary lipid levels significantly higher compared to the ADC for the 16% diets ($53.8 \pm 8.8\%$). Similar results have been reported for the kelp grouper (*Epinephelus bruneus*), rainbow trout (*Oncorhynchus mykiss*), pacu (*Piaractus mesopotamicus*), and grass carp (*Ctenopharyngodon idella*), where, in general, apparent digestibility coefficients (% ADCs) were higher in diets with lower lipid levels (Abimorad and Carneiro 2007; Yoshii et al. 2009; Gumus and Ikiz 2009; Köprücü 2012). Although dietary nutrients such as lipid and protein content can modify ADC of the diets, these effects are species specific (Yoshii et al. 2009) and protein content in the present study did not significantly affect ADC values of the diets. Nonetheless, based on the ADC of the low lipid content diets, the hypothesis that the striped bass requires and performs better with low lipid diets (i.e., 12%) is reinforced, and possibly even lower than the 12% used in the present study and warrants further evaluation.

Both protein and lipid content in the diet and their interaction had a significant effect on the activity of digestive enzymes. Activity for trypsin, chymotrypsin, L-aminopeptidase, and lipases, in both intestinal regions, the pyloric caeca and intestines, was generally higher in fish fed diets with 12% lipid which helps explain the higher ADC at this lipid level. With respect to protein level, fish fed the 48% diets tended to have higher activity at the 12% lipid level but not at the 16% lipid. The relationship between digestive enzyme activity and protein and lipid content in the diet is well-documented in fish studies (Wang et al. 2006; Arslan et al. 2012; Yu et al. 2012; Ma et al. 2019; Wang et al. 2018). However, the significant interaction between both factors might be indicative of an unbalanced P:E ratio, which could suppress amino acid absorption and metabolism (Morais et al. 2007). This would
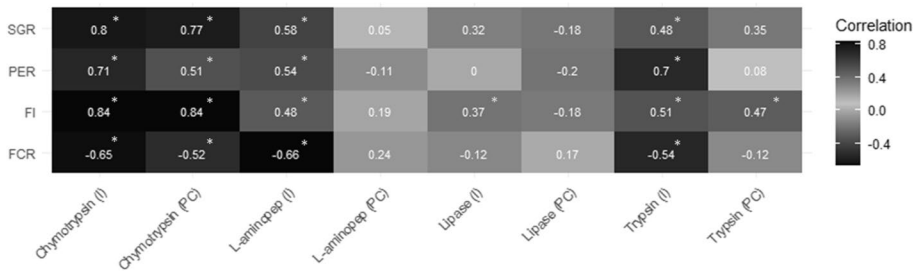
Fig. 2 Heat map illustrating the correlations between enzymatic activities and variables related to food utilization (PER, FCR), FI, and SGR. Colors closer to black indicate a Spearman correlation closer to 1 or −1, while statistically significant correlations ($p < 0.05$) are marked with an *

Table 5 The proximate composition (%) of juveniles of striped bass fillet fed the experimental diets for 12 weeks*

Levels of P:L	Protein	Lipids	Ash	NFE
Initial	63.16±4.77	14.96±0.17	6.39±0.02	15.49±0.2
40:12	69.0±2.8	18.3±2.7	11.1±0.4	1.6±0.4
44:12	68.9±2.9	17.2±2.8	13.6±2.5	1.5±0.9
48:12	69.9±3.2	17.2±2.4	11.1±2.1	0.6±0.8
40:16	68.7±1.8	16.9±2.3	14.1±1.1	0.9±0.3
44:16	68.1±4.9	17.1±2.2	13.6±0.3	1.2±0.4
48:16	68.2±5.0	15.6±1.8	13.3±1.2	2.9±1.2
		$p$values**		
Protein	0.06	0.54	0.36	0.60
Lipids	0.34	0.32	0.06	0.38
P*L	0.06	0.80	0.39	0.06

*If an interaction between both factors is observed, differences were assessed using Duncan's multiple range test

**If a significant main effect occurred, the p value is reported, and within parentheses, the differences between levels of the factor are indicated

explain why, in some cases, there are no significant differences between treatments with different P:L ratios. For example, trypsin activity (I) in treatments 40:12 (1113.7±33.7), 44:12 (1062.9±39.5), and 48:12 (1087.3±55.5) were not significantly different from the estimated activity for fish fed the 48:16 diet (1223.9±16.2).

Digestive enzymes such as chymotrypsin (PC and I), L-aminopeptidase (I), and trypsin (I) were positively correlated with FI, suggesting that an increase in feed consumption stimulated the activity of these enzymes (Fig. 2). In addition, as their activity increased, indices such as PER (strong positive correlation) and FCR (strong negative correlation) improved significantly (Fig. 1). It has been demonstrated that enzymes such as trypsin and chymotrypsin are positively related to feed utilization and fish growth and are reliable indicators for assessing diet quality (Rungruangsak-Torrissen et al. 2006), confirming that a feed formulation with 48% protein and 12% lipids is adequate for this species under our cultured conditions.

Contrary to what was expected, no significant differences were observed in fish muscle lipid content among dietary treatments, but low lipid diets tended to increase lipid content in the muscle (Table 5). It is well documented for many species that the higher the lipid content of the diet, the higher the lipid content in the muscle, as reported for the Atlantic salmon (*Salmo salar*) (Sheehan et al. 1996; Hemre and Sandnes 1999), tambatinga (*Colossoma macropomum* x *Piaractus brachypomus*) (Welengane et al. 2019), hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus polyphekadion*) (Xie et al. 2020) and Japanese seabass (*Lateolabrax japonicus*) (Xie et al. 2021). The lower feed intake and lower digestibility of the high lipid diets could help explain this discrepancy; nonetheless, further research is warranted to elucidate the lipid metabolism for striped bass with low and high lipid diets. Another important observation is that as the protein content in the diet increased, the proportion of liver size relative to intraperitoneal fat decreased. This may indicate an excess of energy relative to the amount of protein provided in the diets, which likely results in excessive accumulation of intraperitoneal fat in lieu of muscular tissue. Therefore, it is recommended to reduce lipid levels in the diets for this species grown at 21 °C in seawater (Webster et al. 1995; Mohanta et al. 2008; Gómez-Requeni et al. 2013; Han et al. 2014).

In the present study, the HSI tended to decrease as the lipid concentration in the diets increased and the protein concentration decreased. Similarly, the LSI decreased as protein content increased in fish fed the 12% lipid diets, while the opposite was observed at the 16% lipid level. Previous studies have reported an increase in LSI and HSI in fish fed high-lipid diets. This trend has been reported in juvenile haddock (*Melanogrammus aeglefinus* L.) (Nanton et al. 2001; Lee and Kim 2005; Tibbets et al. 2005) and in juvenile cobia (*R. canadum*) (Wang et al. 2005). However, other studies have reported a decrease in these indices for the giant trevally (*Caranx ignobilis*), grass carp (*C. idella*), turbot (*Scophthalmus maximus*), and sea bass (*Dicentrarchus labrax*) (Peres and Oliva-Teles 1999; Du et al. 2005; Liu et al. 2014; Nguyen et al. 2022), which is consistent with the results obtained in the present study. Furthermore, Nguyen et al. (2022) and Peres and Oliva-Teles (1999) suggested that the decrease in this index may be related to a positive correlation between hepatic glycogen and dietary carbohydrate levels. In the present study, cornmeal was used as a filler ingredient, which is rich in carbohydrates (68 g/100 g). The increase in carbohydrates in low-lipid and low protein diets could explain the decrease in HSI observed.

Conclusion

In conclusion, based on the results from the present study, it appears that juvenile striped bass raised at 21 °C and in saltwater can meet their nutritional requirements with a diet containing 48% protein and 12% lipids. The low lipid diets (12%) and high protein content (48%) promote increased FI (g/fish/day), ADC (%), FCR, and PER, and their interaction enhances feed utilization and biological performance. A strong relationship between P:E ratios of the diets and SGR, PER, and FCR was observed which helps explain the interactions between protein and lipid observed. An estimated P:E ratio of 11–12 is suggested for efficient protein and feed utilization for this species under our culture conditions. Additionally, reducing the lipid content in the feed does not significantly decrease the lipid content in the fish muscle at the end of the experimental period but rather tends to increase it, which is crucial for market acceptance of this species. Evaluating lower lipid levels in the diet is warranted, as we hypothesize that the requirement may be even lower than evaluated

in the present study. Additionally, the effect of P:E ratios when fish are cultured in lower temperatures (i.e., 16 °C) reflecting winter conditions needs to be evaluated.

Declarations

Ethics approval This research meets the ARRIVE, European Union Council (2010/63/EU), and Mexican Government (NOM-062—ZOO-1999) guidelines for the production, care, and use of experimental animals. Fish were humanely euthanized according to CICESE's fish culture animal-care standards and protocols (Fuentes-Quesada et al., 2018).

Competing interests The authors declare no competing interests.

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