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Effects of dietary protein-energy ratio on the growth and body composition of the white mullet *Mugil curema* (Valenciennes, 1836) (Pisces: Mugilidae)

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Abstract: The white mullet (*Mugil curema)* is an important resources exploited by coastal artisanal fishing in the Mexican Central Pacific. There is still not enough information about its nutritional requirements, in particular the appropriate protein-energy ratio of the diet, which has a significant influence on health and growth. The present study aimed to determine the protein-energy ratio of inert diets associated with greater growth in juveniles of *M. curema* under captive conditions. Eight experimental diets with different concentrations of proteins and lipids (P/L, %) were designed: 35/8, 40/8, 45/8, 50/8, 35/12, 40/12, 45/12, 50/12. Eight juveniles of *M. curema*, with an average weight of 5.7 ± 2.0 g and 7.9 ± 0.9 cm in length, were randomly placed in 24 experimental units of 0.5 m^2 each to evaluate the eight diets in triplicate. The fish were fed to satiety three times a day for 60 days. Growth parameters, diet digestibility and survival rate were measured for each treatment to determine the protein-lipid ratio with the best effect on growth. Results suggest that 40% crude protein and 8% crude fat promote adequate growth in juveniles of *M. curema*. Higher levels of protein and/or lipids tend to generate a negative effect on growth.

Key words: Protein efficiency, Energy efficiency, Digestibility, Meat quality, Tropical aquaculture.

Efectos de la relación proteína-energía sobre el crecimiento y la composición corporal de la Lisa blanca, *Mugil curema* Valenciennes, 1836 (Mugilidae). Resumen: La lisa blanca o criolla *Mugil curema* es un recurso importante de la pesca artesanal ribereña en el Pacífico Central Mexicano. Existe una carencia de información sobre sus requerimientos nutricionales, y en la relación proteína–energía, que es un indicador que se refleja en salud y crecimiento. El objetivo de este estudio fue determinar la relación proteína–energía en dietas inertes, que promueva un mayor crecimiento en juveniles de *M. curema*. Se diseñaron ocho dietas experimentales con diferentes niveles de proteína y lípidos (P/L, %): 35/8, 40/8, 45/8, 50/8, 35/12, 40/12, 45/12, 50/12. Se colocaron aleatoriamente ocho juveniles con un promedio 5.7 ± 2.0 g de peso y 7.9 ± 0.9 cm de longitud, en 24 unidades experimentales de 0.5 m^2, alimentando a saciedad tres veces al día durante 60 días. En cada tratamiento se midieron los parámetros de crecimiento, la digestibilidad de la dieta y la supervivencia, para determinar la relación proteína – lípidos con mejor efecto sobre el crecimiento. Los resultados sugieren que un 40% de proteína cruda y 8% de grasa cruda, promueven un adecuado crecimiento en juveniles de *M. curema*, los mayores niveles de proteína y lípidos tienden a generar un efecto negativo en el crecimiento.

Palabras clave: Eficiencia proteica, Eficiencia energética, Digestibilidad, Calidad de carne, Acuicultura tropical.

Introduction

Mugil curema (Valenciennes, 1836), known as white mullet, is a euryhaline and eurythermal fish that belongs to the Mugilidae family, which lives mainly in coastal waters of temperate and tropical regions throughout the world (Cabral-Solís *et al.* 2010; Ruiz-Ramírez *et al.* 2017). It is a catadromous species, with a high capacity to adapt to brackish and freshwater environments (Gallardo-Cabello *et al.* 2005). *M. curema* inhabits lagoon systems and bays in several areas of the Mexican central Pacific, representing one of the most important resources exploited by coastal no industrial fishing (Ruiz-Ramírez *et al.* 2017). In the wild, these organisms feed mainly on microalgae, diatoms, copepods, detritus and sediment particles. The variable availability of these resources causes fluctuations in the growth of the species throughout the year (Sánchez-Rueda 2002; El-Dahhar 2007; Mondal *et al.* 2015); i.e., in 1999 Mexico reported an annual catch of 219 tons of white mullet in the Mexican Pacific with average size larger than 30 cm of length (Cabral-Solís *et al.* 2010), and more recent reports suggest that during the 2010's, annual catch of this fish averaged 12,000 tons with maximum length of 28 cm per fish in the same area (Salgado-Cruz *et al.* 2021). There is still no industrialized aquaculture production for this species, but its reproduction in captivity has recently been mastered (Salgado-Cruz *et al*. 2021), and its production in polycultures with white shrimp *Litopenaeus vanammei* is being analyzed in Mexico (Viera *et al*. 2021).

Protein is one of the most important nutrients for growth. It is also the most expensive dietary macroconutrient, usually accounting for more than 60% of the diet costs (Tacon & Metian 2008). It is worth noting that the experiments conducted to determine the nutritional requirements of aquatic species seek to establish the minimum amount of nutrients required for the synthesis of muscle and tissue and for maximizing growth (Rueda-López *et al.* 2011; NRC 2011). Dietary energy can also be a critical factor since high energy levels can reduce nutrient intake, while low energy levels can cause protein to be used as an energy source to meet the energy requirements of basal metabolism (Pérez-Velázquez *et al.* 2015). It is greatly important to optimize the ratio between these nutrients, minimizing the use of proteins while also meeting the energy requirements of growth to reduce production costs and prevent the quality of culture water from deteriorating.

The objective of the present study was to evaluate the effect of different proteins to lipids ration in the diet of *M. curema* on growth, survival rate, and body composition.

Materials and Methods

Collecting fish specimens: Two-hundred juveniles of *Mugil curema* were collected from the "El Quelele" lagoon in the state of Nayarit, Mexico, with an average weight of 5.4 ± 1.5 g and an average length of 7.5 ± 0.5 cm. The fish were collected through artisanal means using a cast net. They were later put in a 1000 L container with water from the lagoon (salinity at 33 UPS) and constant aeration for transfer to the University Center of the Coast of the University of Guadalajara (UdeG) in Puerto Vallarta, Jalisco, Mexico.

After the transfer, they were placed inside a 1700 L container with clean water at 33 UPS (Unit practice of salinity) in the Laboratory of Water Quality and Experimental Aquaculture (LACUIC). The health status of the fish and their adaptability to conditions of overcrowding and controlled feeding. The fish stayed in the container for a quarantine period of 20 days, during which the health status of the fish and their adaptability to conditions of overcrowding and controlled feeding were observed. During the quarantine, the fish were fed a commercial pelleted diet for shrimp (Azteca®) with a content of 35% protein and 5% lipids.

Experimental design: The bioassay was carried out at LACUIC, using 24 plastic experimental units (EU) with a capacity of 600 L, connected to a seawater recirculation system (salinity at 33 UPS). Each container had its own aeration system and continuous water flow.

Eight juveniles of *M. curema*, with an average weight of 5.7 ± 2.0 g and a length of 7.9 ± 0.9 cm, were placed inside each EU. The experimental diets were distributed manually three times a day (09:00, 12:00, 18:00 h) for 60 days, offering them until apparent satiety. Each treatment was evaluated in triplicate. The temperature and oxygen of each container were recorded daily with an oximeter (YSI550®), salinity with a refractometer, and pH with a potentiometer (Ohaus®). During the 60 days of the bioassay, temperature was maintained at an average of 29.0 ± 1.0°C, salinity was maintained within a range of 30 to 35, dissolved oxygen in the water was maintained at an average value of 4.3 ± 0.5 mg L^{-1} and pH was maintained at an average value of 7.3 ± 0.8, all parameters with exception of temperature (±7°C higher) were similar to Benetti & Fagundes-Netto (1991).

Experimental diets: Eight diets were formulated using four concentrations of protein (35, 40, 45 and 50%), each with two concentrations of lipids (8 and 12%). Fishmeal was used as a protein source and fish oil as a lipid source. All diets had equal inclusion levels of a premix of vitamins and minerals (Table I). To prepare the diets, the macronutrients were first mixed in a blender (Blazer® 5 L). The micronutrients, fish oil and corn starch (previously hydrated) were then added. Gelatin was added at the end until obtaining a homogeneous mixture. A meat grinder (Torrey®) with a 3 mm die to make the pellets, which were then placed in metal trays in a drying oven (Novatech®) at a temperature of 60°C for 24 h. Afterward, the pellets were left to cool at room temperature, placed in previously labeled plastic bags and kept under refrigeration at 2°C until later use.

Table I. Ingredients and proximate composition of the experimental diets with different concentrations of proteins (35, 40, 45 and 50%) and lipids (8 and 12%) during 60 days of experimental feeding of *Mugil curema* juveniles.

	Experimental treatments (protein/lipids)							
INGREDIENTS	35/8	40/8	45/8	50/8	35/12	40/12	45/12	50/12
Fish Meal (FM)*	440.4	516	591.5	667.1	440.4	516	591.5	667.1
Corn flour	160	160	160	160	160	160	160	160
Fish oil**	43.7	38	32	26	83.3	77.5	71.7	65.9
Cornstarch	268.5	198.6	129.1	59.5	229	159.1	89.4	19.6
Gelatin	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Mix of vitamins & minerals (Rovimix)***	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Vitamin C	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sodium benzoate	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Alpha Tocopherol	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (% of dry matter)								
Crude protein (%)	35.6	41	46.1	51.2	35.2	40.8	46.5	51
Crude fat (%)	7.8	8.5	8	7.5	11.9	12	11.4	11.3
Ash (%)	14	13.2	18.6	18.8	14.3	15.4	16.2	16.1
ELN ▲	42.5	37.3	27.3	22.8	38.6	31.8	26	21.6
Digestibility****	79.1 ± 1.8[a]	82.4 ± 3.2[a]	81.7 ± 2.9[a]	76.1 ± 2.4[a]	67.8 ± 3.5[b]	67.5 ± 4.2[b]	62.5 ± 5.5[b]	51.3 ± 6.3[c]
Net energy (Mj kg^{-1})*****	19.0	19.7	19.0	19.3	19.9	20.1	20.2	20.5
P/E (g MJ^{-1})	8.2	10.7	14.3	17.7	7.8	10.5	13.6	16.6

*Fishmeal (59.9% crude protein and 6.9% crude fat) from Proteínas Marinas y Agropecuarias, Guadalajara, Mexico.
**Sardine oil, Marine and Agricultural Proteins, Guadalajara, Mexico.

*** Rovimix® 25% For tropical fish; Vitamin-C, SeenZoo animal nutrition Guadalajara, Mexico. In summary: (mg kg^{1}) inositol, 256.39; choline chloride, 149.78; niacin, 51.28; riboflavin, 30.0; aminobenzoic acid, 25.53; pantothenic acid, 17.92; b-carotene, 9.39; menadione, 6.11; thiamine–HCl, 3.85; pyridoxine, 3.06; folic acid, 0.96; biotin, 0.39; cholecalciferol, 25,793 IU; a-tocopherol, 25,643 IU; vitamin B_{12}, 5.59; $MgSO_4.7H_2O$, 284; $FeSO_4$, 40; $CuSO_4$, 26; $MnSO_4$, 6.6; $ZnSO_4$, 132; CoC_{l2}, 0.04; KI, 0.6; $NaSeO_3$ 0.02; $Ca(H_2PO_4)_2$, 203.2; diatomaceous earth, 307.54.
▲The free nitrogen extract (ELN) was calculated as follows: free nitrogen extract (%) = 100-(A+B+C) where: A: crude protein content (%) B: crude lipid content (%) C: ash content (%).
****Different superscript letters indicate statistical differences (P<0.05).
*****Based on protein 23.4 MJ kg^{-1}, lipid 39.2 MJ kg^{-1}, and ELN 17.2 MJ kg^{-1}.

Chemical composition of the diets and fish: The chemical composition of the diets and of the initial and final whole fish samples was evaluated in triplicate on dry matter in the LACUIC, following the method proposed by the Association of Official Analytical Chemists (AOAC 1995).

Total nitrogen content was determined using the Kjeldahl method, while the percentage of crude protein was calculated as %N × 6.25. Lipid content was determined using the Soxhlet extraction method with hexane as extraction solution. Ash content was determined by calcining the sample in a muffle (Terlab®) at 550°C for 8 h. The nitrogen-free extract was calculated by the difference in dry matter, including soluble and insoluble carbohydrates (NFE (%) =100 – (% crude protein + % total lipids + ash)).

Digestibility of the diets: In the last 15 days of experimentation, feces were collected, 120 min after feeding, from each of the EUs to measure the digestibility of the food. Feces were collected from the bottom of the EU and placed in an aluminum tray. They were rinsed with distilled water to remove salts, then placed in a freezer (-4 °C) until later use. Uneaten food was processed in the same way but it was collected 30 min after feeding. The apparent digestibility coefficient (ADC) of the nutrients in the feed and in the feces was determined using the technique described by Tejeda de Hernández (1992) and modified by Montaño-Vargas *et al.* (2002), in which acid-insoluble ash (AIA) is used as an internal marker in *in vivo* assays.

- Acid-insoluble ash (AIA) was determined using the following formula:
- AIA (%) = (ash weight g − crucible weight g) / dry sample g) × 100
- Apparent digestibility (AD) was determined using the following formula:
- AD (%) = 100 − (100 × % ash in feed / % ash in feces)

Growth parameters: To determine growth, the organisms were anesthetized with an infusion of clove oil (1 g L^{-1}) following the method proposed by Javahery *et al.* (2012). They were individually weighed on a digital scale (accuracy of ± 0.05 g). The total length was determined using an ichthyometer.

The following indexes were calculated:

- Weight increase (WI, g): WI (%) = final weight × 100 / initial weight
- Specific growth rate (SGR, % d^{-1}); SGR = (ln final weight – ln initial weight) / time × 100
- Thermal growth coefficient (TGC); TGC = (($\sqrt[3]{}$ (final weight(g))-$\sqrt[3]{}$ (initial weight(g))) / (mean water temperature (°C) × days)) × 1000
- Survival rate (S, %): S = N° of final fish × 100/N° of initial fish.

Statistical analysis: The normality and homogeneity of the results were verified, and they were subjected to a factorial analysis (two-way ANOVA) using the statistical program SigmaPlot11. When significant differences were found between the treatments, a Tukey's multiple range test was applied. The statistical significance of all applied tests was = 0.05.

Results

The evaluated treatments were associated with significant differences (P < 0.05) in the final weight of the fish. The 40/8 treatment (protein and lipids, respectively) was the one that yielded the highest growth. No statistical differences in the SGR were observed between treatments (P > 0.05). Regarding the after 60 days, the 50/12 treatment was the worst with 77.7% (Table II). WI showed statistical differences (P < 0.05) between treatments. The treatment with the highest WI was the one with 40% protein and 8% lipids and 34.3 ± 1.7 g ind^{-1} (Table II). An increase in the content of crude protein from 45 to 50% was associated with a decrease in WI and the concentration of lipids (Table II).

Statistical differences were observed (P < 0.05) in the digestibility of the diets between treatments with different lipid concentrations. In other words, diets with 8% lipid content and different concentrations of protein content showed higher digestibility values, compared to diets with

Table II. Biological indexes of *Mugil curema* juveniles fed with different concentrations of proteins (35, 40, 45 and 50%) and lipids (8 and 12%) during 60 d of experimentation. WI: weight increase; SGR: specific grown rate; TGC: Thermal growth coefficient. Different superscript letters indicate statistical differences (P<0.05).

Treatments	Initial weight (g ind^{-1})	Final weight (g ind^{-1})	Initial length (cm)	Final length (cm)	WI (%)	SGR (% d^{-1})	TGC	Survival (%)
35/8	5.7 ± 2.0	30.4 ± 3.4^{a}	7.9 ± 0.9	13.1 ± 1.0	533.1 ± 34.0^{a}	2.7	0.07	100
40/8	5.7 ± 2.1	34.3± 1.7^{a}	7.9 ± 0.1	13.6 ± 0.6	601.5 ± 23.8^{a}	3.0	0.08	100
45/8	5.7 ± 2.2	30.5± 3.5^{a}	7.9 ± 0.1	13.3 ± 0.6	534.6 ± 14.4^{a}	2.5	0.07	100
50/8	5.7 ± 2.3	29.2± 4.5^{ab}	7.9 ± 0.1	13.3 ± 0.9	512.4 ± 52.2^{ab}	2.6	0.07	100
35/12	5.7 ± 2.4	25.1± 4.0^{b}	7.9 ± 0.1	12.7 ± 0.8	444.0 ± 33.0^{b}	2.5	0.06	100
40/12	5.7 ± 2.5	24.8± 6.6^{b}	7.9 ± 0.1	12.9 ± 1.1	425.3 ± 74.6^{b}	2.4	0.06	100
45/12	5.7 ± 2.6	21.1± 4.6^{b}	7.9 ± 0.1	12.3 ± 1.0	368.0 ± 64.6^{b}	2.1	0.05	83.3
50/12	5.7 ± 2.7	23.7± 4.7^{b}	7.9 ± 0.2	12.7 ± 0.8	406.9 ± 72.3^{b}	2.3	0.06	77.7

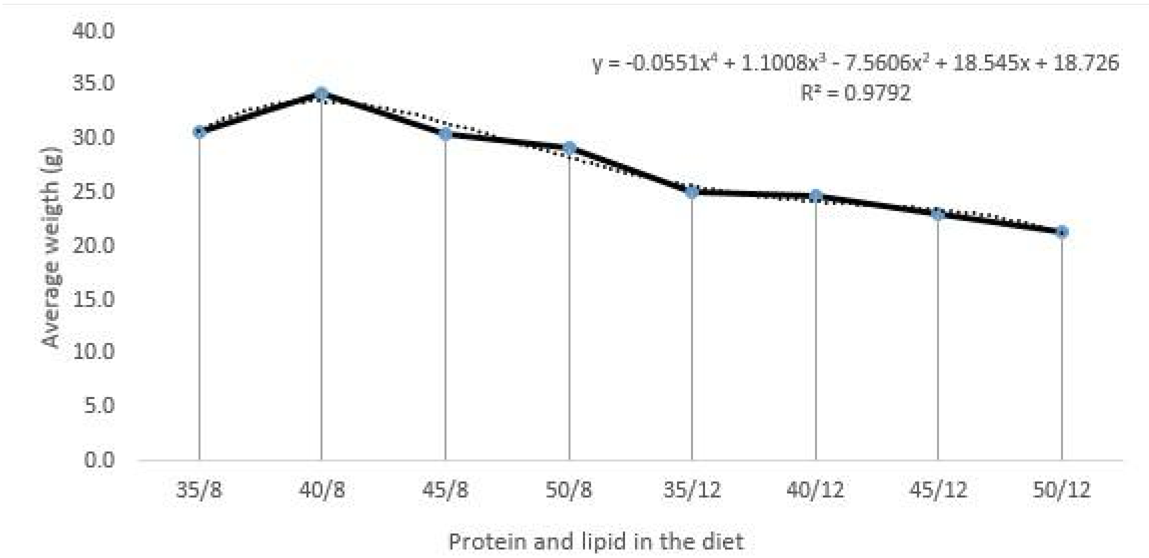

Figure 1. Relationship between average weight and the content of protein and lipids in the diet for juveniles of *Mugil curema* during 60 days of experimentation.

12% lipids, which explains the deficient growth promoting effect of the latter (Table I, Fig. 1).

Regarding the protein content in whole fish, there were statistically significant differences (P < 0.05) between treatments. The 35/12 treatment was associated with lower protein content, with 53.7 ± 3.0%, compared to the other treatments (Table III). Regarding the fat content in whole fish specimens, there were also statistically significant differences (P < 0.05) between treatments. The treatment with 50/12 was associated with the highest total body fat composition (35.6 ± 1.0%) (Table III).

Discussion

Many studies have analyzed the effect of using different concentrations of proteins and lipids in diets for the Mugilidae family, evaluating the optimal ratio for promoting growth (Papaparaskeva-Papoutsoglou & Alexis 1986; Tamaru *et al.* 1993; El-Dahhar 2000, 2007, El-Dahhar *et al.* 2011; Carvallo *et al.* 2010; Gisbert *et al.* 2016). However, few studies focused on *Mugil curema* (Benetti & Fagundes-Netto 1991) have evaluated its nutritional requirements under controlled laboratory conditions. The present study is one of the first to do so.

In the first experiments with this family of fish and with juveniles of *M. capito*, Papaparaskeva & Alexis (1986) used concentrations of protein of

Table III. Proximate composition of initial and final whole fish samples of *Mugil curema* juveniles fed with concentrations of protein (35, 40, 45 and 50%) and lipids (8 and 12%) during 60 d of experimentation. Different superscript letters indicate statistical differences (P<0.05).

Experimental treatments	Crude protein (%)	Crude fat (%)	Ash (%)	ELN
Initial	63.6 ±1.7	20.3 ± 1.0	10.4 ± 0.5	5.7
35/8	55.9 ± 2.7[b]	22.6 ± 3.2[c]	13.5 ± 1.5	8.0
40/8	61.3 ± 3.6[a]	23.7 ± 3.4[c]	12.8 ± 2.3	2.2
45/8	58.5 ± 4.9[a]	23.6 ± 4.3[c]	13.6 ± 2.1	4.3
50/8	56.1 ± 3.4[ab]	32.7 ± 2.1[b]	9.5 ± 1.8	1.7
35/12	53.7 ± 3.0[b]	32.6 ± 1.0[b]	8.2 ± 1.6	5.5
40/12	57.9 ± 3.2[ab]	32.0 ± 1.2[b]	9.1 ± 1.4	1.0
45/12	58.2 ± 3.3[ab]	32.4 ± 1.0[b]	8.7 ± 2.5	0.7
50/12	53.5 ± 3.4[b]	35.6 ± 1.0[a]	8.6 ± 2.8	2.3

12, 24, 36, 48 and 60% and a concentration of lipids of 8%. They concluded that 24 and 36% protein was adequate to optimize growth and that higher levels of protein in the diet reduced growth and affected the health of organisms, increasing mortality. El-Dahhar (2000), studying *M. cephalus* larvae, evaluated its protein requirements (14, 18, 22, 26, 30, 34 and 38%) and 7% lipids, and found a positive relationship between weight increase and crude protein content increase from 14 to 26%. However, when protein content increased from 30 to 38%, fish growth decreased slightly compared to the growth associated with 26% crude protein. Both authors mention that the optimal concentration of protein in the diet can be lowered if the concentration of energetic nutrients in the diet is increased.

When comparing growth as a function of protein content with other species of the *Mugil* genus, the results of the present study showed that *M. curema* had higher protein requirements (40%), compared to the range of 30-36% for *M. cephalus* (El Dahhar 2000; De *et al*. 2012; Yones *et al.* 2019), *M. capito* (Papaparaskeva & Alexis 1986) and *M. platanus* (de Carvalho *et al.* 2010), all at different energy levels. Benetti & Fagundes (1991), also evaluated the effect on the growth of *M. curema* of balanced diets containing 32 and 36% crude protein. Their results showed that the fish grew 20% less with the diet containing 32% protein and that the best result was obtained with 36% protein. However, the authors suggested that their results could be incomplete due to the lack of experimentation with higher levels of inclusion of protein and lipids in the diet. The present study included higher protein levels in some treatments and found that fish growth was optimized at a slightly higher concentration (40% protein) than the level reported by Benetti & Fagundes (1991).

Results obtained about diet digestibility in this work agree with those of De *et al.* (2012), who found that diets for *M. cephalus* with 30 and 35% protein and 8% lipids had higher digestibility. Moniruzzaman *et al*. (2019) and Kasiga *et al.* (2020) mentioned that assessing the apparent digestibility of dietary nutrients is of great importance since this factor reflects the percentage of nutrients that are actually assimilated by the organisms. However, they also mentioned that few studies evaluate digestibility due to the difficulty of collecting feces.

High energy levels could cause a reduction in feed efficiency and increase the deposition of fat in the viscera, which could lead to fatty liver, as occurs in other species. El-Dahhar (2007), mentions that when mullets are subjected to overcrowding conditions in cultivation, they require certain levels of physicochemical parameters in the water, but the living space and the diet have a greater influence on growth and survival. Gisbert *et al.* (2016), evaluated the effect of substituting fishmeal with vegetable meals (mixture of corn gluten, wheat gluten and soybean meal) containing 36% protein and 16% lipids in the diet of juveniles of *M. cephalus*. They found that the content of protein and lipids did not affect growth and that up to 75% of fishmeal could be substituted with the mixture of vegetable meals.

The results obtained in the present study suggest that 40% crude protein and 8% crude fat are sufficient to promote adequate growth in juveniles of *Mugil curema*. Higher concentrations of protein and/or lipids can have a negative effect on the growth of juveniles of this species.

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Ethical statement

All authors express that collection of fish and the experimental trial was carried out in accordance with the code of ethics of the University of

Guadalajara and in accordance with national and international ethical guidelines.

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