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Research Article

Total or partial replacement of fishmeal with soybean meal in the diet of the Pacific fat sleeper *Dormitator latifrons* juveniles

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ABSTRACT. Groups of *Dormitator latifrons* in triplicate (4.1 ± 2.0 g and 6.2 ± 1.0 cm) were fed experimental diets containing four levels of substitution of fishmeal (FM) by soybean meal (SM) (0, 40, 70, and 100%, respectively). The diets were formulated to be isoproteic (35% crude protein) and isolipidic (8.0% crude lipids). The effect of each treatment on growth was evaluated and its implications on the cost of feeding. After 60 days of feeding, there were no significant differences in the fish's proximate composition ($P < 0.05$). There were no significant differences between the diets ($P < 0.05$) in the evaluated biological indices: total growth increase (TGI), specific growth rate (SGR), thermal growth coefficient (TGC), and survival (%). Feeding costs decreased significantly as the proportion of soybean meal in the diet increased. The results indicated that substituting FM by up to 100% of SM can promote adequate growth in *D. latifrons* without affecting body composition and survival while also reducing operative costs during the fattening process.

Keywords: *Dormitator latifrons*; Eleotridae; native fish; nutrition; growth; bioeconomics; aquaculture

INTRODUCTION

In Mexico, most studies of species with aquaculture potential have focused on marine species. In contrast, the inland aquaculture industry relies mostly on exotic species such as tilapia (*Oreochromis* spp.) and carp (Cyprinidae family). Native freshwater species with high productive potential have been poorly studied. One of these species is *Dormitator latifrons* (Richardson 1844), also called fat sleeper, chame, puyeque, popoyote, chococo, or chalaco. Belongs to the diadromous fish group, particularly to the amphidromous fishes, spawns in freshwater bodies; the larvae migrate to the sea and then return to freshwater, where they will spend the rest of their development (Milton 2009). It is distributed along with Pacific coastal areas and estuaries, from California (USA) to southern Peru. In Ecuador, *D. latifrons* is considered a fishery and aquaculture resource. That country has accumulated the greatest wealth of empirical knowledge on this species, as it is a key ingredient of traditional gastronomy and is cultivated from wild hatchlings. (Arriaga & Martínez 2003, Schwarz 2007, Rodríguez et al. 2012). In Mexico, there have been studies on the cultivation of *D. latifrons* (Basto-Rosales et al. 2019), on the quality of its meat (López-Huerta et al. 2018, Basto-Rosales et al. 2020), and the nutritional requirements of juvenile fish (Badillo-Zapata et al. 2018). However, alternative diets on the growth and production costs of this species have not been studied yet. Regarding its nutritional value, it was demonstrated that *D. latifrons* has good essential amino acid.

Numerous studies have focused on finding alternative ingredients as the main source of protein in fish feed, some of vegetable origin (soybeans, wheat, peas, grains or oilseeds, among others), others of animal origin (poultry meal, blood meal, feather meal) (Li et al. 2011, 2020, Badillo et al. 2014, Pares-Sierra et al. 2014, Tangendjaja 2015, Wang et al. 2017, Tran et al. 2019, Ye et al. 2019). Soybean meal (SBM) has

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been recognized as one of the most appropriate alternative protein sources for fishmeal in aquafeed because of its wide availability, sustainable supply chain, reasonable price, high protein content (45-50%), and adequate amino acid profile (Lin & Luo 2011). Soybean meal is a great candidate for substituting fishmeal for cultivated fish species (Wang et al. 2017, Ye et al. 2019). Finding cheaper alternative diets that do not affect the growth during cultivation is essential to consolidate the production of native fishes (Tran et al. 2019). Highlighting the importance of cost reduction by substituting some diet formulation ingredients with less expensive alternatives, but obtaining acceptable nutrition and growth results with partial or total replacement (Poot-López et al. 2010).

With the aim of beginning to lay the foundations for the future establishment of *D. latifrons* as a species with a commercial interest and sustainable rural production in Mexico, the present study evaluates the effect of the substitution of fishmeal by soybean meal on the growth, body composition, digestibility, survival, and feed costs associated with the juveniles of this native fish.

MATERIALS AND METHODS

Collection of specimens

Juveniles of *Dormitator latifrons* (200) were captured using a fishing net in the El Quelele Lagoon (20º43'25.43'' N, 105º18'03.63'' W), located in the state of Nayarit, Mexico. The fish were transported under constant aeration (4 mg O_2 L^{-1}) in a 400 L container to the Laboratory of Water Quality and Experimental Aquaculture (LACUIC) of the Centro Universitario de la Costa (CUCosta), in the University of Guadalajara (UdeG) in Puerto Vallarta, Jalisco, Mexico. Before distributing the fish in the culture tanks, they were subjected to an ectoparasite removal treatment with Dimilin® (diflubenzuron 22%), at a concentration of 300 µg L^{-1} diflubenzuron, in a 200 L container for 10 min. The fish were kept in a seven-day quarantine in 500 L reservoir tanks with constant recirculation. During this period, the fish were fed once a day with commercial feed containing 30% protein and 8% lipids (Purina®), dissolved oxygen was monitored daily using an oximeter (YSI® 550A).

Preparation of the diets

A completely randomized experimental design with four treatments (diets), each in triplicate, was used with the iso-nitrogenous (crude protein concentration, 35%) and iso-lipidic (lipid 8%) requirements proposed by Badillo-Zapata et al. (2018). Fishmeal was substituted by 0, 40, 70, and 100% of soybean meal (SM0, SM40, SM70, SM100, respectively) (Table 1). The ingredients were weighed on a precision balance (Nimbus® NBL8201e) and then mixed and homogenized (with the appropriate amount of water) in a mixer (Blazer Torrey® model B10); the resulting mixture was pelletized (3 mm pellets) in a mill (Torrey® model M-12-FS). The material was then dried in an oven (Novatech®) at 60°C for 24 h, packed in plastic bags, and stored at -20°C until use.

Design and experimental system

Fish were randomly distributed in 12 experimental units (EU), consisting of a 300 L plastic tank with constant aeration and 30% weekly water exchange. Each EU contained ten specimens with an initial weight of 4.1 ± 2.0 g and an initial size of 6.2 ± 1.0 cm. During 60 days, the organisms in each EU were fed twice a day (9:00 and 16:00 h) with the corresponding experimental diet; the amount of feed provided corresponded to 5% of each EU's total biomass. Feces and non-consumed food were removed daily (using a siphon) during the feeding period. Feces were later used to test the apparent digestibility of each diet. The photoperiod was set to 12:12 h (light:dark) using timers. Temperature, dissolved oxygen, and pH were daily monitored using an oximeter (YSI® 550A), averaging 27.7 ± 1.4°C, 3.84 ± 0.86 mg L^{-1}, and 7.87 ± 0.24, respectively, throughout the experiment. Two biometric measurements were performed. The total weight (g) was recorded at the beginning and the end of the experimental period, with a digital scale (Ohaus® PR2201) and total length (cm) with an ichthyometer; the length of each fish, was measured from the most anterior part of the head to the most posterior part of the tail fin.

Chemical analysis

Proximate composition of diets and whole organism were measured (in triplicate 10-15 g) for each treatment and experimental unit and expressed on dry matter basis according to standard procedures of the Association of Official Analytical Chemists (AOAC 1990). After 60 days of experimentation, all the fish in the EU were captured and subjected to a thermal shock by cold (Díaz-Villanueva & Robotham 2015). Six whole organisms aleatory per tank were pooled and blended for analysis. Total nitrogen content was determined using the micro-Kjeldahl method (Novatech® model KJR), and percent crude protein was then calculated as % N and multiplied by a factor of 6.25. Total lipids concentration was determined by the Soxhlet extraction method (Novatech®) using hexane as the carrier solvent, and the crude fat was calculated gravimetrically. Ash content was estimated by heating samples to 550°C for 6 h, using a muffle digital

Table 1. Ingredients, cost of the diets, feed expenses, proximate composition, and digestibility coefficient of the experimental diets with different substitution levels of fishmeal by soybean meal. The diets were used to feed juvenile *Dormitator latifrons* fish. Different letters in the same row indicate significant differences ($P < 0.05$). [1]Proteínas Marinas y Agropecuarias, S.A. de CV, Guadalajara, Jalisco, México. [2]Sistemas en Zootecnia S.A. de C.V., Tlajomulco de Zuñiga, Jalisco, México. [3]Input prices quoted by sellers and updated to 2020. *Based on 23.4 KJ g^{-1} of protein, 39.2 KJ g^{-1} of lipids, and 17.2 KJ g^{-1} of NFE. Digestibility coefficient five days before the end of the experimental period (%), values are presented as mean ± standard deviation (SD) (n = 3). For costs, total feed consumed, the total cost of feed and digestibility coefficient, different superscripts letter in the same raw indicate statistical differences ($P < 0.05$).

Ingredients	Experimental treatments			
	HS0	HS40	HS70	HS100
Fishmeal[1]	35.2	21.1	10.6	0
Cornmeal	8.6	8.6	8.6	8.6
Soybean meal[1]	0	20	35.1	50.1
Fish oil[1]	5.3	4.8	5.5	5.2
Corn oil	0	1.2	1.2	2.2
Cornstarch	41.1	34.4	29.3	24.2
Gelatin	6	6	6	6
Vitamin C[2]	0.4	0.4	0.4	0.4
Vitamins and minerals[2]	3	3	3	3
Sodium benzoate	0.2	0.2	0.2	0.2
Choline chloride[2]	0.1	0.1	0.1	0.1
Tocopherol	0.01	0.01	0.01	0.01
Total (g)	100	100	100	100
Cost per 100 g of diet (USD)[3]	0.27[a]	0.23[ab]	0.19[b]	0.16[b]
Total feed consumed (g)	594[ab]	621[b]	544.5[a]	580.5[a]
Total cost of feed (USD)	1.60[a]	1.41[a]	1.06[b]	0.94[b]
Proximate composition (% of dry matter)				
Total proteins (%)	35.1	35.3	36	34.6
Total fat (%)	7.9	7.6	7.6	7.9
Ash (%)	13.3	10.6	8.2	6.6
NFE	43.7	46.5	48.2	50.9
Net energy (KJ g^{-1})*	19.1	19.6	20.2	20.3
Digestibility coefficient (%)	64.0 ± 2.6[a]	63.3 ± 0.6[a]	93.2 ± 0.2[c]	85.9 ± 0.6[b]

(Novatech®). The nitrogen-free extract was calculate by differences of dry matter and include soluble and insoluble carbohydrates [NFE (%) = 100 – (% crude protein + % total lipids + % ash)].

Apparent digestibility of the diets

Each EU's bottom was cleaned after feeding, using a filtered siphon to avoid collecting food remnants to determine the diets' coefficient of apparent digestibility. Two hours later, feces were collected from the bottom of each EU and dried in an oven at 60°C for 24 h. The dried feces were then placed in plastic containers and refrigerated at -20°C until analysis. This method was repeated for 15 days until the end of the assay.

The apparent digestibility (AD) was determined using the insoluble acid ash (AIA) technique of Van Keulen & Young (1977), modified by Montaño-Vargas et al. (2002). The content of acid-insoluble ash was determined in both feed and feces, using the following formulas:

$$AD\ (\%) = 100 - \left(\frac{100 \times \%\ AIA\ feed}{\%\ AIA\ feces}\right)$$

$$AIA\ (\%) = \left(\frac{sample\ weight + ashes\ (g) - crucible\ weight\ (g)}{dry\ sample\ weight\ (g)}\right) \times 100$$

Growth indices

Growth indices were determined for each treatment, evaluating weight increase, survival, and the following biological indices, calculated using the formulas specified below:

Total growth increase (TGI) (%): $TGI = \left(\frac{weight_{final}(g)}{weight_{initial}(g)}\right) \times 100$

Growth rate (GR) (g d^{-1}): $GR = \left(\frac{weight_{final}(g) - weight_{initial}(g)}{Time(d)}\right)$

Specific growth rate (SGR) (% d^{-1}):

$$SGR = \left(\frac{\ln weigth_{final}\ (g)\ -\ \ln weight_{initial}\ (g)}{Time(d)}\right) \times 100$$

Thermal growth coefficient (TGC):

$$TGC = \left(\frac{\sqrt[3]{weight_{final}(g)} - \sqrt[3]{weight_{initial}(g)}}{mean\ water\ temperature\ (°C) \times time\ (day)}\right) \times 1000$$

Feeding costs

Feeding costs (Fc) were evaluated for each treatment to relate the effect on growth and production cost for the substitution of fishmeal by soybean meal on juveniles of *D. latifrons*. The prices of the inputs and the quantities required for the elaboration of the diets were quantified. The amount of feed provided to each treatment during the culture period was also quantified using the 5% of total biomass daily. The cost per gram of feed (Hc) was calculated for each treatment and multiplied by the amount of food (f) provided and the time (t). The formula used was as follows:

$$Fc = Hc \times (f \times t)$$

Statistical analysis

The data were subjected to homogeneity tests to decide whether to use parametric or nonparametric methods. One-way analysis of variance (ANOVA) was used to determine differences between treatments. When significant differences (P < 0.05) were found, a *post-hoc* Tukey's test was used to identify statistical differences in digestibility and the proximate analysis between treatments. A means contrast test was also performed. Statistical significance was set at P < 0.05, and the results are presented as mean ± standard deviation. All statistical analyzes were done using StatSoft® Statistica 12.0.

RESULTS

The biological indices used in this experiment did not show statistical differences between treatments (P < 0.05) (Table 2). However, fish fed with the HS40 diet showed the greatest increase in weight (9.7 ± 2.4 g) and whole carcass protein content (61.3 ± 0.9%), while fish treated with the HS0 diet was associated with the highest survival rate (96.7 ± 5.8%) (Table 2).

Regarding fat and ash content, fishes treated with the HS70 and HS100 diets showed significant statistical differences. The HS0 and HS40 diets were associated with the lowest fat percentages in fish (12.3 ± 0.3 and 12.3 ± 0.2%, respectively), while the lowest percentage of ash content was associated with the HS100 diet (12.3 ± 0.1%). The nitrogen-free extract (NFE) found in the fish did not show statistical differences; however, the HS0 diet was presented the highest percentage (13.7%), as shown in Table 3.

Significant differences were also found in the coefficient of apparent digestibility (P < 0.05). At the end of the 60 days the experimental feeding period, the HS0 and HS40 diets (64.0 ± 2.6 and 63.3 ± 0.6%, respectively) showed significantly different results than the other diets, with the HS70 and H100 diets showing the highest digestibility values (93.2 ± 0.7 and 85.9 ± 0.6%, respectively) (Table 1).

Feeding costs decreased significantly as the percentage of soybean meal increased (Table 1). The fish under the HS40 treatment consumed the largest feed during the experimental feeding period (621 g), while the lowest feed consumption corresponds to the fish under the HS70 treatment (544.5 g).

DISCUSSION

Generally, freshwater fish species have been associated with good results in terms of growth, survival, and feed yield when provided with vegetable protein sources at substitution levels of 80% or even 100% (Zhao et al. 2016), while marine species tolerate only low replacement levels (Wang et al. 2017).

Soybean meal is widely used as the primary protein source in different formulations due to its nutritional composition, ample availability, and low cost (Gatlin et al. 2007, Tangendjaja 2015, Wang et al. 2017, Bruce et al. 2018, Ye et al. 2019). Numerous studies have demonstrated the feasibility of substituting fishmeal in the diet of various fish species of commercial interest without affecting their growth or productivity (Elangovan & Shim 2000, Heikkinen et al. 2006, Bruce et al. 2018, Zhang et al. 2018). However, the availability and utilization of nutrients in fish feed vary with the type of processing methods used to produce soybean meal (Tangendjaja 2015, Bruce et al. 2018). These differences might be associated with anti-nutritional components such as trypsin inhibitors, phytic acid, and saponins, among others, which can have adverse effects on nutrient digestibility and assimilation, affecting fish growth (Gatlin et al. 2007, Wang et al. 2017).

One method to verify the quality of a diet ingredient is to carry out controlled feeding studies with the species of interest, testing the effects of increasing amounts of the ingredient (Tangendjaja 2015). Like *Dormitator latifrons* in this research, omnivorous fishes had shown great acceptance to fishmeal substitution with soybean meal. Studies like those of Lin & Luo (2011) on *Oreochromis niloticus*, with 100% substitution; and Zhang et al. (2018) on *Lateolabrax japonicus*, with 70% substitution; showed an inverse

Table 2. Biological indices of juvenile *Dormitator latifrons* fish fed with different levels of substitution of fishmeal by soybean meal. GR: growth rate, SGR: specific growth rate, TGC: thermal growth coefficient. Different letters in the same row indicate significant differences ($P < 0.05$). Values on the same line with different letters show statistically significant differences ($P < 0.05$).

Biological indices	Experimental treatments			
	HS0	HS40	HS70	HS100
Initial weight (g)	4.1 ± 2.0	4.1 ± 2.0	4.1 ± 2.0	4.1 ± 2.0
Final weight (g)	9.1 ± 3.0	9.7 ± 2.4	8.0 ± 1.0	8.8 ± 1.6
Weight gained (%)	222.7 ± 72.9	236.2 ± 58.5	194.3 ± 24.4	215.4 ± 38.5
GR (g d^{-1})	0.1 ± 0.1	0.1 ± 0.05	0.1 ± 0.02	0.1 ± 0.04
SGR (% d^{-1})	2.5 ± 0.9	2.7 ± 0.9	2.2 ± 0.5	2.5 ± 20.6
TGC	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Survival (%)	96.7 ± 5.8	93.3 ± 11.5	86.7 ± 15.3	90.0 ± 10.0

Table 3. Proximate analysis of whole juvenile *Dormitator latifrons* fish fed different substitution levels (0, 40, 70, and 100%) of fishmeal by soybean meal. Different letters in the same row indicate significant differences ($P < 0.05$). Values are shown as mean ± standard deviation (n = 3). Super indices indicate statistically significant differences ($P < 0.05$).

Proximate analysis	Experimental treatments				
	Initial fish	Final fish			
		HS0	HS40	HS70	HS100
Crude protein (%)	57.6 ± 0.6	58.8 ± 1.4	61.3 ± 0.9	59.9 ± 1.0	60.7 ± 3.4
Crude fat (%)	13.4 ± 0.4	12.3 ± 0.3^a	12.3 ± 0.2^a	13.8 ± 0.7^b	15.8 ± 0.3^c
Ash (%)	15.4 ± 0.4	1 5.3 ± 0.1^c	14.7 ± 0.2^c	13.6 ± 0.1^b	12.3 ± 0.1^a
Nitrogen-free extract	13.4	13.7	10.5	11.3	11.2

relationship between the percentage of substitution and growth rate and sustained good growth results when replacing fishmeal by 50% or higher. In carnivorous fish, the replacement of fishmeal with soybean meal has proved to affect the growth. Tibaldi et al. (2006) found that substituting FM by more than 50% SBM affected intestinal enzymatic activity, directly associated with the digestibility and bioavailability of nutrients. Similar results were reported by Elangovan & Shim (2000), substituted FM by a maximum of 50% of SBM for *Barbodes altus* affected the growth of the organisms under study.

The use of soybean meal as a substitute for fishmeal in the feed of *D. latifrons* had no negative growth effects after 60 days. Concerning the digestibility coefficient, the diets containing between 70 and 100% of SBM had better nutrient digestibility values (80-90%) compared to the diet with 100% FM (Table 1). Kasiga et al. (2020) mention that the digestibility of a food or an experimental diet is a crucial factor and that few studies evaluate when the use of a diet is being evaluated, this factor reflects the percentage of a food sample that is absorbed in the intestine of an animal.

Diets with the same percentage of proteins and lipids, but with different levels of substitution of fishmeal by soybean meal, positively affected the proximate composition of juvenile *D. latifrons* fish; protein and lipids' content increased as the percentage of substitution with soybean increased. Kaushik et al (2004), studying the European sea bass (*Dicentrarchus labrax*), obtained good growth and nitrogen retention results with only 5% of fishmeal and 95% of vegetable protein sources in the diet, including soybean meal; however, the authors found that the fish had a significantly increased content of fat (15.6%) compared to the fish fed with the control diet containing 100% of fishmeal. The present study recorded a similar trend.

The fact that, in the present study, total fat showed a tendency to increase as fishmeal was replaced by soybean meal contrasts with the results of other studies that have shown that high soybean meal levels tend to reduce fat digestibility in fish with generally carnivorous habits (Tibaldi et al. 2006). Due to the origin of the SBM use during our research, some authors suggest the SBM's production process may affect the performance of digestibility for commercial fish (Tangenjadja 2015, Bruce et al. 2018). Olli & Krogdahl (1995) evaluated the effect of including different levels of a concentrate of alcohol-soluble soybean meal (soy molasses) in fishmeal-based diets used to feed Atlantic salmon (*Salmo salar*). They reported that the SBM's alcohol-soluble components contained antinutrients that negatively affected fat digestibility and may have been responsible for a significant part of the negative effects of the standard soybean meal.

Regarding feeding costs, the complete substitution of FM by SBM makes a considerable difference compared to 100% FM (Table 1). The present study results showed that it is cheaper to produce the HS100 diet, but the fish fed with it contain more protein and are thus of better nutritional quality. The latter can improve the species' market performance based on the idea that more protein may diversify the presentation of the product (fillet, fish medallion), and the lowered feeding costs mitigate new marketing costs by this process. Ajani et al. (2016) obtained *O. niloticus* of better nutritional quality using SBM as a protein source in their diet, resulting in lower input costs improving income and utility on the farm.

The reduction of costs obtained in the present work, a result of substituting animal protein with plant protein in the diet of juvenile *D. latifrons* fish, coincides with the results obtained by several authors in similar studies. Robinson & Li (2010) found a reduction in production costs by feeding *Ictalurus punctatus* fish with a diet in which fishmeal was partially or entirely substituted by corn gluten or cottonseed meal. Poot-López et al. (2010) obtained a considerable reduction in management and operating costs, with no negative effect on growth, by partially substituting a balanced diet fed to *O. niloticus* with tree spinach (*Cnidoscolus chayamansa*) waste at satiety. According to Subedi et al. (2019), the minimization of costs in aquaculture resulting from the substitution of FM by SBM would allow for optimization of the cost-benefit ratio when scaling up production to commercial scale based on bio-economic models of tropical fish production that indicate that food represents the most significant expense in aquaculture (Okwu & Acheneje 2011, Hernández et al. 2014).

The commercial value of wild *D. latifrons* fish in rural areas of the Mexican Pacific coast is still relatively low, USD 1.3 on average for a dozen fish (whole), ranging from 13 to 16 cm in size, which corresponds to approximately 1 kg. The average price of the fillet is USD 2.2 per kg. (Basto-Rosales et al. 2020). If feeding costs could be reduced without affecting the protein content of the meat, it might be possible to find a broader market for farmed *D. latifrons* fish with a marketing strategy focused on the nutritional qualities and lower price of this fish, as has been done with other native species in regions such as Africa (Tran et al. 2019). Promoting the consumption of *D. latifrons* would help revert the displacement of this native species by introduced species, similar to what has been done in the Mediterranean with marine fish (Katsanevakis et al. 2014).

It is important to study the nutritional value of alternative ingredients to formulate diets based on a mixture of vegetable proteins that can partially or replace FM in the cultured fish diet (Poot-López et al. 2010, Li et al. 2020). Using higher levels of substitution of fishmeal by plant protein sources has shown to be feasible by using mixtures of ingredients (Kaushik et al. 2004). The possible effects of soybean meal on fish nutrition are associated with the method of presentation of the feed to the fish, the methods used to process the soybeans, and the age, species, and dietary habits of the cultured fish (Zhou et al. 2017).

The results obtained in the present work indicate that fishmeal can be substituted by up to 100% soybean meal in the diet of juvenile *D. latifrons* fish. This substitution yields adequate growth, high survival rates, good nutritional qualities in the cultured fish, and reduced production costs.

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