

INCORPORATION OF THE NATIVE CICHLIDS TENGUAYACA, *PETENIA SPLENDIDA*, AND CASTARRICA, *CICHLASOMA UROPTHALMUS* INTO SUSTAINABLE AQUACULTURE IN CENTRAL AMERICA IMPROVEMENT OF SEEDSTOCK QUALITY AND SUBSTITUTION OF FISH MEAL USE IN DIETS

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INTRODUCTION

To achieve fish culture at a commercial scale, basic and applied research need to be carried out in order to allow the closure of the cycle of life of the species. However, in Mexico the focus for this activity has been for introduced species such as tilapia and trout, where its technology is already known (Rojas y Mendoza, 2000). For this reason the research for the culture of native species has reached only a minimum scale of production. In this sense, Southeast of Mexico has a lot of native species including cichlids which represent a potential resource for human consumption. The most important species are the castarrica (*Cichlasoma urophthalmus*) and tenguayaca (*Petenia splendida*) which have high potential for culture (Steeffens, 1987; Luna, 1997).

The studies with these species began in the 80's, and now allows implementation of their culture, although only in an extensive scale in the region (Martínez-Palacios and Ross, 1994).

Some of the most important investigations for *C. urophthalmus* were conducted by Martínez-Palacios (1987) that include topics as nutrition and culture method and the handle of broodstock management and larval quality (Mendoza and Navarro, 1994). We have also started studying the acceptance of commercial feeds and growth performance of this

species. So far we have compared growth performance of fry fed *Artemia* nauplii against fry fed commercial feed. We have observed a significant difference in growth if the fish are fed nauplii during the first 30 days of feeding even though fry accepted inert food. Fish fed 30 days with nauplii grew more than twice that those that received nauplii. Empirical information indicates that males grow faster and larger than females. On the other hand, Real (2003) obtained 95 to 100% of male monosex juveniles feeding larvae with *Artemia* nauplii enriched with a concentration of 15 mg/L of 17 α -Methyl testosterone.

For *P. splendida* several studies that include ecology, habitat, feeding content and distribution were conducted (Reséndez and Salvadores, 1983; Ferreira et al., 1988; Caro et al., 1994; Domínguez and Rodiles, 1998). For culture, the advances include, García (2003), Jiménez (2004), and Chan (2004), whom determine the maximum interval of temperature resistance (30.68 and 31.95 Celsius), and the thermal preferendum (28-30 °C) for the best metabolism and growth of juveniles. Contreras (2003) and Vidal-López (2004), obtained 95 to 100% mono sex population feeding larvae with trout commercial diet and *Artemia* nauplii enriched with 17 α -Methyl testosterone using a concentration of 30 mg/L for 45 days. For larviculture the morphological development was described (Martínez, 2004). In the case of nutrition Uscanga (2006) determined the protein requirement for masculinized and non-masculinized juveniles using semi-purified diets.

This information is promising; however, although the culture of these species are already known, at the moment a commercial food for trout has been used. This food contains a high quantity of protein and lipids, which increases the cost for large-scale production. In addition the ingredients of the formula are unknown, for this reason we consider that the growth and the survival of the species is not optimum.

During the formation of broodstock it is fundamental to incorporate the highest genetic diversity as possible, both at the intra- and inter-population level. This is particularly important when planning genetic selection programs. Family structure is also an important issue that may affect inbreeding and consequently affect productivity.

Genetic improvement in fish has had a large impact during the last years (Martínez y Neira, 1995; Hulata, 2001; Gjedrem, 2005; Martínez, 2005) focusing on growth enhancement, diseases resistance, transfer of desirable characters, combination of benefic characters from two species, avoid unwanted reproduction, improving food conversion rates, and tolerance to cold and bad quality water (Gjedrem, 1985, Bartley y Hallerman, 1995; Hallerman y Kapuscinsky, 1995) and applying different techniques such as selection, intra and inter-specific breeding, sex inversion, chromosomal manipulation and polyploidy (Foresty, 2000; Hulata, 2001).

Artificial selection consists of selecting organisms with the best traits and potential application allowing modification of the genetic pool of one population in respect to one or more characters in such a way that generation after generation, the groups move towards the desired goals (Kirpichnikov, 1981; Rodríguez-Gutiérrez y Marañón-Herrera, 1993; Gjedrem, 2005). The main objective is to produce stocks that are more efficient in economic terms improving growth and viability under culture conditions (Álvarez-Jurado, 1987; Gjedrem, 1997).

We have seen genetic improvement as an option for the culture of the native cichlids *P. splendida* and *C. urophthalmus* which have demonstrated high potential for inclusion in

aquaculture; however, no information exists about how these species may respond to a selection program or current differences among wild populations that may enhance the selection program.

METHODS AND MATERIALS

Objective 1. To improve Seedstock quality based on a genetic improvement program for P. splendida and C. urophthalmus.

Experiment 1. Genetic improvement of *Petenia splendida*, and Castarrica, *Cichlasoma urophthalmus* Using Total Length and Condition Factor.

This experiment was initiated late due to two consecutive flooding events in Tabasco. The first one occurred in 2007 and the second one in 2008. In both cases our facilities were not severely affected; however, our investigations were delayed due to fish loss or systems damaged.

This experiment was divided in two parts. The first one consisted in the capture of adult organisms from the wild, acclimation to captivity and fry production; the activities were conducted at the Laboratorio de Acuicultura Tropical; División Académica de Ciencias Biológicas. The second part was the grow-out of fish in hapas and floating cages the activities were conducted at the Laboratorio de Acuicultura, División Académica de Ciencias Agropecuarias at Universidad Juárez Autónoma de Tabasco.

Fish Capture and acclimation to captivity

Adult castarricas were collected from three different localities of Tabasco: Centla, Centro and Comalcalco. These localities were selected based on the historical presence of the species in the area. Forty-five adult fish were caught from each locality, then transferred to the laboratory and kept in quarantine. A prophylactic treatment was performed the first day of arrival. From each locality, fish in best conditions were selected as broodstock: 36 fish from Centla (24 females and 12 males); 24 from Centro (16 females and 8 males) and 24 from Comalcalco (16 females and 8 males). All fish were kept in 2000-L plastic tanks connected to a recirculating system equipped with a sand filter, a biofilter and a centrifugal pump that returned the water to the system. Fish were fed three times per day, providing 3% of the total biomass using pelleted trout food “El Pedregal” (Silver Cup™) containing 45% protein.

Adult tenguayacas were collected from three different localities of Tabasco: Nacajuca, Centro, and Centla; and one locality from Chiapas: Mal Paso. These localities were selected based on the historical presence of the species in the area. However, due to overfishing, forming the lot of broodstock for this species was more difficult. From each locality, fish in best conditions were selected as broodstock: 36 fish from Nacajuca (24 females and 12 males); 36 from Centro (24 females and 12 males); 12 from Centla (8 females and 4 males); and 21 from Mal Paso, Chiapas (14 females and 7 males).

All fish were individually sexed, weighed, measured and identified using a microchip injected intra-muscularly. Morphometric measurements were taken (total length, standard length, height, perimeter, length of dorsal, pectoral and pelvic fins, height and length of caudal peduncle, head length, superior jaw length, mouth and eye diameter) as well as meristics (number of scales in the lateral line, superior and inferior, number or rays in the caudal and dorsal fins, and number of spines in dorsal and anal fins). Condition factor of each fish was also calculated.

Hematologic measurements

To evaluate fish health, the following parameters were evaluated: Microhematocrit, hemoglobin, total plasmatic protein, red blood cell count and white blood cell count. Hematologic procedures followed procedures proposed by Hesser (1960) and Houston (1990). Fish were first anesthetized with Tricaine methanesulfonate (MS-222) and blood drawn from the caudal vein using heparinized syringes.

Reproduction

Gender proportion used in all tanks was 1:2 (male:female) at a density of 12 fish per tank (4 males : 8 females). Each 2000-L tanks was equipped with 8 plastics sheets placed forming an angle against the tank wall to serve as refuge and potentially as nest. Water quality in each tank was monitored daily and 50% water exchange was performed every other day.

Fry collection and rearing

Once spawning occurred, both parents were captured and identified reading the pit tag. Both fish were placed back into the tank to allow parental care of the fertilized eggs. Once hatching occurred, all the fry were siphoned-out of the spawning tanks and placed in a 100-L rearing tank. All rearing tanks were connected to a recirculation system equipped with a sand filter, a biofilter and a centrifugal pump that returned the water to the system. Fry were kept in the rearing tanks for 60 days and fed five times per day with 1.5 hours difference between feeding times. *Artemia nauplii* were supplied during the first 15 days and trout commercial feed afterwards (Silver Cup™) containing 45% protein.

Grow-out phase

After the rearing period, families were moved to our facilities located in the Agronomical Sciences Division, each family was placed in a 2 m³ mosquito mesh hapa (2x1x1.20 m) using a density of 150 fish/. m³. Biometric measures were taken every 15 days (weight and Total length) in each hapa. After 120 days of grow-out, fish were moved to 2 m³ floating cages (2x1x1.20 m) with ½ inch of mesh size and kept there until first selection was conducted.

Line Selection

The inter- and intra-family selection method was used. After 225 days of culture, fish were separated by sex and total length and weight measured. 26% of the fish with the best weight were selected; 40 males and 40 females were stocked in 2 m³ floating cages (2x1x1.20 m) with 1 inch of mesh size. A control group from each family was formed using fish randomly selected (40 males and 40 females). During final grow-out, fish growth was evaluated every 15 days.

Water quality in ponds

Water quality parameters were measured daily or weekly. Temperature and Dissolved Oxygen were measured using a multifunction DO meter (YSI 55™). pH was measured using a portable pH meter (CONDUCTRONIC™). Ammonia, Nitrates and Nitrites were measured weekly using the multiparametric colorimeter (HANNA™).

Statistical Analyses

A descriptive analysis was performed to all hematologic data. Relations between morphometric and meristics variables were analyzed using a discriminate analysis. Relationships between condition factor (k), weight of the female and number of eggs

produced were determined using a multiple regression analysis. Differences in weight between families were detected using a one-way ANOVA. All statistical procedures were conducted using the statistical package STATGRAPHICS Plus[®] V5, and statistical differences were indicated using a 95% confidence level.

Objective 2. To determine the effect of the substitution of fish meal for poultry meal on growth, survival, apparent digestibility and chemical composition of P. splendida and C. urophthalmus juveniles.

Experiment 1: Effects of the substitution of fish meal with pork meal on P. splendida and C. urophthalmus juveniles.

This study was conducted at the Laboratory of Tropical Aquaculture at UJAT, Tabasco, Mexico. Growth of *P. splendida* and *C. urophthalmus* juveniles was evaluated using practical isocaloric and isoproteic diets containing 0, 25, 50, 75 and 100% substitution of fish meal with pork meal.

Masculinized juveniles were selected from a group of 2,000 fry produced in the Laboratory of Aquaculture at UJAT. The production of masculinized juveniles consisted on feeding larvae with 17 α -Methyltestosterone (20 mg/L) enriched-artemia nauplii for 15 days and 17 α -Methyltestosterone (60 mg/Kg of diet) enriched commercial trout diet (Silver Cup) for 30 additional days. From this batch, 270 fish were selected and randomly distributed among the 18 experimental units at a density of 15 fish/tank. Water for grow-out was recirculated using bio-filters, 25% of the volume was exchanged twice a week.

Sampling schedule for Tenguayaca and Castarrica juveniles consisted in collecting all fish in each tank at the beginning of the experiment and every 14 days. Total length and weight were measured to the nearest 0.001 mm or g. Mortality was recorded daily. Growth and food quality indexes were calculated at the end of the experiment: Feed conversion rate (FCR), specific growth rate (SGR), condition factor (CF), protein efficiency rate (PER), and percentile weight gain (WG %). Mortality was recorded daily. Feces recollection for the measure of apparent digestibility was conducted daily. Samples of fish were taken at the beginning and the end of the experiment to determine chemical composition in whole fish (AOAC, 1995). Experimental treatments were as follows:

- 1) Tenguayaca and castarrica juveniles fed with a diet containing 0% fish meal and 100% pork meal: (0%FM-100%PorM).
- 2) Tenguayaca and castarrica juveniles fed with a diet containing 25% fish meal and 75% pork meal (25%FM-75%PorM).
- 3) Tenguayaca and castarrica juveniles fed with a diet containing 50% fish meal and 50% pork meal: (50%FM-50%PorM).
- 4) Tenguayaca and castarrica juveniles fed with a diet containing 75% fish meal and 25% pork meal: (75%FM-25%PorM).
- 5) Tenguayaca and castarrica juveniles fed with a diet containing 100% fish meal and 0% pork meal: (100%FM-0%PorM).
- 6) Tenguayaca and castarrica juveniles fed with a trout commercial diet (CD).

Each treatment was run in triplicate. First feeding juveniles were fed with experimental diets four times a day (8:00, 12:00, 16:00, and 20:00 h). Fish received daily rations containing 10 percent of the total biomass in the tank. Daily rations were estimated using a spread-sheet constructed with previous growth data for each species.

Growth data were compared using a one-way ANOVA for each species. Survival was compared using a Chi-square test.

Experiment 2: Effects of the substitution of fish meal with poultry meal on *P. splendida* and *C. urophthalmus* juveniles.

All experimental conditions and measurements were similar as those used in experiment one. Treatments were as follows:

- 1) Tenguayaca and castarrica juveniles fed with a diet containing 0% fish meal and 100% poultry meal: (0%FM-100%PouM).
- 2) Tenguayaca and castarrica juveniles fed with a diet containing 25% fish meal and 75% poultry meal: (25%FM-75%PouM).
- 3) Tenguayaca and castarrica juveniles fed with a diet containing 50% fish meal and 50% poultry meal: (50%FM-50%PouM).
- 4) Tenguayaca and castarrica juveniles fed with a diet containing 75% fish meal and 25% poultry meal: (75%FM-25%PouM).
- 5) Tenguayaca and castarrica juveniles fed with a diet containing 100% fish meal and 0% poultry meal: (100%FM-0%PouM).
- 6) Tenguayaca and castarrica juveniles fed with a trout commercial diet (CD).

RESULTS

Objective 1. To improve Seedstock quality based on a genetic improvement program for *P. splendida* and *C. urophthalmus*.

Experiment 1. Genetic improvement of *Petenia splendida*, and Castarrica, *Cichlasoma urophthalmus* Using Total Length and Condition Factor.

Acclimation to captivity took more time for *P. splendida* than for *C. urophthalmus*. It is possible that the carnivorism of *P. splendida* may have played a significant role on this, delaying significantly adaptation to foods provided in the laboratory. Despite early capture of adults of this species in the year, first spawnings were obtained until August of 2009; therefore, evaluation of growth is still ongoing in this species.

In *C. urophthalmus*, comparison of morphometric and meristic characters among localities showed a similar pattern indicating that no significant differences were found among the adults captured for establishing our broodstock lots. The discriminant analysis indicates that the number of scales in the superior lateral line, condition factor, length of dorsal and pectoral fins, total length and length of the caudal peduncle may allow separation of the three populations sampled with an 86.08% percent of cases correctly classified. Fish from Centla had the best condition factor (K; 2.42 ± 0.30) when compared against Comalcalco (2.25 ± 0.52) and Centro (1.99 ± 0.16). No statistical differences were found ($P > 0.05$; table 1) for the hematologic variables from the adults. However, the highest values for microhematocrit, hemoglobin, total plasmatic protein, red blood cell count and white blood cell count were found in the fish obtained in Comalcalco, while Centla showed the lowest values. These types of comparisons were not possible with *P. splendida*, because fish were not collected at the same time.

Reproduction of Castarrica was evaluated from January to November 2009. During this time, Castarricas from Centla had the largest amount of spawnings (53) and a total of 260,554 fry. Fish from Centro had 30 spawnings with a total of 96,833 fry and 21

spawnings were obtained from Comalcalco with a total of 86,845 fry. This species actively spawned between March and August. No significant differences in the average number of fry per spawning event were found between Centla (4825.07 ± 1402.77) and Comalcalco (4342.25 ± 1871.75) but these values were significantly higher ($p < 0.001$) than the average number of fry obtained for Centro (3339.07 ± 993). Same results were obtained when the average number of fry was estimated per gram of female: Centla (21.47 fry/g), Comalcalco (18.95 fry/g) and Centro (12.23 fry/g). These results are interesting since females from Centro had larger average weight ($263.83 \text{ g} \pm 38.65$) than those from Centla ($229 \text{ g} \pm 64.38$) and Comalcalco ($218.27 \text{ g} \pm 52.33$).

In the spawning tanks, water quality conditions were maintained optimal, average temperature, dissolved oxygen and pH were $29 (\pm 2^\circ\text{C})$, $7.5 (\pm 0.15)$ and $7.8 (\pm 0.12)$, respectively. Average values for ammonia, nitrates and nitrites were $0.30 \text{ mg/L} (\pm 0.04)$, $1.3 \text{ mg/L} (\pm 0.03)$ and $0.03 \text{ mg/L} (\pm 0.003)$, respectively.

For line selection, families that hatched within a lapse of time of 2 weeks were used to avoid differences caused by age and/or environmental conditions. Eleven families were used (5 from Centla, 3 from Centro and 3 from Comalcalco). Average fry weight at hatching was 5.5 mg with 7.0 mm in total length. Castarrica fry accepted artemia nauplii during the first days and weaning was successfully achieved feeding them with artificial food in few days. Survival at this stage was 97% reaching 41.1 mg and 35.89 mm in 60 days.

No significant differences were found between families or locations during the grow-out phase ($P > 0.05$; Fig. 1). During selection, all fish were separated by sex. Females from Centla showed no significant differences in weight between families ($13.50 \text{ g} \pm 4.88$; $P > 0.05$), but differences in length were detected ($9.00 \text{ cm} \pm 0.93$; $P < 0.05$). Males from this locality had significant differences between families both in weight and in length averaging $19.10 \text{ g} (\pm 5.62)$ and $11.10 \text{ cm} (\pm 0.89)$; $P < 0.001$, respectively. Females from Centla had significant differences in weight and length between families (average: $12.20 \text{ g} \pm 4.88$ and $8.75 \text{ cm} \pm 1.03$; respectively). The same was observed for males which had significant differences between families both in weight and in length averaging $14.13 \text{ g} (\pm 5.18)$ and $9.33 \text{ cm} (\pm 1.13)$; $P < 0.001$, respectively. Fish from Comalcalco also had significant differences between families for females and males. In females fish averaged $13.20 \text{ g} (\pm 5.18)$ and $9.10 \text{ cm} (\pm 1.13)$ in length. Males averaged $20.45 \text{ g} (\pm 5.43)$ and $11.2 \text{ cm} (\pm 0.87)$; $P < 0.001$).

When females from the three localities were compared, no significant differences were found in weight or length ($P > 0.05$). However, highly significant differences were found between males from the three localities both for weight and length ($P < 0.001$). Significant differences ($P < 0.001$) were found between all families selected when compared against the control group (Figs. 2, 3 and 4).

Objective 2. To determine the effect of the substitution of fish meal for poultry meal on growth, survival, apparent digestibility and chemical composition of P. splendida and C. urophthalmus juveniles.

Experiment 1: Effects of the substitution of fish meal with pork meal on P. splendida and C. urophthalmus juveniles. The analysis of variance indicated that exist statistical significant differences in weight and total length ($P < 0.05$) between treatments for P.

splendida after 42 days of feeding (Fig. 1a and b). At day 84, fish fed 100%SM-0%PorM and 75%SM-25%PorM substitution were larger than the rest of the treatments (Fig. 1c and d). Food conversion rate (FCR), weight gain (WG%) and survival (Sur) showed significant differences ($P<0.05$) between treatments. For survival, fish fed with 100%HS-0%PorM, CD, 0%FM-100%PorM, and 25%FM-75%PorM had higher values compared with 50%FM-50%PorM and 75%FM-25%PorM. Fish fed with 100%FM-0%PorM, CD, 50%FM-50%PorM, and 0%FM-100%PorM had statistically higher FCR compared with the other treatments. Finally, fish fed with 100%FM-0%PorM were significantly different to fish fed 25%FM-75%PorM and 75%FM-25%PorM (Table 1).

For *C. urophthalmus*, no significant differences ($P>0.05$) were detected at any time for weight, total length and food quality indexes (Fig. 2 and Table 2).

Experiment 2: Effects of the substitution of fish meal with poultry meal on *P. splendida* and *C. urophthalmus* juveniles. For *P. splendida*, significant differences in growth and total length were detected ($P<0.05$) after 56 days of experimentation (Fig. 3a and b). After 84 days of feeding significant differences ($P<0.05$) were detected for fish fed 75%FM-25%PouM and CD compared with the rest of the treatments (Fig. 3c and d). On the other hand, survival showed statistical differences ($P<0.05$) for fish fed CD and 0%FM-100%PouM compared only with 100%FM-0%PouM. For specific growth rate (SGR) and weight gain (WG%) significant higher value ($P<0.05$) were detected only for fish fed 75%FM-25%PouM compared with fish fed 100%FM-0%PouM (Table 3).

For *C. urophthalmus*, significant differences were detected ($P<0.05$) after 42 days of experimentation for weight and total length (Fig. 4 a and b); at the end of the experiment, the highest weight and total length were detected only for fish fed with CD compared with the rest of the treatments (Fig. 4 c and d). For daily fed intake (DFI) and daily protein intake (DPI) significant differences ($P<0.05$) were detected only for fish fed with 0%FM-100%PouM with the highest values compared with fish fed with 25%FM-75%PouM (Table 4).

DISCUSSION

According to our results, morphologic and meristic characters did not show significant differences in *C. urophthalmus*; however, fish from Centro and Comalcalco showed more affinity among themselves, than fish from Centla. Cheng *et al.* (2005) compared morphometric characters of *Coilia* (a teleost) from four populations, observing that Only four of the characters measured helped identifying closely related populations at a 88-100% accuracy level. Sumantadinata and Taniguchi (1990) found morphologic differences among adult carps, indicating that morphologic and meristic values provide valuable information that can be useful for genetic improvement in aquaculture.

Average values obtained for microhematocrit, hemoglobin, protein concentration in blood and red and white cells counts in *C. urophthalmus* found in this study differ from those reported by Güemez y Sima (1998) y Pelaez (2009) for this species under culture conditions. These differences can be attributed to different factors such as: origin of the fish -mainly due to differences in environmental conditions- (Atencio-García and Genes, 2007) as well as nutritional status, sexual maturation, and even differences between sexes Ranzani-Paiva, 1991; Fernández *et al.*, 2002).

In a period of time of 10 months of evaluation, fish from Centla had the highest Condition factor, number of spawnings events and fry produced than those from Centro and Comalcalco. Morgan (2004), did not find significant differences when comparing Condition factors from three populations of *Hippoglossoides platessoides*, but did find a relationship between the condition of the fish and reproductive activity. On the other hand, we found no relationship between fecundity and weight of the females, particularly due to the fact that small females from Centla spawned more times and produced more eggs than those from Centro, which presented larger sizes. Age, nutrition and environmental conditions at the place of origin may play a role in these results, since several researchers have reported that fecundity decreases with age in fish (Musa and Salam, 2007). Adult fish from Centro presented the lowest Condition Factor and were the group that lasted longer in initiating reproduction in captivity (approximately six months). These fish were captured in a lagoon with a very large population of castarricas, but impacted by urban development.

During the first stage of evaluation, all families performed similarly; however, significant differences have been found once the fish are in the grow-out phase. Regarding to this, different authors have reported similar results during the early stages of growth evaluation (Mair, *et al.*, 2004; Liti *et al.*, 2005) who did not find differences in growth in tilapia lines. These authors suggested that food availability may play an important role at this age. So far we have found significant differences among the fish used as controls for each family (randomly selected) and the selected fish. Another interesting result is that no differences have been found so far for females from the different localities, but males have very different growth patterns between localities. In few months, we should be able of selecting the fish that will form our broodstock for the first selected line of *C. urophthalmus*. More time will be needed for *P. splendida* due to the late start in the selection.

The results obtained with the diets experiments demonstrate that the fish meal can be replaced in a practical diets by the pork and poultry by-product meals up to 25%, without adverse effects on growth and survival for *Cichlasoma urophthalmus* and *Petenia splendida* which is in agree with results obtained for Hasan *et al.* (1997) who obtained a substitution of 20% of the fish meal by hydrolyzed feather meals in the carp *Labeo rohita*. Warith *et al.* (2001) were able of replacing 40% of fish meal by poultry by-product meal in juveniles of African catfish *Clarias gariepinus*. In the same way, Millanema (2002) and Van and Yu (2003) reported satisfactory results in growth with an 80% substitution of the fish meal by meat and bone meal in *Epinephelus coioides* and *Pangasianodon hypophthalmus* respectively. In another study, Brown (2005) reduced the cost of food production by 20% with a substitution of 45% of meat and bone meal in black bass *Micropterus salmoides*. Peters *et al.* (2006) and Zhang *et al.* (2006) substituted 20% of fish meal with meat and bone meal with favorable growth in *Carassius auratus gibelio* and *Oreochromis sp.* juveniles. Qinghui *et al.* (2006) were able to replace a 45% fish meal protein by meat and bone meal (MBM) in yellow croaker *Pseudosciaena crocea*. Additionally, Shapawi *et al.* (2007), reduced 50% of fish meal with poultry by-product meal in *Cromileptes altivelis*, and Zhang *et al.*, (2008), replaced 26% of the fish meal with a mix of vegetable and animal protein meal with good results in growth and survival of *P. crocea*. Finally, Menghong *et al.* (2008) reported that only 6% fish meal substitution could be done with combinations of the poultry by-product, meat-bone and blood meals.

Best food quality indexes for *C. urophthalmus* and *P. splendida* were obtained when 25% fish meal was substituted for pork and poultry meals. Similar values were obtained by Warith *et al.* (2001), who obtained and FCR=1.63, SGR=3.56 and PER=1.72 substituting

40% of the fish meal by poultry byproducts meal when feeding *Clarias gariepinus*. These authors found very good growth, even higher than the values obtained here for *C. urophthalmus*. Millamena (2002), studied inclusion of 80% bone and blood meals in diets for *Epinephelus coioides*, obtaining values of 1.04 for FCR and 2.92 for SGR. These values are similar to the ones obtained in our study.

Other studies have reported better results with other species than the ones obtained by us in this study. Zhang et al. (2006) obtained higher values for WG% (155.16) when studying inclusion of 20% bone and blood meals in diets for tilapias (*Oreochromis sp.*). Ávalos (2006), determined higher values for Nile tilapia, *Oreochromis niloticus* (FCR=1.91; SGR=3.18; DPI=386.3; PER=1.64; DFI=0.19; WG=494.1%) using a 35% substitution of fish meal for pork meal. Tilapias as omnivorous fish may adapt easily to different ingredients in the diets while *C. urophthalmus* is considered opportunistic carnivorous and *P. splendida* is carnivorous (Chávez et al., 1989). In a different study, Zhang et al. (2008) reported a SGR of 3.5 when replacing 6% of the fish meal by a mixture of protein sources for *P. crocea*.

Survival in our study was good, reaching 95% for *C. urophthalmus* and 77 - 100% for *P. splendida*. Best results were obtained when 25% of the fish meal was replaced by either pork or poultry meal. Almeida (2008) obtained 96% survival for *P. splendida* when using 25% wheat gluten indicating that some mortality is present when changes to the amount of fish meal used are made. Since *P. splendida* is a carnivorous species, its ability to digest diets containing vegetal ingredients is low. This situation is clear in our study since the diet with 100% FM had the highest survival. More research is needed to determine other options for replacing fish meal without compromising survival.

In general, partial or total substitution of fish meals in diets for fish or crustaceans - particularly carnivorous- for alternative ingredients has a series of advantages such as diversification of the industry when products that may be considered waste by-products are used. Some of these by-products are currently generated in the pork, bovine and poultry meat industry. The current way of processing these products are designed to be highly efficient and pathogen free; therefore, the by-products are high-quality from the sanitary point of view as well as from the quality of the meals obtained (El-Sayed, 1999). Fish meal used in diets for aquaculture is a very efficient ingredient with high protein and excellent amino acid profiles has become a highly valued ingredient with a price that continuously rise. This situation has created disadvantages for using fish meals since the price of diets are making aquaculture not affordable in several countries (Zaldívar, 2002). The constant fluctuation of fish meal quality has become another important issue; the fact that most of the fish meal is made of sardine, herring and hake, made the industry strongly dependant of the wild captures for these species. Environmental conditions and stock fluctuations severely impact commercial captures and therefore the price, quality and availability of fish meals. The use of alternative ingredients (such as pork, poultry and bovine by-products) will be adequate, either through national or international enterprises that certify quality of the fabrication procedures and the meals obtained. Impacts to the feeds used in aquaculture will be substantial since an important decrease in the price of the feeds will be achieved by decreasing the use of fish meals (Robaina, 1998; Allan et al., 2000).

Previous results regarding nutrient requirements, practical diet evaluation and digestive capacity for *C. urophthalmus* (Martínez-Palacios and Ross, 1994; López, 2008) indicate that this species is an opportunist carnivorous making feasible the substitution of fish meal

in the diets. However, this species requires a high amount of protein (45%) which can be easily achieved using pork meal. Pork meal has amino acid profiles similar to the one found in fish meals, with the difference that lipids and ashes are higher in pork meals. These characteristics may make pork meal difficult for fish to digest.

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Tabla 1. Average values for hematologic parameters measured in adult castarricas (*C. urophthalmus*) from the different localities.

	Centla	Comalcalco
Microhematocrit (%)	22.03 ± 4.26	28.55 ± 3.88
Hemoglobin (g/100 mL)	4.10 ± 0.93	4.78 ± 0.77
Total protein in plasma (g/dl)	4.98 ± 2.98	6.04 ± 1.58
Erythrocytes (cel/mm ³)	1.16 ± 0.43	1.65 ± 1.09
Leucocytes (cel/mm ³)	31.85 ± 14.01	31.13 ± 21.38

Tabla 2. Fry production during January-October 2009 from females used as broodstock .

Females	Weight (g)	Jan	Feb	March	April	May	June	July	Aug	Sep	Oct	Total # Fry
J1: Centla	159				5,702	6,758	7,540					20000
J2: Centla	179	4,332		5,500		6168						16000
J3: Centla	206	2,544			4,927		3349	3,727	2200			16747
J4: Centla	281	2,238			4,833	5,500	4607	4,000	2,822			24000
J5: Centla	309			5,823	6,813	6,738	5,933	6,275	5764			37346
J6: Centla	141				6,190		5,810					12000
J7: Centro	267				4,555		3,954					8509
J8: Centro	240			3,857	3,714	3,074		3,489	2,979	2,637	2,197	21947
J9: Centro	252				3,947	4,710		4,510	3,923		2,645	19735
J10: Comalcalco	300				7,927							7927
J11: Comalcalco	222	2,283	2,667		3,050							8000
J12: Comalcalco	227				5,589				5,850	5,261		16700
J13: Comalcalco	181			5,750		7,150						12900

Table 3. Food quality indexes and survival of *P. splendida* juveniles feed with diets substituting fish meal for pork meal.

Indexes	Substitution					CD
	0%SM- 100%PorM	25%SM- 75%PorM	50%SM- 50%PorM	75%SM- 25%PorM	100%SM- 0%PorM	
FCR	1.71 ± 0.70a	2.44 ± 0.38b	1.49 ± 0.45a	2.33 ± 0.40b	0.84 ± 0.25a	1.41 ± 0.45a
Sur	100.00 ± 0.00a	95.56 ± 3.85a	86.67 ± 6.67b	88.89 ± 10.18ab	97.78 ± 3.85a	100.00 ± 0.00a
SGR	3.14 ± 0.28	2.65 ± 0.29	3.08 ± 0.20	3.03 ± 0.56	3.69 ± 0.22	3.25 ± 0.42
CF	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.00	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
DFI	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.00	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
DPI	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00
PER	1.52 ± 0.81	0.92 ± 0.15	1.58 ± 0.44	1.32 ± 0.96	1.87 ± 1.03	1.70 ± 0.56
WG%	814.02 ± 165.79ab	550.33 ± 137.92b	901.67 ± 154.30ab	578.91 ± 289.51b	1241.51 ± 292.25a	900.56 ± 308.25ab

Table 4. Food quality indexes and survival of *C. urophthalmus* juveniles feed with diets substituting fish meal for pork meal.

Indexes	SUBSTITUTION					CD
	0%FM- 100%PorM	25%FM- 75%PorM	50%FM- 50%PorM	75%FM- 25%PorM	100%FM- 0%PorM	
FCR	2.94 ± 0.82	2.17 ± 0.29	2.72 ± 1.04	2.43 ± 0.84	3.11 ± 0.79	2.40 ± 0.07
Sur	100.00 ± 0.00	100.00 ± 0.00	97.78 ± 3.85	97.78 ± 3.85	97.78 ± 3.85	100.00 ± 0.00
SGR	1.25 ± 0.19	1.53 ± 0.11	1.44 ± 0.32	1.59 ± 0.35	1.22 ± 0.09	1.19 ± 0.15
CF	1.81 ± 0.09	2.07 ± 0.24	1.76 ± 0.02	1.76 ± 0.09	1.67 ± 0.06	2.03 ± 0.26
DFI	0.19 ± 0.01	0.19 ± 0.00	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.00	0.17 ± 0.01
DPI	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
PER	0.79 ± 0.19	1.04 ± 0.14	0.93 ± 0.46	1.02 ± 0.43	0.74 ± 0.17	0.93 ± 0.03
WG%	146.51 ± 36.19	192.61 ± 23.96	161.49 ± 81.50	183.03 ± 72.25	121.56 ± 38.15	130.93 ± 23.82

Table 4. Food quality indexes and survival of *P. splendida* juveniles feed with diets substituting fish meal for poultry by-product meal.

Indexes	Substitution					CD
	0%FM-100%PouM	25%FM-75%PouM	50%FM-50%PouM	75%FM-25%PouM	100%FM-0%PouM	
FCR	0.87 ± 0.12	0.95 ± 0.20	1.39 ± 0.44	0.83 ± 0.29	1.43 ± 0.40	0.85 ± 0.18
	100.00 ±	86.67 ±	95.56 ±	97.78 ±	77.78 ±	100.00 ±
Sur	0.00a	0.00ab	3.85ab	3.85ab	10.18b	0.00a
	3.69 ±	3.81 ±	3.47 ±			3.85 ±
SGR	0.02ab	0.27ab	0.10ab	3.97 ± 0.09a	3.25 ± 0.32b	0.09ab
CF	1.13 ± 0.08	1.10 ± 0.04	1.05 ± 0.01	1.08 ± 0.02	1.07 ± 0.09	1.23 ± 0.06
DFI	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
DPI	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
PER	2.60 ± 0.33	2.42 ± 0.54	1.72 ± 0.60	2.87 ± 0.83	1.65 ± 0.50	2.71 ± 0.67
	1221.41 ±	1352.09 ±	1038.86 ±	1513.07 ±	875.77 ±	1378.82 ±
WG%	15.73	258.06	82.61	104.22	196.15	89.91

Table . Food quality indexes and survival of *C. urophthalmus* juveniles feed with diets substituting fish meal for poultry by-product meal.

Indexes	SUBSTITUTION					CD
	0%FM-100%PouM	25%FM-75%PouM	50%FM-50%PouM	75%FM-25%PouM	100%FM-0%PouM	
FCR	2.29 ± 0.16	2.14 ± 0.35	1.96 ± 0.20	1.78 ± 0.35	1.99 ± 0.19	1.33 ± 0.13
	100.00 ±	100.00 ±	100.00 ±			100.00 ±
Sur	0.00	0.00	0.00	97.78 ± 3.85	97.78 ± 3.85	0.00
SGR	2.27 ± 0.26	2.12 ± 0.09	2.19 ± 0.15	2.26 ± 0.21	2.33 ± 0.12	2.71 ± 0.07
CF	1.80 ± 0.11	1.75 ± 0.12	1.92 ± 0.29	1.97 ± 0.28	1.83 ± 0.23	1.84 ± 0.22
		0.19 ±	0.18 ±		0.19 ±	0.18 ±
DFI	0.20 ± 0.00a	0.00ab	0.00ab	0.17 ± 0.01b	0.00ab	0.01ab
		0.08 ±	0.08 ±		0.09 ±	0.08 ±
DPI	0.09 ± 0.00a	0.00ab	0.00ab	0.08 ± 0.00b	0.00ab	0.00ab
PER	0.97 ± 0.07	1.05 ± 0.16	1.14 ± 0.12	1.28 ± 0.25	1.12 ± 0.11	1.68 ± 0.15
	395.36 ±	341.52 ±	366.25 ±	388.86 ±	411.77 ±	567.35 ±
WG%	93.19	27.94	50.72	69.99	42.97	31.21

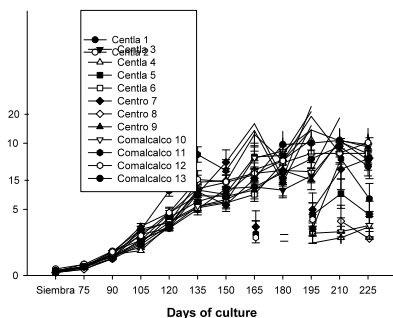


Figure1. Average weight from 13 castarricas (*C. urophthalmus*) families from the three localities evaluated.

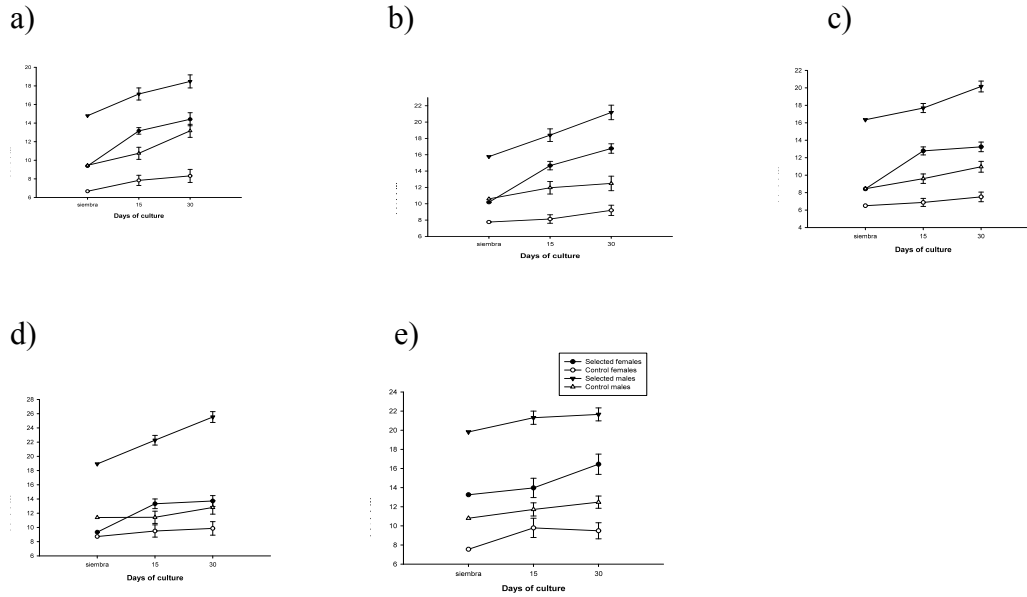


Figure 2. Average growth in weight (g) of males and females selected compared against control groups of *C. urophthalmus* families from Centla: a) Family 1; b) Family 2; c) Family 3; d) Family 4 and e) Family 5.

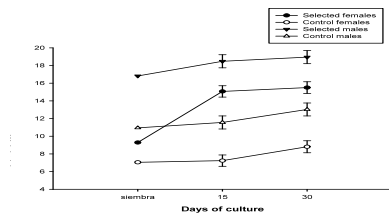


Figure 3. Average growth in weight (g) of males and females selected compared against control groups of one *C. urophthalmus* family from Centro

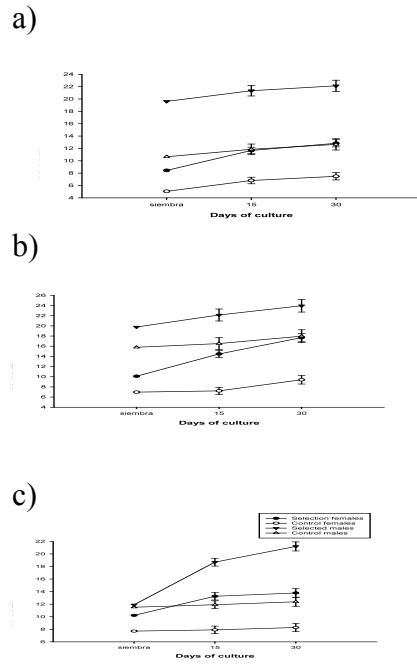


Figure 4. Average growth in weight (g) of males and females selected compared against control groups of *C. urophthalmus* families from Comalcalco: a) Family 1; b) Family 2 and c) Family 3.

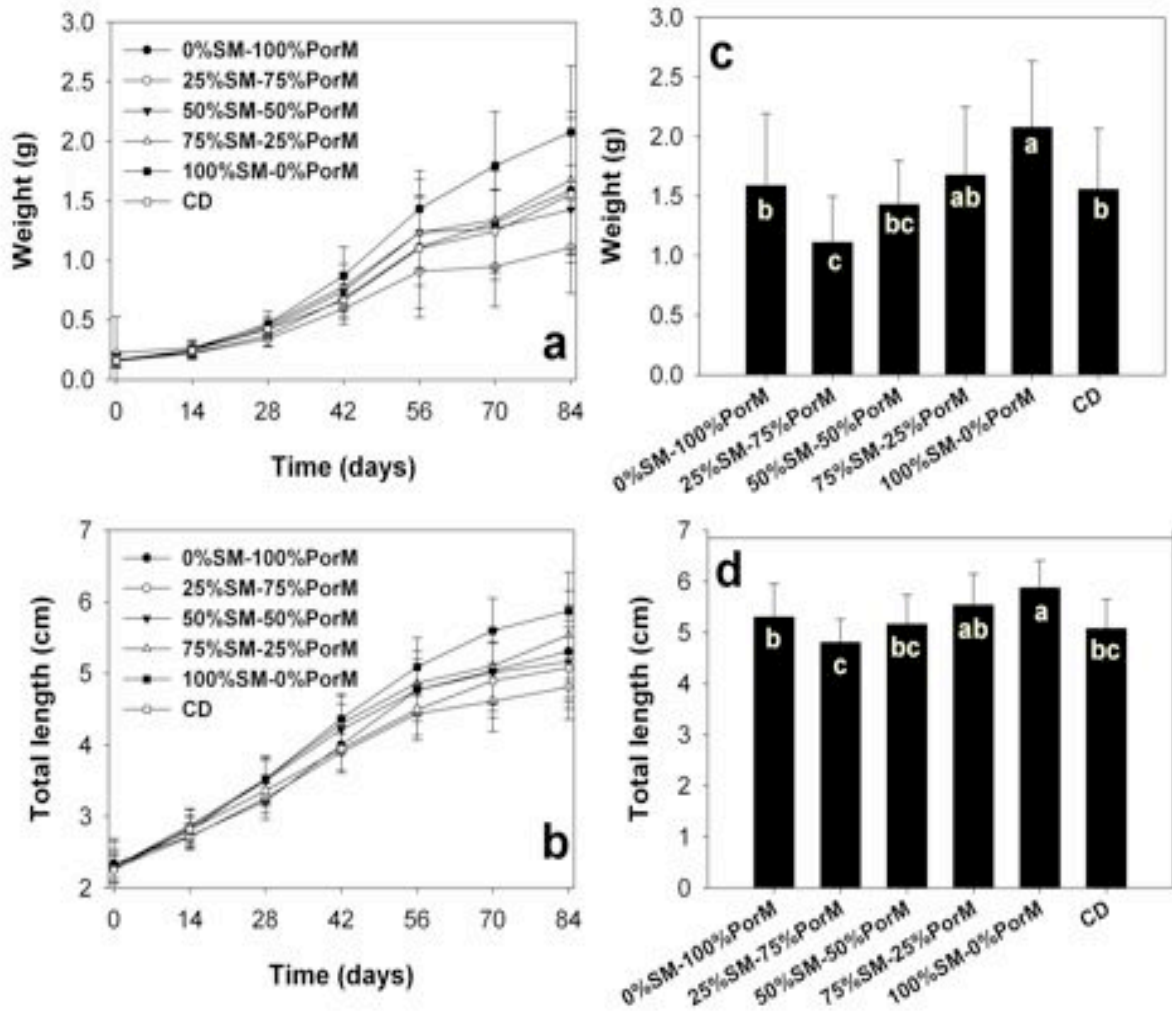


Figure 5. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *P. splendida* juveniles feed with diets substituting fish meal for pork meal.

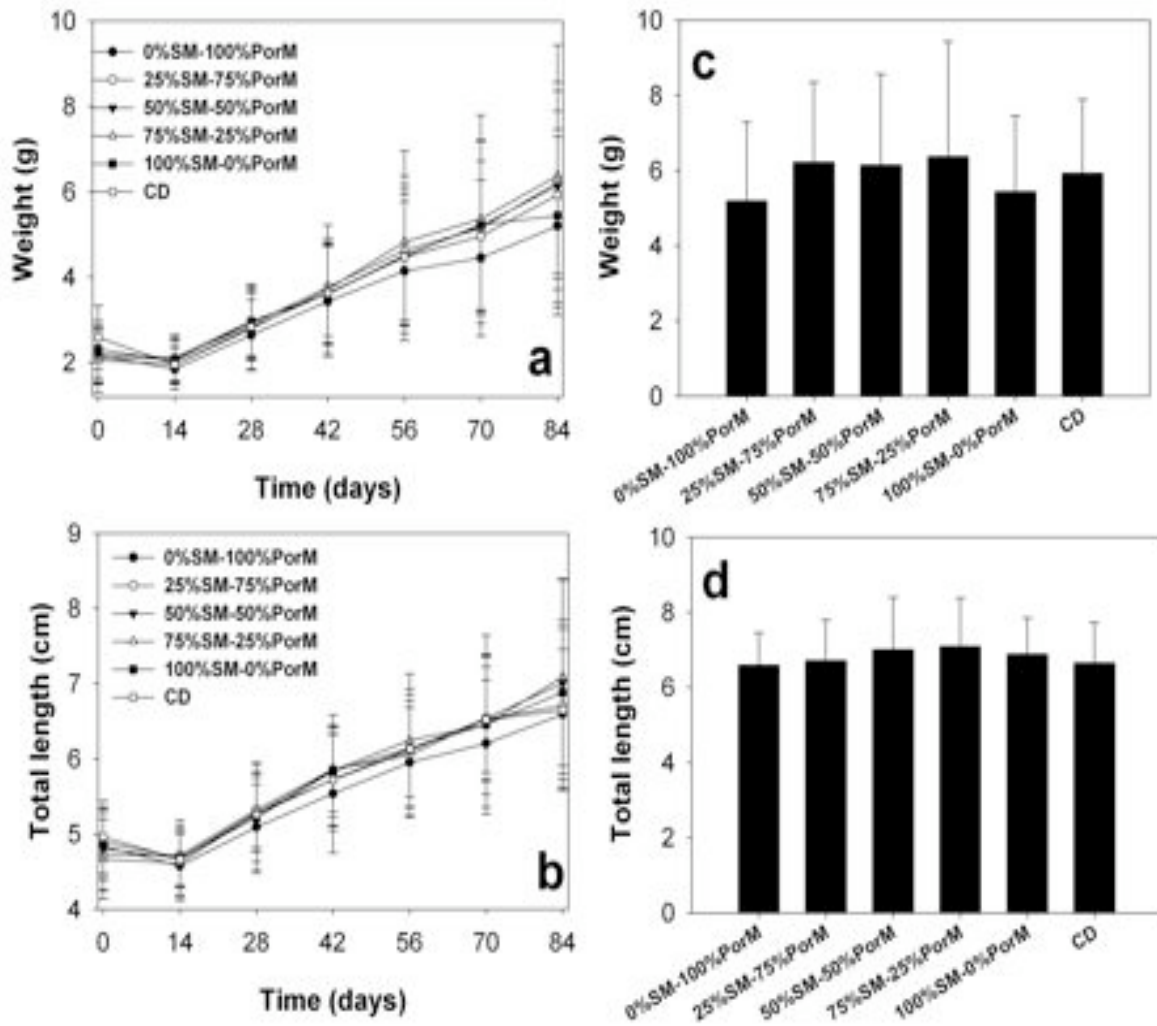


Figure 6. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *C. urophthalmus* juveniles feed with diets substituting fish meal for pork meal.

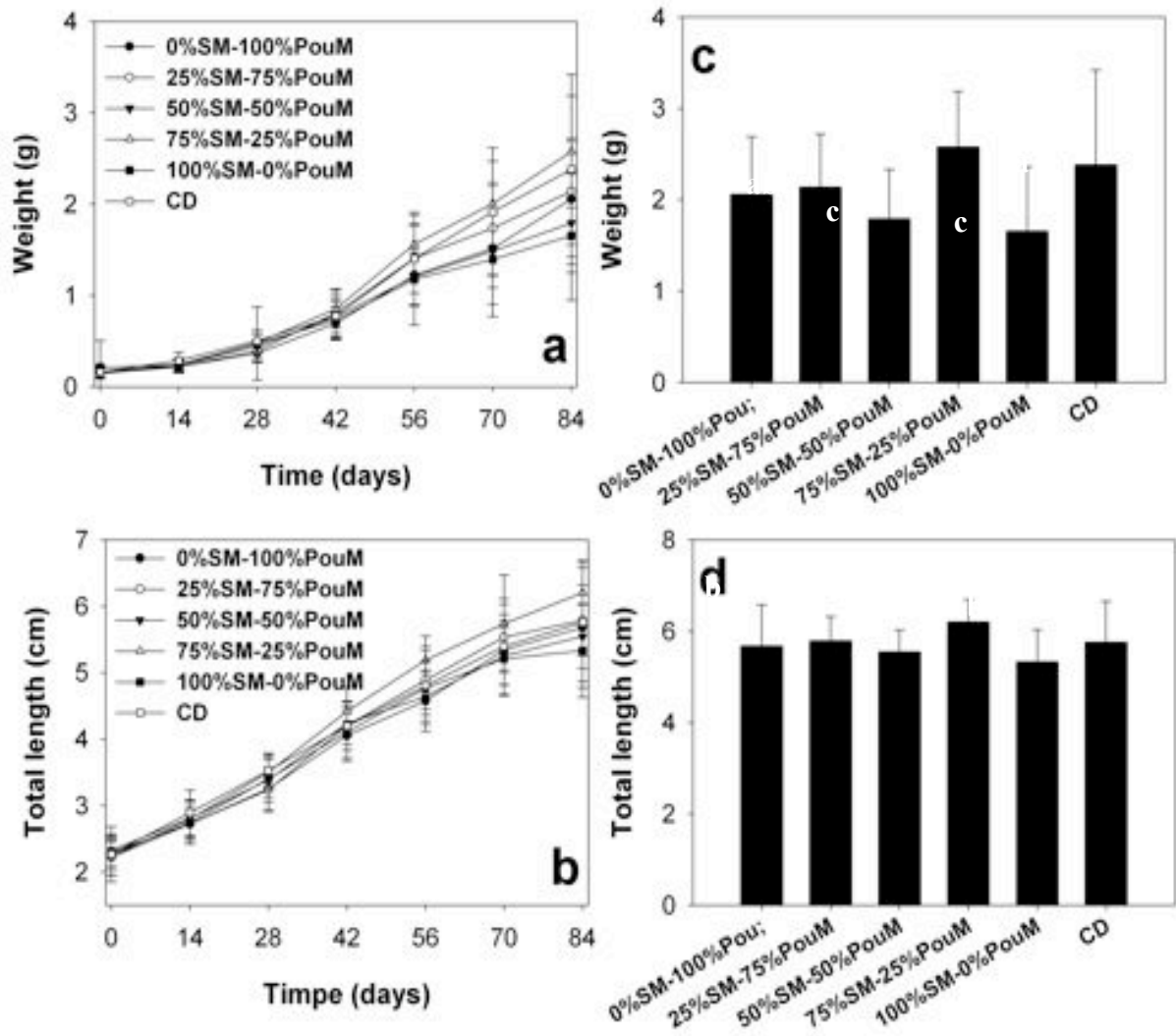


Figure 7. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *P. splendida* juveniles feed with diets substituting fish meal for poultry by-product meal.

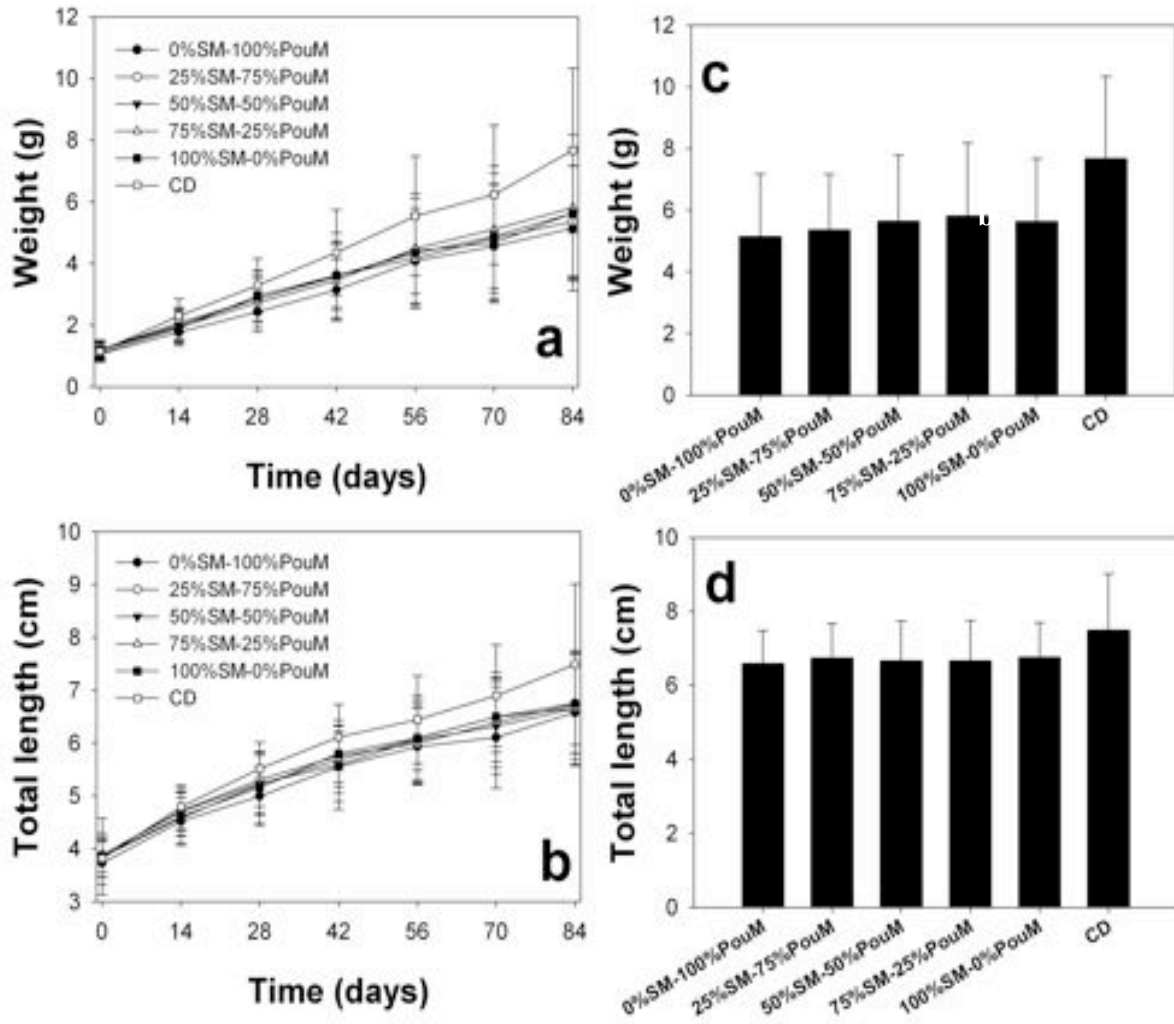


Figure 8. Growth in weight (a) and total length (b), and final weight (c) and total length (d) of *C. urophthalmus* juveniles feed with diets substituting fish meal for poultry by-product meal.