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# Evaluation of Chaya Leaf Meal *Cnidoscolus chayamansa* McVaugh in the Diets for Blue Shrimp *Litopenaeus stylirostris* Stimpson

A. Rocha Estrada, Ma. G. Alanis Guzman, D. Ricque Marie, L.E. Cruz Suarez,
M.A. Alvarado Vazquez and C.L. Garcia Diaz
Facultad de Ciencias Biologicas, Universidad Autonoma de Nuevo Leon, UANL, A.P.F-2,
66451, San Nicolas de los Garza N.L., Mexico

**Abstract:** To understand the nutritional value of chaya leaf meal (*Cnidoscolus chayamansa*) as an alternative diet for blue shrimp (*Litopenaeus stylirostris*), four separate diets that contained 0, 10, 20 or 30% chaya leaf meal were created. These diets replaced a mix of soybean meal/wheat meal (45.63-54.36%) and the effects of these diets were compared to those observed with a commercial diet. The diets were evaluated after *ad libitum* feeding of juvenile blue shrimp (initial weight of 0.250±0.04 g) for 28 days using a completely randomized design (4 replicates with 6 shrimp per aquarium). Bioassay results from the evaluated parameters demonstrated that the best diets for feeding rate and weight gain were the 20% chaya diet (1.36 g and 269.98%) and the commercial diet (1.46 g and 247.36%). For the feed conversion rate parameter, the best results were obtained from the 20% (2.06) and 0% (2.09) chaya diets. There were no significant differences in survival rates ($p>0.05$) but shrimp on the commercial diet had the lowest survival rate (88.88%).

**Key words:** Meal, protein, shrimp, chaya, Mexico

## INTRODUCTION

In aquaculture feeding, fish, shrimp and squid meal have been traditionally used as primary protein sources because of their high nutritional value and palatability. However, their high cost and relative unavailability has necessitated their partial or complete substitution with plant-based ingredients (Lim and Dominy, 1990). In recent decades, the use of plant-based proteins has become quite important in aquaculture because plant-based proteins are cheaper than animal-based proteins. Earlier studies have evaluated proteins from the following sources as feed for shrimp and other aquatic organisms: soybean (Gallagher, 1994; Davis *et al*., 1995; Jimenez-Yan *et al*., 2006), cotton meal (Lim, 1996), Bermuda grass protein extract (Buentello *et al*., 1997), ipil-ipil (Penaflorida *et al*., 1992; Osman *et al*., 1996), sunflower meal, peanut and rapeseed meal (Haiqing and Xiqin, 1994), amaranth, potato protein concentrate (Xie and Jokumsen, 1997), papaya and yam, moringa leaf meal (Richter *et al*., 2003) and corn meal (Kissil and Lupatsch, 2004). Several of these studies have indicated that the use of plant-based proteins can result in poor growth, low survival rates and poor feed conversion rates for several reasons including anti-nutritional factors and/or limiting aminoacids (lysine, tryptophan, threonine, or methionine). *Cnidoscolus chayamansa* (also known as chaya) is a native plant of Mexico and the Yucatan Peninsula that has been used since pre-Hispanic times. Ancient Mayans favored this plant for both food and medicinal purposes, especially its leaves which have high protein content (Kuti and Torres, 1996; Kuti and Kuti, 1999; Figueroa-Valverde *et al*., 2009; Miranda-Velasquez *et al*., 2010). Moreover, chaya is resistant to pests and is easy to propagate because it is drought-resistant and has high productivity (Nagy *et al*., 1978; Kuti and Torres, 1996). Chaya is a bush distributed throughout the Southeastern and Northern areas of Mexico and Central America and it is currently being introduced in the United States (primarily in Southern Texas and Florida). Chaya is an attractive, succulent plant that is 2-3 m in height and has slender branches and white pith. Chaya is a thick plant with few stinging hairs and has one or two glands at the apex of the petiole. Chaya plants have bright green truncate-cordate leaves that are tri-lobed, roughly wavy-toothed and wider than they are long (10-16 cm in width and 4-8 cm in length). Chaya plants have petioles that are 8-15 cm long and usually have stinging hairs. The flowers are tubular, white and unisex with male flowers measuring 6-7 mm with 10 stamens and female flowers measuring 9-10 mm with capsular 3-seeded fruit (Kuti and Torres,

**Corresponding Author:** A. Rocha Estrada, Facultad de Ciencias Biologicas, Universidad Autonoma de Nuevo Leon, UANL, A.P.F-2, 66451, San Nicolas de los Garza N.L., Mexico

1996). There are no studies on the use of chaya leaf meal (*Cnidoscolus chayamansa*) in aquaculture. Thus, this study sought to determine the utility of chaya in aquaculture by comparing the weight gain, survival, feeding rate and feed conversion rate of *Litopenaeus stylirostris* juveniles fed diets with different levels of chaya substituted into a mix of commonly used plant-based protein (soybean meal and wheat meal).

## MATERIALS AND METHODS

Chaya leaves were obtained from a family garden in Monterrey, Nuevo Leon and were analyzed to determine their proximate composition, dietary fiber and protein digestibility *in vitro* with pepsin (AOAC, 1997). In addition, the levels of their protein nitrogen, non-protein nitrogen and nitrates were measured (Tokoro *et al*., 1987) (Table 1). The Mixit-2 computational program was used to create 4 experimental diets based on the nutritional requirements of shrimp (Akiyama *et al*., 1991). These diets were later compared to a commercial diet (Table 2). To compensate for the protein contribution of chaya, varying amounts of a mixture of soybean meal/wheat meal was incorporated (45.63-54.35%) to have the same protein content as chaya (27.65%). In addition, chaya lipids were replaced with soybean oil. Sodium monophosphate was used to maintain the calcium: phosphorous ratio (1:1 or 1.5:1) in the diets. The feed was pelletized in a meat grinder to obtain pellets with a diameter of 2 mm that were dried in an oven at 100°C for 8 min (Lim and Dominy, 1990). The pelletized feed was broken up and stored in refrigerated plastic containers during the course of the bioassay. Experimental diets were analyzed to determine their chemical composition using methods described by the AOAC (1997).

Table 1: Approximate chemical composition of chaya leaf meal (g/100 g of sample)

| Measure | Percentage |
|---|---|
| Moisture | 3.27±0.94 |
| Protein | 27.65±0.65 |
| Ether extract | 7.05±0.52 |
| Ash | 10.38±1.26 |
| Crude fiber | 7.41±0.41 |
| Nitrogen Free Extract (NFE) | 44.24±0.73 |
| Dietary fiber | 31.51±0.22 |
| Total nitrogen | 4.42±0.73 |
| Protein nitrogen | 4.31±0.04 |
| Non-protein nitrogen | 0.09±0.00 |
| Nitrates | 1.84±0.44 |
| *In vitro* protein digestibility | 89.89±0.97 |

*Mean±standar deviation, n = 3

Table 2: Experimental diets composition

| Ingredients (%) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Chaya leaf meal | - | 10.00 | 20.00 | 30.00 |
| Fish meal | 25.09 | 25.09 | 25.09 | 24.93 |
| Soybean meal/wheat meal | 64.50 | 54.79 | 44.02 | 33.89 |
| Fish oil | 1.00 | 1.00 | 1.00 | 1.00 |
| Soybean oil | 1.58 | 1.03 | 0.44 | - |
| Solvay2 (INVE) | 0.18 | 0.18 | 0.18 | 0.18 |
| Attractant (FP) | 0.10 | 0.10 | 0.10 | 0.10 |
| Soybean lecithin (F-100) | 2.50 | 2.50 | 2.50 | 2.50 |
| Sodium monophosphate | - | 0.29 | 1.36 | 2.22 |
| Sodium alginate (SIGMA) | 3.00 | 3.00 | 3.00 | 3.00 |
| Hexametaphosphate (Aldrich) | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamins (INVE) | 0.25 | 0.25 | 0.25 | 0.25 |
| Minerals (INVE) | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine (Tecnicas Nutricionales) | 0.12 | 0.09 | 0.25 | 0.25 |
| Antioxidant (ETQ) | 0.02 | 0.02 | 0.20 | 0.02 |

1: 0% chaya; 2: 10% chaya; 3: 20% chaya and 4: 30% chaya

Stability tests were performed for each of the diets using the AQUACOP (1978) Method to determine the loss of dry material (leaching) after an hour of immersion in seawater (35 g $L^{-1}$). The growth study lasted for 28 days. Six *L. stylirostris* juvenile shrimps (0.250±0.04 g) from the Aquastrat farm (Escuinapa, Sinaloa) were added to each aquarium and 4 replicates (i.e., 4 separate aquariums) were used to evaluate each diet. Shrimp were acclimatized for 1 week in 500 L aquariums and fed a base feed before starting the experiment. During this period, the shrimp were separated into groups based on size to select shrimp with the closest possible size range. The shrimp were then distributed to the different aquariums and an analysis of variance of their initial weights was conducted to avoid significant differences between different treatment groups. Shrimp from these groups were randomly distributed among aquariums and were subsequently fed one of the four designated appropriate experimental diets. The temperature (29.85°C) and salinity (33.82 g $L^{-1}$) were measured daily and the pH (8.23), nitrites (0.49 ppm), nitrates (20 ppm) and total ammonia (0.42 ppm) were measured weekly. The shrimp were given *ad libitum* access to their experimental diets which were administered twice a day (between 9:00 and 10:00 am and between 5:00 and 6:00 pm) during the 1st 2 weeks and 3 times a day (between 9:00 and 10:00 am between 2:00 and 3:00 pm and between 5:00 and 6:00 pm) during the following 2 weeks. Each day, leftover food, mortality and molts were documented and the leftover food and feces were removed. During the bioassay, the individual weight, aquarium biomass, weight gain, feeding rate (per treatment group), feed conversion rate and survival rate were determined.

The average weight, weight gain, survival rate and feed conversion rate were analyzed by one-way Analysis of Variance (ANOVA) with multiple comparisons of means (Duncan) to determine significant differences between treatment groups.

## RESULTS AND DISCUSSION

Proximate analysis (Table 1) revealed that chaya leaf meal had higher protein content, ash and ether extract (27.65, 10.38 and 7.05, respectively) than did ipil-ipil leaf meal (*Leucaena leucocephala*) (26.8, 8.4 and 5.7%). Only the crude fiber content was lower in chaya meal (7.41%) compared with ipil-ipil (11.6%) (NRC, 1983).

Proximate diet analysis (Table 3) showed moisture values in the experimental diets in the range between 6.3 and 6.5% and the commercial diet had a high moisture value of 7.6%. All experimental diets were isoproteic and isolipidic however, the amount of crude fiber varied between the diets with the lowest value being found in the 0% chaya diet (0.47%). In the remaining diets, the amount of fiber increased with increasing percentages of chaya meal and 3.12% crude fiber was found in the commercial diet. The ash content showed similar behavior to that of the crude fiber content. In contrast, there was much less variation in the caloric content in the diets which ranged from 4.45-4.53 kcal $g^{-1}$ for the experimental diets and was 4.16 kcal $g^{-1}$ for the commercial diet.

There were significant differences ($p<0.05$) in the stability of each diet type in water with a greater loss in dry material observed in the experimental diets which together averaged a 12.87% loss. The 30% chaya diet showed the highest leaching rate (18.49%) and the commercial diet exhibited the smallest loss of dry material at 5.12% (Table 3). As expected based on the shrimp distribution procedure used at the beginning of the experiment, there were no significant differences in individual shrimp weight between the aquariums ($p>0.05$). At 28 day, the results showed that only the survival rate had no significant differences ($p>0.05$) among diets. With regard to feeding rate, the commercial diet was the most rapidly consumed, followed by the 20% chaya diet. The least consumed diet was the 0% chaya diet. A comparison of means revealed that the base diet (0% chaya) was not significantly different than the 10 or 30% chaya diets and that the 20% chaya diet was not significantly different than the 30% chaya diet or the commercial diet. The percent weight gain ranged from 195-270% and the greatest growth was observed with the 20% chaya diet. Significant differences in weight gain among the diets ($p<0.05$) were found. The comparison of means demonstrated that the 0% chaya diet showed similarity to the 20% chaya diet, the 30% chaya diet and the commercial diet. In contrast, the survival rates were not significantly different in the various treatment groups ($p>0.05$) and survival rates between 89 and 95% were observed for all treatments. For feed conversion rates, the best results were found with the diets containing 20% (2.06) and 0% (2.09) chaya. The diets containing 10 and 30% chaya showed the highest values (2.75 and 2.66, respectively). There were significant differences among diets where the 0% chaya diet showed similarity to the 20% chaya diet, the 30% chaya diet and the commercial diet. The 10% chaya diet showed similar results to that of the 30% chaya diet. No significant differences in biomass were observedbetween diets ($p>0.05$). However, the 20% chaya diet had the highest biomass (5.20 g) and the 10% chaya diet had the lowest biomass (3.86 g) (Table 4).

The results of the bromatological analysis for chaya leaf meal were similar to the results reported by Perez, Zouza, Barrera and Garza. Chaya has a protein content that is comparable to that of ipil-ipil meal (27.65 vs. 26.80%) but higher than the values reported for alfalfa (17.3%), oats (11.8%), sorghum (11.1%) and wheat (12.2%)

Table 3: Approximate analysis of diets (wet basis)

| | Diets | | | | |
|---|---|---|---|---|---|
| Parameters | 1 | 2 | 3 | 4 | Commercial |
| Moisture | 6.53±0.08 | 6.32±0.32 | 6.37±0.10 | 6.49±0.26 | 7.65±0.31 |
| Ash | 6.69±0.73 | 7.82 0.92 | 9.79±0.82 | 10.45±0.68 | 12.01±0.07 |
| Protein | 35.50±0.30 | 35.70±0.10 | 35.10±0.70 | 35.50±0.40 | 35.40±0.40 |
| Ether extract | 8.07±0.50 | 7.94±0.31 | 8.32±0.37 | 8.45±0.26 | 8.62±0.49 |
| Fiber | 0.47±0.08 | 1.19±0.20 | 1.65±0.20 | 2.99±0.93 | 3.12±0.88 |
| NFE* | 42.90±0.10 | 42.30±1.00 | 42.30±1.00 | 37.90±1.50 | 33.20±0.60 |
| LDM** | 6.70±0.56 | 12.11±1.05 | 12.11±1.05 | 18.49±0.79 | 5.12±0.56 |
| Energy (kcal $g^{-1}$)*** | 4.53±0.06 | 4.48±0.50 | 4.48±0.50 | 4.53±0.09 | 4.16±0.93 |

1: 0% chaya; 2: 10% chaya, 3: 20% chaya, 4: 30% chaya; *NFE obtained based on difference; **Loss of dry material from immersion in seawater; ***5.6 for protein, 9.5 for lipids and 4.1 kcal $g^{-1}$ for carbohydrates were for energy levels

Table 4: Results of biological evaluation after 28 days

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| Parameters | 1 | 2 | 3 | 4 | Commercial | p-values |
| Initial weight (g) | 0.25±0.00 | 0.25±0.00 | 0.240±0.00 | 0.25±0.00 | 0.25±0.00 | 0.960 |
| Final weight (g) | 0.84±0.27 | 0.70±0.22 | 0.910±0.28 | 0.75±0.17 | 0.88±0.17 | 0.005 |
| Weight gain (%) | 237.00±54.0$^{bc}$ | 180.00±150$^{a}$ | 270.000±22.0$^{c}$ | 195.00±2100$^{ab}$ | 247.00±5.00$^{c}$ | 0.004 |
| Individual consumption (g) | 1.20±0.04$^{a}$ | 1.23±0.15$^{a}$ | 1.360±0.07$^{b}$ | 1.31±0.09$^{a}$ | 1.46±0.06$^{b}$ | 0.010 |
| FCR (g) | 2.09±0.41$^{a}$ | 2.75±0.44$^{b}$ | 2.063±0.20$^{a}$ | 2.66±0.20$^{b}$ | 2.35±0.10$^{ab}$ | 0.010 |
| Survival rate (%) | 91.70±9.62 | 91.70±9.60 | 95.800±8.30 | 95.80±8.30 | 89.00±19.2 | 0.890 |
| Biomass (g) | 4.62±0.81 | 3.86±0.54 | 5.200±0.31 | 4.32±0.51 | 4.67±1.03 | 0.110 |

1: 0% chaya, 2: 10% chaya, 3: 20% chaya, 4: 30% chaya; FCR: Feed Conversion Rate

(NRC, 1983) (Table 1). The percentage of protein and non-protein nitrogen was determined from the total nitrogen and the proportion of protein nitrogen (4.31%) was much higher than that of non-protein nitrogen (0.09%). These results are not consistent with the distribution of percentages of protein and non-protein nitrogen reported by Tokoro *et al*. (1987) in 12 other plant species where the level of protein nitrogen was reported to be lower than that of non-protein nitrogen (leaf protein). The crude fiber content was low when compared to the total dietary fiber. It is worth mentioning that results obtained using the crude fiber method is likely to underestimate the dietary fiber content. The enzymatic method provides more exact measures for reporting total fiber content. The nitrate content present in chaya meal was 1.8% and the nitrate inclusion levels amounted to 0.18, 0.36 and 0.54 g nitrates/100 g of feed for the 10, 20 and 30% chaya diets, respectively. Nevertheless, in calculating the ratio of nitrates with respect to grams of feed consumed by shrimp over the 28 day bioassay, the quantity was lower for the 10% chaya diet (1.23 g of feed), the 20% chaya diet (1.36 g of feed) and the 30% chaya diet (1.31 g of feed) which had nitrate contents of 0.002, 0.004 and 0.007 g, respectively. These nitrate quantities are considered nontoxic for shrimp. It is thought that certain vegetables such as spinach, chinese cabbage and lettuce, contain a high quantity of nitrates (9379, 3830 and 2426 ppm, respectively) which could result in serious health consequences. Nitrate quantities increase when vegetables are produced in soils fertilized with products containing large quantities of nitrogen (Tokoro *et al*., 1987).

The proximate composition of the experimental diets was similar and met the established nutritional requirements for shrimp. Crude fiber content increased proportionally along with increases in the rate of inclusion of chaya, an effect that can be explained by the greater fiber content of chaya compared to soybean meal/wheat. The average energy contained in the feed was 4.39 kcal $g^{-1}$ which is similar to the reported standards for shrimp feed (between 2.08 and 4.31 kcal $g^{-1}$ with an average of 3.80 kcal $g^{-1}$) (Devresse, 1985). The higher levels of leaching observed in diets with 10, 20 and 30% chaya indicated that the loss of dry material increases with increased fiber content. Fiber is difficult to grind finely, so the remaining fiber acts as a conduit for water which can create fractures and destabilize the stability of feed in aquatic media (Akiyama *et al*., 1991). However, the leaching values found in the present study were low in comparison to results reported by Rodriguez who used *Salicornia europea* in feed (32% in the control diet, 31% in a diet with straw meal and 30% in a diet with pasta meal). Romero Alvarez analyzed 3 mexican commercial brand diets and found that leaching ranges from 3-5% per hour. Regarding the use of plant-based nutritional sources for shrimp, Lim and Dominy (1990) reported that 28% is the maximal inclusion level of soybean meal in the diet of *Litopenaeus vannamei* that does not reduce their growth, survival or feeding rate. In the present study, the percentage of soybean meal in the diets decreased from 30-20% when 20% chaya was included in the formula which may be one of the reasons for the beneficial effects of this diet. Other researchers have also obtained good results when using other plant-based proteins such as ipil-ipil meal (*L. leucocephala*) to feed *Penaeus monodon* juveniles with an 87% survival rate (Penaflorida *et al*., 1992). Penaflorida reported that the inclusion of 16% papaya meal can substitute for fish meal when supplemented with β-carotene in the *P. monodon* species and Lim (1996) found that cotton meal at an inclusion level of 26.5% can be used in diets for *L. vannamei* without affecting growth or survival rates.

## CONCLUSION

Regarding the use of *C. chayamansa* for feeding *L. stylirostris*, the results obtained in this study demonstrate that these shrimp are capable of using chaya as a protein source. For the 20% chaya meal diet, a feed conversion rate of 2.06, a weight gain of 270% and a survival rate of 95.8% were observed after testing animals that had an initial weight of 250 mg. These results fall within commercial standards (Akiyama *et al*., 1991). Therefore, it is concluded that chaya leaf meal has satisfactory protein quality compared to other meals used in aquaculture and that the values for the evaluated biological parameters in this study are comparable to those of the control and commercial diets.

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