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Passion fruit (*passiflora edulis*) seed cake as a feed ingredient for jaraqui (*semaprochilodus insignis*) and tambaqui (*colossoma macropomum*)

Abstract

Passion fruit seed cake was evaluated as a fish feed ingredient at graded levels (0,5,10,15,20%), employing jaraqui and tambaqui fingerlings. Jaraqui of av. length 3.12-3.21 cm and av. wt. 2.59-2.76 g when fed on these diets attained a final av. length of 9.80-10.51 cm and av. wt. of 22.75- 26.11g at the end of 120 days, while tambaqui of initial av. length 5.06-5.16 cm and av. wt. 4.65-4.74 g grew to a final av. length of 15.28-16.76 cm and av. wt. of 81.22-118.34 g. A progressive decline in protein and an increase in lipid contents were noticed in the formulated diets with increasing passion fruit seed cake incorporation. Replacement of fishmeal with passion fruit seed cake in the test diets did not affect jaraqui growth at all the levels tested, whereas tambaqui growth was affected at higher levels (15 and 20%). The apparent digestibility values of dry matter, protein and lipid in the diets did not differ significantly. Diets affected fish carcass composition. The highest protein and lipid values were recorded with T_0 and T_5 diets that had 30.15% protein and 5.33% lipid respectively. While FCR did not vary significantly between treatments, PER of fish receiving diet T_5 was significantly higher with both the species of fish. The results show that the diets developed using passion fruit seed cake can be used in the culture of jaraqui and tambaqui with economic advantage.

Keywords: Fish meal substitution, Passion fruit, Jaraqui, Tambaqui, Growth, Carcass composition

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Introduction

Passiflora edulis (passion fruit) belongs to the family Passifloraceae and is cultivated commercially in tropical and subtropical areas, mainly for the edible fruit. Brazil stands out as the world's largest producer of passion fruit, producing approximately 9,20,000 tonnes.[1] Locally known as maracujá, passion fruit is rich in vitamin C, calcium and phosphorus. The waste, including the seeds, that represents over 60% of the fruit is usually discarded after extraction of juice from the fruit, and is generally treated as organic waste.[2] An alternative to by-products from passion fruit industrialization would be their use in animal feed. The seeds of passion fruit are rich in fiber, minerals and lipids, with good amount of protein.[3-5] Oil is extracted from the seeds as they contain over 23% oil, rich in PUFA. The resultant by-product oilcake can form a useful feed ingredient. The shortage and rising cost of commercial fish meal has prompted researchers to investigate the production of cost effective feed formulations for cultured fish species.[6,7] Several plant protein sources/agricultural by-products have been tested as replacement for fish meal in fish diets[8-14] with a view to achieve cost reduction in fish production. However, studies with passion fruit seed cake are lacking. Therefore, it was felt worthwhile exploring the suitability of this cheaper nutrient source as a fish feed ingredient.

The two test species of fish were selected based on their economic importance. Jaraqui plays an important social role by catering to the needs of low income population in the Amazon, accounting for approximately 50% of fish landings in the port of Manaus.[15] Tambaqui is one of the most popular cultured species in the Amazon, representing almost half of the total fish sold in Manaus.[16] The present study aimed at investigating the feasibility of utilizing passion fruit seed cake as a fish feed ingredient, replacing the fish meal component at different levels.

Materials and methods

Two experiments were carried out in the wet laboratory of the Coordination of Research in Aquaculture (CPAQ) of National Institute of Research in the Amzon (INPA), employing jaraqui and tambaqui seed obtained from a local farm in Manaus. The seed were separately acclimated for 15 days in two 1000 L tanks, hand feeding them to satiation with the control diet twice daily. The percentage of feeding was standardized at 4% based on the consumption during the acclimatisation period.

Diets

Oil extracted passion fruit (*Passiflora edulis*) seed cake was procured locally; it contained 14.47% protein and 9.89% lipid. Five experimental diets (T_0-T_5) were formulated incorporating 0, 5, 10, 15 and 20% seed cake, by replacing the fish meal component (Table 1). The diet without seed cake (T_0) served as the control. The feed mixture was hand kneaded with 300 ml water per kg and processed through a pelletiser to obtain pellets of 2 mm diameter. They were dried in a thermostatic oven at 40°C, packed in air-tight plastic bags and kept at room temperature until use.

Values with the same superscript in each row are not statistically different (P> 0.05).

Experimental set-up

The experiments were conducted in a flow through system consisting of 15 circular tanks of 500 L each, containing 300 L of water. In the first experiment, 20 fingerlings of jaraqui (*Semaprochilodus insignis*) of av. length 3.12-3.21 cm and av. wt. 2.59-2.76 g were stocked, while in the second experiment tambaqui (*Colossoma macropomum*) fingerlings (av. length 5.06-5.16 cm and av. wt. 4.65-4.74 g) were stocked at 20 per tank. The tanks were continuously aerated from a central aerator, using one aerator stone per tank. The fish were fed twice daily (09.00 hr and 16.00 hr) six days a week, at 4% of body weight in 2 equal halves, over the experimental duration of 120 days. Fish length, weight and total biomass were assessed through sampling every 15 days and the quantity of feed given was readjusted based on the total fish biomass at each sampling.

Table 1 Ingredient proportion and proximate composition (%) of diets

Ingredients	T_0	T_1	T_2	T_3	T_4
Fishmeal	25	20	15	10	5
Soybean meal	20	20	20	20	20
Wheat bran	14	14	14	14	14
Wheat flour	20	20	20	20	20
Maize	18	18	18	18	18
Passion fruit seed cake	0	5	10	15	20
Vitamin-mineral premix*	2	2	2	2	2
Chromic oxide	1	1	1	1	1
Proximate composition (Mean± SD, %)					
Moisture	9.30± 0.04[b]	8.78± 0.08[a]	9.34± 0.06[b]	9.62± 0.03[b]	8.96± 0.09[a]
Crude protein	30.15± 0.09[e]	28.30± 0.14[d]	27.18± 0.12[c]	26.64± 0.16[b]	25.35± 0.18[a]
Lipid	4.76± 0.06[a]	4.89± 0.07[a]	5.13± 0.02[b]	5.24± 0.05[b]	5.33± 0.03[b]
Ash	10.16± 0.10[a]	10.22± 0.08[a]	10.68± 0.05[b]	11.03± 0.16[c]	11.21± 0.09[c]
Crude fibre	11.88±0.08[a]	11.97±0.11[a]	12.11±0.14[ab]	12.32±0.10[b]	12.43±0.18[b]
NFE	33.75	35.84	35.56	35.15	36.72
Gross energy (Kcal g^{-1})	353.69	353.08	347.87	344.18	344.21

*kg mixture contains Vitamins: 6000000 IU A, 5000 mgB_1, 1120 mg B_2, 30000 mg B_3, 30000 mg B_5, 8000 mg B_6, 2000 mg B_8, 3 000 mg B_9, 20000 mcg B_{12}, 500 mgC, 2250000 IU D_3, 3000 mg K_3, 75000 mg E. Minerals: 150000 mg $ZnSO_4$, 60000 mg $MnSO_4$, 4500 mg KI, 100000 mg$FeSO_4$, 2000 mg$CoSO_4$, 400 mg Na_2SeO_3

Water quality monitoring

Water quality parameters viz. dissolved oxygen (DO), electrical conductivity (EC), temperature and pH were measured on a weekly basis, whereas alkalinity, free carbon dioxide (CO_2), nitrite nitrogen (NO_2) and total ammonia (NH_3) were analysed every 15 days. A combined digital YSI 85 meter (YSI incorporated Yellow Springs, Ohio, USA) was used to monitor DO and EC; temperature and pH were measured with a digital YSI 60 meter. Alkalinity, CO_2, NO_2 and NH_3 were estimated following standard procedures.[17]

Digestibility measurement

On termination of the growth experiments, 5 fish each from the replicate tanks were held in 15 cylindrical 200 L fibre glass tanks and fed once daily at 09.00 hr. with the respective 5 diets *ad libitum* in triplicate for 30 days, 6 days per week. Faecal matter was collected every morning and then dried in an oven. The faecal matter collected over the entire period from the respective tanks was pooled and analysed for proximate composition, with chromic oxide as the marker.

Chemical analyses

Ingredients, diets, faecal samples and fish carcass were analysed for proximate composition as follows.[18] Moisture content by oven drying at 105°C for 24 h; crude protien (Nx6.25) by micro Kjeldal digestion and distillation after acid digestion using a Kjeltec 1026 distilling unit together with a Tecator digestion system (Tecator, Sweden); lipid by Soxlet extraction; crude fibre by acid/alkali digestion; ash by ignition at 550°C in a muffle furnace to constant weight. Nitrogn-free extract (NFE) was computed by subtracting the sum values of crude protein, lipid, ash, crude fibre and moisture from 100.[19] Gross energy was calculated by using the conversion factors 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE[20] respectively. Chromic oxide content in the diets and faeces was determined by the acid digestion method[21] using absorption spectrophotometer. Three fish from each tank were sampled on termination of the growth experiments for carcass proximate analysis.

Growth and feed utilization

Fish performance in terms of specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent nutrient digestibility was calculated using the formulae:

Specific growth rate

where Wt is weight of fish at time t, Wi is weight of fish at time 0, and T is the culture period in days

Feed conversion ratio (FCR) = Feed consumed (g) / Weight gain (g)

Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g)

$$Apparent\ nutrient\ digestibility\ (\%) = 100 - 100[100\ x\ (Id\ /\ If\ x\ Nf\ /\ Nd]$$

Where Id represents chromic oxide in diet and If , chromic oxide in faeces, Nd is nutrient in diet, and Nf , nutrient in.

Statistical analysis

The data were analysed by one-way analysis of variance (ANOVA) using Origin 6.1 software. Mean differences between treatments were tested for significance at P<0.05 by Tukey test and comparison was made by Duncan's multiple range test.[22]

Results

Water quality

Water quality parameters varied narrowly between treatments during the experimental period. The range of values were: water temperature 25.56-26.21/26.12-26.81°C, dissolved oxygen 7.20-7.38/7.33-7.65mg/L, pH 5.10-5.33/5.22-5.64, conductivity 29.24-32.38/28.12-34.52 µS/cm², free CO_2 0.94-1.23/0.89-1.37 mg/L, alkalinity 6.42-8.56/6.18-9.03 mg $CaCO_3$/L, NO_2 0.17-0.22/0.15-0.24 mg/L and NH_3 0.09-0.21/0.07-0.19 mg/L in experiments 1 and 2.

Feed

Fish meal replacement in the formulated diets with passion fruit seed cake resulted in a progressive decline in protein and an increase

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in lipid contents. Moisture and ash levels differed significantly ($P<0.05$) between diets (Table 1). However, despite the lower protein level, diets with passion fruit seed cake had almost similar energy content as that of the control; the values ranged from 344.18 (T_4) to 353.69 Kcal/g (T_0).

Fish growth

On termination of the growth experiment, final av. length of jaraqui varied from 9.80 (T_4) to 10.51 cm (T_1) and av. wt. from 22.75 (T_5) to 26.11g (T_2), while tambaqui fingerlings grew to a final av. length of 15.28 (T_5)-16.76 cm (T_0) and av. wt. of 81.22 (T_5)-118.34 g (T_0), there being no significant difference between any of the treatments with the former species. However, growth of tambaqui under T_3 and T_4 treatments was significantly lower compared to T_0 and T_1 treatments. Fish survival was 100% in all the treatments. FCR varied from 2.13 to 2.25 in the case of jaraqui and 2.23 to 2.35 with tambaqui. PER ranged from 1.22 to 1.47 with jaraqui and 1.15 to 1.38 in the case of tambaqui (Tables 2 & 3).

Feed digestibility and carcass composition

The apparent digestibility values of protein, dry matter and lipid did not differ significantly between fish receiving different diets. But, fish carcass composition was affected by the diets in both the species. The values (%) of protein and lipid ranged from 60.37 to 62.47 and 9.15 to 10.68 in jaraqui (Table 2) and 59.33 to 61.56 and 10.35 to 11.85 in the case of tambaqui (Table 3).

Table 2 Growth parameters, diet digestibility and body composition of jaraqui (Mean+ SD, Experiment 1)

Treatment **Parameter**	T_0	T_1	T_2	T_3	T_4
Initial mean length (cm)	3.21 ± 0.14^a	3.24 ± 0.11^a	3.12 ± 0.10^a	3.16 ± 0.16^a	3.18 ± 0.09^a
Final mean length (cm)	10.51 ± 0.92^a	10.37 ± 0.84^a	10.18 ± 0.59^a	9.80 ± 0.77^a	10.23 ± 0.46^a
Initial mean wt. (g)	2.76 ± 0.27^a	2.67 ± 0.21^a	2.74 ± 0.12^a	2.71 ± 0.17^a	2.59 ± 0.27^a
Final mean wt. (g)	25.37 ± 2.29^a	26.11 ± 3.42^a	25.44 ± 2.18^a	23.33 ± 3.10^a	22.75 ± 2.95^a
Net wt. gain (g)	22.61 ± 1.98^a	23.44 ± 2.09^a	22.70 ± 1.83^a	20.62 ± 2.21^a	20.16 ± 2.02^a
SGR (% day^{-1})	1.85 ± 0.09^a	1.90 ± 0.04^a	1.86 ± 0.05^a	1.79 ± 0.02^a	1.71 ± 0.10^a
FCR	2.13 ± 0.10^a	2.17 ± 0.13^a	2.15 ± 0.07^a	2.21 ± 0.11^a	2.25 ± 0.09^a
PER	1.22 ± 0.06^a	1.30 ± 0.09^{ab}	1.34 ± 0.11^{ab}	1.39 ± 0.10^{ab}	1.47 ± 0.12^b
Feed Digestibility (Mean± SD, %)					
Dry matter	89.15 ± 0.52^a	88.24 ± 0.45^a	89.06 ± 0.57^a	88.52 ± 0.63^a	88.34 ± 0.70^a
Protein	90.06 ± 0.65^a	90.12 ± 0.71^a	90.14 ± 0.45^a	89.22 ± 0.78^a	89.33 ± 0.62^a
Lipid	92.10 ± 0.45^a	92.25 ± 0.23^a	91.66 ± 0.52^a	92.23 ± 0.31^a	91.44 ± 0.54^a
Carcass Composition on Dry Weight Basis (Mean± SD, %)					
Dry matter	88.36 ± 0.25^a	89.12 ± 0.16^b	88.23 ± 0.31^a	89.35 ± 0.32^b	88.48 ± 0.12^a
Crude Protein	62.47 ± 0.48^b	61.18 ± 0.54^a	62.45 ± 0.35^b	60.39 ± 0.29^a	60.37 ± 0.33^a
Lipid	9.15 ± 0.10^a	9.26 ± 0.06^a	9.72 ± 0.15^b	10.25 ± 0.09^c	10.68 ± 0.08^d
Ash	13.29 ± 0.18^a	13.67 ± 0.27^a	14.18 ± 0.12^b	14.41 ± 0.23^{bc}	14.74 ± 0.15^c

Values with the same superscript in each row are not statistically different ($P>0.05$).

Table 3 Growth parameters, diet digestibility and body composition of tambaqui (Mean+ SD, Experiment 2)

Treatment **Parameter**	T_0	T_1	T_2	T_3	T_4
Initial mean length (cm)	5.16 ± 0.21^a	5.06 ± 0.24^a	5.14 ± 0.12^a	5.09 ± 0.16^a	5.11 ± 0.15^a
Final mean length (cm)	16.76 ± 0.82^a	16.24 ± 0.68^a	16.08 ± 0.42^a	15.93 ± 0.54^a	15.28 ± 0.76^a
Initial mean wt. (g)	4.67 ± 0.15^a	4.71 ± 0.11^a	4.74 ± 0.09^a	4.69 ± 0.12^a	4.65 ± 0.16^a
Final mean wt. (g)	118.34 ± 7.14^b	114.85 ± 9.21^b	100.38 ± 7.44^{ab}	92.53 ± 6.40^a	81.22 ± 8.46^a
Net wt. gain (g)	113.67 ± 8.32^b	110.14 ± 6.14^b	95.64 ± 8.21^{ab}	87.84 ± 7.12^a	76.57 ± 7.28^a
SGR (% day^{-1})	2.69 ± 0.17^a	2.66 ± 0.05^a	2.54 ± 0.07^a	2.48 ± 0.05^a	2.38 ± 0.11^a
FCR	2.23 ± 0.08^a	2.26 ± 0.14^a	2.30 ± 0.11^a	2.33 ± 0.09^a	2.35 ± 0.12^a
PER	1.15 ± 0.07^a	1.22 ± 0.10^{ab}	1.26 ± 0.05^{ab}	1.33 ± 0.11^{ab}	1.38 ± 0.08^b
Feed Digestibility (Mean± SD, %)					
Dry matter	90.12 ± 0.67^a	90.24 ± 0.46^a	89.32 ± 0.65^a	89.25 ± 0.58^a	89.46 ± 0.47^a
Crude protein	90.16 ± 0.62^a	90.02 ± 0.78^a	89.25 ± 0.45^a	89.32 ± 0.52^a	89.78 ± 0.54^a
Lipid	91.32 ± 0.45^a	91.25 ± 0.49^a	90.26 ± 0.56^a	90.44 ± 0.42^a	90.12 ± 0.65^a
Carcass Composition on Dry Weight Basis (Mean± SD, %)					
Dry matter	89.31 ± 0.38^b	90.12 ± 0.56^{bc}	88.23 ± 0.35^a	90.35 ± 0.28^c	89.48 ± 0.22^b
Crude protein	61.56 ± 0.32^b	60.97 ± 0.25^b	60.11 ± 0.34^a	59.79 ± 0.24^a	59.33 ± 0.38^a
Lipid	10.35 ± 0.15^a	10.62 ± 0.08^a	11.25 ± 0.18^b	11.56 ± 0.11^b	11.85 ± 0.05^c
Ash	14.23 ± 0.18^a	14.54 ± 0.25^a	15.27 ± 0.16^b	15.45 ± 0.20^b	15.61 ± 0.19^b

Values with the same superscript in each row are not statistically different ($P>0.05$).

Discussion

The water quality parameters monitored during the two experiments were within the acceptable limits for fish culture as has been reported by earlier researchers,[23,24] varying narrowly between treatments and without any drastic fluctuations. Inadequate conditions of water quality affect growth, reproduction, health, survival and quality of fish life, jeopardizing the success of aquaculture.[25] Water temperature recorded in the present study was in the range 25.56-26.21°C. Jaraqui is found in nature in both lotic and lentic ecosystems, where the minimum temperature is 24°C and maximum 40°C, indicating high temperature tolerance.[26] The optimal level of oxygen for tropical fish species ranges from 4 to 6 mg/L.[27] Tambaqui has morphological adaptations to survive in hypoxic environments and can survive in waters with less than 1 mg O2/L.[28,29] Dissolved oxygen was above 7 mg/L throughout this study; however, pH remained slightly acidic. In nature, jaraqui tolerates large ionic plasticity and survives well even in acidic waters.[26] Low alkalinity of 10 mg/L has been reported in tambaqui production systems in the Amazon water when liming is not done.[30] In the present study, alkalinity was relatively low because of the flow through system. For fish, nitrite is toxic in water when the concentration crosses 0.5 mg/L[31] and ammonia, when higher than 2 mg/L.[24] Nitrogen (NO_2 and NH_3) and free CO_2 levels were low in this study (Table 1) and hence would not have adversely influenced fish growth.

In the test diets, a progressive decline in protein and an increase in lipid contents were noticed due to passion fruit seed cake supplementation which can be attributed to its proximate composition. It had 14.47% protein and 9.89% lipid as against 52.61% protein and 6.72% lipid in fish meal. Moisture and ash levels differed significantly ($P<0.05$) between diets. However, their energy content did not vary drastically, despite the lower protein level in diets incorporated with passion fruit seed cake (Table 1).

The best growth of jaraqui was obtained with T1 diet (26.11 g) at the end of 120 days of the feeding experiment, there being no significant difference between treatments. Tambaqui recorded the highest growth with the control (T0) diet (118.34 g); its growth under T3 and T4 treatments was significantly lower compared to T0 and T1 treatments. Genetically, these two species of fish have different growth rates. Jaraqui is a slow growing fish, while tambaqui is a fast growing one. Thus, tambaqui attained 4 times the growth of jaraqui on termination of the feeding experiment. Varied response of the two species to the test diets reflects the difference in feed utilization by them. Increasing replacement of fish meal impacted the quality of the diets due to declining protein levels. However, jaraqui growth was unaffected even with 20% substitution of fish meal with equal amount of passion fruit seed cake; only marginal decrease in its growth was observed under T3 and T4 treatments (Table 2). Thus, 25% protein with 5% fish meal was found sufficient for jaraqui. In tambaqui, 27% protein with 15% fish meal appears desirable, since with lesser protein and fish meal levels its growth was significantly lower (Table 3). Fishes utilize fish meal very well from the diet as its nutrient content is very close to their body composition. Fish meal is a well balanced source of high quality protein. It increases feed efficiency and growth through better food palatability and higher nutrient uptake, digestion, and absorption. Further, it is an effective feed attractant[32] and contains some unknown factors[33,34] which enhance fish growth. Cent percent survival recorded in all the treatments indicates that the fish received sufficient amount of nutrients from the diets provided and the quality of water was conducive.

FCR was marginally better with jaraqui, compared to tambaqui. In contrast, PER was marginally superior with tambaqui than jaraqui, indicating the former's ability to utilize protein more efficiently from artificial diets. While FCR did not vary significantly between treatments, PER of fish receiving diet T5 was significantly better with both the species, compared to the control (T0) diet (Tables 2 & 3). This reflects better utilization of protein from diets containing lower protein level. The protein level in T0 and T4 diets was 30.15 and 25.35% respectively. Higher protein utilization from low protein diets has been reported earlier with other species.[35,38] Better FCR and PER was obtained in salema porgy (*Sarpa salpa*) juveniles receiving lower protein (37 and 30%) diets than higher protein (40-57%) diets.[39] Even though passion fruit seed is known to contain antinutrients like phytates and oxalates,[40] the apparent digestibility values of dry matter, protein and lipid in the diets did not differ significantly with the two fish species. Fish carcass composition was affected by the diets. In jaraqui, the highest protein (62.47%) and lipid (10.68%) values were recorded with T0 and T5 diets, reflecting the influence of dietary nutrients on body composition. Similar was the case with tambaqui which recorded the highest protein (61.56 %) and lipid (11.85%) levels with these diets. The influence of diets on fish carcass composition is well documented.[41-43]

Based on the present results, it may be concluded that passion fruit seed cake can be utilized as a feed ingredient in the diets of jaraqui and tambaqui to the extent of 20 and 10% respectively, by substituting equal amount of fish meal, without affecting the growth performance of fish. The findings have economic significance.

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Conflicts of interest

None.

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