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# Seeds of sacha inchi (*Plukenetia volubilis*, Euphorbiaceae) as a feed ingredient for juvenile tambaqui, *Colossoma macropomum*, and matrinxã, *Brycon amazonicus* (Characidae)

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## ABSTRACT

Sacha inchi (*Plukenetia volubilis*) is a plant native to the Peruvian Amazon with seeds that contain 24 to 29% protein, and levels of vitamin A and E and omega 3 polyunsaturated fatty acids that are suitable for fish nutrition. Thus, this study aimed to evaluate the use of sacha inchi seeds as an ingredient in the diets of tambaqui (*Colossoma macropomum*) and matrinxã (*Brycon amazonicus*). The acceptance and effect of three levels of sacha inchi seed meal (0, 15 and 30%) in fish feed was evaluated in a randomized experiment, testing groups of 12 juvenile tambaquis (29.8 ± 1.0 g, 11.0 ± 1.4 cm) and groups of eight juvenile matrinxãs (34.8 ± 1.3 g, 13.7 ± 1.0 cm). Growth performance and body composition of the fish were determined at the end of the experiment. An effect of the sacha inchi diet was only observed for feed conversion of tambaquis, which was worst at the highest level of sacha inchi seed. In conclusion, sacha inchi can be added to feed juvenile tambaquis at a level of 15%, and juvenile matrinxãs at 30% without compromising growth performance and body parameters of the fish.

**KEYWORDS:** alternative diet, growth performance, nutrition, fish

# Sementes de sacha inchi (*Plukenetia volubilis*, Euphorbiaceae) em rações para juvenis de tambaqui, *Colossoma macropomum*, e matrinxã, *Brycon amazonicus* (Characidae)

## RESUMO

Sacha inchi (*Plukenetia volubilis*) é uma planta nativa da Amazônia peruana cujas sementes contém 24-29% de proteína, além de vitamina A e E e ácidos graxos polinsaturados ômega 3 em níveis adequados para a nutrição de peixes. Dessa forma, este estudo teve como objetivo avaliar o uso de sementes de sacha inchi como ingrediente em rações para o tambaqui (*Colossoma macropomum*) e matrinxã (*Brycon amazonicus*). Níveis de inclusão de sacha inchi nas rações e aceitação foram avaliados em um delineamento experimental inteiramente aleatorizado, avaliando três grupos de 12 juvenis de tambaqui (29,8 ± 1,0 g; 11,0 ± 1,4 cm) e três grupos de oito juvenis de matrinxãs (34,8 ± 1,3 g; 13,7 ± 1,0 cm) alimentados com rações contendo 0, 15 ou 30% de farelo de sementes de sacha inchi. O crescimento e a composição corporal dos peixes foram avaliados ao final do experimento. Um efeito da ração com sacha inchi foi somente observado para a conversão alimentar de tambaquis, que foi pior no maior nível de semente de sacha inchi. Em conclusão, sacha inchi pode ser adicionada na alimentação de juvenis de tambaqui até o nível de 15% e na de juvenis de matrinxã até 30% sem comprometimento do desempenho e dos parâmetros corporais.

**PALAVRAS-CHAVE:** dieta alternativa, desempenho, nutrição, peixes

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## INTRODUCTION

Tambaqui (*Colossoma macropomum*) and matrinxã (*Brycon amazonicus*) are fish species native to the Amazon region that are produced in semi-intensive aquaculture and are important economic alternatives in northern Brazil (Arbeláez-Rojas et al. 2002, Sebrae 2015). Tambaqui is the main native fish farmed in Brazil, for its meat of high quality and good taste. Its production increased from 8,000 to 135,858 tons from 1994 to 2015 and continues to grow. Tambaqui currently accounts for 28.1% of the fish farmed in Brazil. In the Amazonas state, in northern Brazil, 15,000 tons of fish are produced in aquaculture, largely involving rearing and growth of tambaquis in dugout ponds (IBGE 2015). Tambaquis are favorable for aquaculture due to their rusticity and rapid growth. Tambaqui is naturally resilient, tolerates low concentrations of dissolved oxygen and is resistant to sudden changes in pH (Aride et al. 2007; Silva et al. 2007; Dairiki and Silva 2011). About 80% of tambaqui currently commercialized is produced in captivity (Jacometo et al. 2010). The production of matrinxã production was of 9,366,203 tons in 2015 (IBGE 2015). This number may increase since this fish is in high demand by consumers due to its favorable organoleptic characteristics (taste and meat color) and high farming feasibility, with established technology for reproduction, management and growth (Honczaryk and Inoue 2009). Tambaqui and matrinxã are omnivorous and accept ingredients of vegetable origin. However, the feeding behaviour of the two species differs in the acceptance and consumption of diets formulated with unconventional ingredients.

Sacha inchi (*Plukenetia volubilis*) belongs to the plant family Euphorbiaceae, native to Central and South America. It is commercially cropped in the Peruvian Amazon, and Brazil has potential to produce this plant. Its seeds contain 24 to 29% protein, nearly 41.4% oil (Gutiérrez et al. 2011; Souza et al. 2013; Rodriguez et al. 2015) and high levels of vitamin A and E (Fanali et al. 2011), making it suitable for dietary use. In addition, sacha inchi oil is highly nutritious, containing 45% omega 3 polyunsaturated fatty acids (PUFA) (linolenic acid) (Guillén et al. 2003; Céspedes 2006; Clavijo et al. 2015). Beneficial effects of these fatty acids include the ability to prevent cardiovascular disorders, lower glyceride levels and antithrombotic action (Garmendia et al. 2011; Maurer et al. 2012, Catalán et al. 2015). Sacha inchi leaves and oil, as well as the presscake produced using oil extracted from its seeds, are an excellent option of a sustainable ingredient for omnivorous fish feeds. Cold pressed sacha inchi oil has high commercial value and is non-toxic to animals (Gorriti et al. 2010), making it a suitable ingredient for animal nutrition. In Amazonas state, in northern Brazil, tambaqui and matrinxã farming is an important small family business (Almudi and Pinheiro 2015), therefore there is a demand for the use of local, low-cost ingredients in tambaqui and matrinxã feeds, since conventional ingredients are expensive and come from different regions, increasing production costs (Dairiki and Silva 2011; Dairiki et al. 2013). The nutrient and energy balance of fish feeds, which is determined by the proportion of different feed ingredients, are fundamental aspects in fish production that directly affect carcass quality (Portz and Furuya 2012).

Tropical fish in general do not exhibit a high highly unsaturated fatty acids (HUFA) content in the body, but can convert PUFAs such as linolenic acid into HUFA by desaturation and elongation. Fish oil is the fish feed ingredient with the highest PUFA and HUFA levels; however, its high costs makes it difficult to obtain and it can become unsustainable, stimulating the search for novel diet ingredients. Flaxseed oil is a potential candidate for use in tropical fish feed. In fact, the carcass quality of jundias (*Rhamdia quelen*) improved with dietary flaxseed inclusion (Vargas et al. 2008). In addition, vegetable ingredients can be more suitable than those of animal origin. Using sensory analysis Stone et al. (2011) found that acceptance and preference for rainbow trout fillets by testers was higher for fish fed diets containing rapeseed oil as opposed to fish oil. In northern Brazil, flaxseed oil is not a feasible ingredient given its high cost and unavailability. As such, the inclusion of vegetable oils, presscake and/or oilseeds from non-traditional or local plants may be an excellent strategy to provide PUFA for bioconversion and thus improve lipidic quality in the carcass and/or fillets of tropical fishes.

Considering the omnivorous habits of both tambaqui and matrinxã, and that sacha inchi is easily available from the Amazonian biodiversity, we hypothesize that the seeds of sacha inchi may be a convenient and nutritious option for use in the composition of feeds in both species' aquaculture. Therefore, this study aimed to evaluate the effect of different proportions of sacha inchi seed meal in the feed on the growth performance and body composition of juveniles of tambaqui and matrinxã.

## MATERIAL AND METHODS

The experiment was carried out in the Aquaculture Division of Embrapa Western Amazon, Manaus, Amazonas state, Brazil (03º06'07"S, 60º01'30"W), from January to March 2013, in a two-factorial (fish species x feed) completely randomized design. The experimental units (replicates) consisted of a group of 12 juvenile tambaquis (29.8 ± 1.0 g initial weight and 11.0 ± 1.4 cm initial length) and a group of eight juvenile matrinxãs (34.8 ± 1.3 g initial weight and 13.7 ± 1.0 cm initial length). There were three replicates for each species and each of three feeds tested (see below), totaling 18 replicates. Fish were obtained from a commercial hatchery and allowed to adapt to experimental conditions for one week. For initial biometric assessment, fish were anesthetized in benzocaine solution (500 mg $L^{-1}$ water), weighted, measured using the ictiometer and kept during the whole experimental period in eighteen 310 L polypropylene tanks in a closed water recirculation system, with partial water replacement and forced aeration produced by a blower and air stones.

Six test feeds were produced, three for matrinxãs and three for tambaquis. Diets were based on formulations specifically developed for matrinxãs (Izel *et al.* 2004) and tambaquis (Oishi *et al.* 2010). The three feeds for each species contained similar nitrogen and energy levels, but each one contained a different proportion of sacha inchi (0, 15 and 30%) (Table 1). To produce the feed, sacha inchi seeds were ground and sieved to obtain seed meal, which was mixed with the other ingredients. The mixture was homogenized, added to 10% water and pelletized using an industrial grinder. Pellets of 3 to 4 mm were dried in a forced circulation oven (45 ºC for 24 h). Feed was stored at -20 ºC in hermetic containers and portions used in daily feeding were weighed immediately before use and kept in coolers.

Fish were given the test feeds for 60 days, receiving two daily meals (8 h and 15 h) until satiety. Water quality parameters (pH, dissolved oxygen, temperature) were monitored daily. Nitrite, total ammonia, alkalinity and hardness were monitored monthly. The tanks were cleaned weekly by siphoning. To evaluate growth performance, at the end of the experiment fish were assessed to determine final weight, weight gain (WG = final weight – initial weight), feed intake (the total amount of feed consumed after the 60 days per experimental unity), feed conversion ratio (FCR= feed intake/weight gain), specific growth rate [SGR = (ln final weight - ln initial weight) / time x 100] and survival [S = (final number of fish – initial number of fish) × 100]. In addition, four fish from each experimental unity were euthanized using a benzocaine overdose (5,000 mg $L^{-1}$ water). The liver, adipose tissue and viscera were removed by abdominal laparotomy and weighed to determine body composition using the hepatosomatic (HIS = liver weight / carcass weight x 100), liposomatic (LSI = intraperitoneal fat/ carcass weight x 100), and vicerosomatic indices (VSI = viscera weight / carcass weight × 100). Acceptance was estimated by the consumption of the experimental feeds. Data were submitted to exploratory analysis by outlier data test, variance homogeneity, range of the response variable, and analyzed by ANOVA followed by the Tukey test to contrast means ($\alpha$=0.05) using the SAS statistical package.

## RESULTS

Mean water temperature (27.3 ± 0.6 °C), dissolved oxygen (7.1 ± 0.4 mg $L^{-1}$), pH (5.1 ± 0.5), nitrite (0.04 ± 0.11 mg $L^{-1}$), total ammonia (0.19 ± 0.18 mg $L^{-1}$), alkalinity (2.90 ± 0.92 mg $L^{-1}$ $CaCO_3$) and water hardness (5.78 ± 1.55 mg $L^{-1}$ $CaCO_3$) were within the range established by Gomes *et al.* (2005), Gomes and Urbinatti (2005) and Dairiki and Silva (2011) for both species. During the experiment, sacha inchi acceptance and the growth performance of juvenile tambaquis and matrinxãs were satisfactory according to the criteria of Arbeláez-Rojas

**Table 1.** Formulation and calculated composition (%) of test feeds for tambaqui and matrinxã with 0, 15 and 30% sacha inchi levels (as fed basis).

| Ingredient | Tambaqui feed | | | Matrinxã feed | | |
|---|---|---|---|---|---|---|
| | 0% | 15% | 30% | 0% | 15% | 30% |
| Soybean meal | 46.59 | 43.50 | 37.82 | 36.93 | 30.90 | 33.43 |
| Ground corn | 30.27 | 20.00 | 0.00 | 19.65 | 0.00 | 10.00 |
| Wheat meal | 10.00 | 1.85 | 5.91 | 30.00 | 34.82 | 0.72 |
| Meat and bone meal | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Soybean oil | 0.04 | 0.00 | 0.00 | 0.32 | 0.00 | 0.00 |
| Dicalcium phosphate | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Mineral Premix(1) | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin Premix (2) | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Common salt | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Inert (kaolin) | 0.00 | 6.55 | 13.17 | 0.00 | 6.18 | 12.75 |
| Sacha inchi(3) | 0.00 | 15.00 | 30.00 | 0.00 | 15.00 | 30.00 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| Ash (%) | 9.41 | 15.85 | 22.04 | 8.63 | 14.36 | 21.55 |
| Crude energy (kcal $kg^{-1}$) | 3,894 | 3,894 | 3,894 | 3,900 | 3,900 | 3,900 |
| Ether extract (%) | 3.18 | 10.17 | 17.25 | 3.63 | 10.42 | 17.36 |
| Crude fiber (%) | 4.37 | 4.95 | 6.29 | 5.39 | 6.78 | 5.76 |
| Crude protein (%) | 30.00 | 30.00 | 30.00 | 28.00 | 28.00 | 28.00 |

(1) Mineral mix (Nutreco®) per kg of product: Mg, 25,000 mg; Fe, 35,000 mg; Cu, 20,000 mg; Zn, 80,000 mg; I, 1,000 mg; Mn, 50,000 mg; Se, 200 mg; and Co, 480 mg.
(2) Vitamin mix (Nutreco®) per kg of product: vitamin A, 5,000.000 UI; vitamin D3, 1,600,000 UI; vitamin E, 60,000 UI; vitamin K3, 6,000 mg; thiamine (B1), 10,000 mg; riboflavin (B2), 10,000 mg; niacin, 60,000 mg; pyridoxine, 10,000 mg; pantothenic acid, 20,000 mg; biotin, 300 mg; folic acid, 3,000 mg; cobalamin, 12,000 µg; inositol, 40,000 mg. Ascorbic acid (vitamin C), 350,000 mg.
(3) Chemical composition of the Sacha inchi seeds: 40 % of ether extract (18.7 % of linolenic acid; 2.3 % of ash; 26.3 % of crude protein and 94.87 % of dry matter.

*et al.* (2002). No statistical differences were detected among treatments in the evaluated growth parameters, except for FCR in tambaquis, which was significantly lower for the 30% sacha inchi feed in relation to the sacha inchi-free feed (0%). No statistical differences were detected among treatments for survival, HSI, LSI and VSI (Table 2).

## DISCUSSION

Feed intake parameters showed that both tambaquis and matrinxãs accepted the feeds containing sacha inchi, and survival, growth and body composition of fish generally did not differ significantly from feeds without sacha inchi (0%). These results indicate that sacha inchi does not affect negatively nor improve significantly the development of tambaqui and matrinxã over commercially available feed. Thus, considering the cost-benefit relation, sacha inchi has the potential of being a cost-effective and alternative as a fish feed ingredient in the Amazon region.

Even in laboratory conditions, the growth performance of the matrinxã and tambaqui juveniles was satisfactory according to Izel *et al*. (2004) and Dairiki *et al.* (2013), respectively. Juvenile matrinxãs submitted to feed deprivation exhibited full compensatory growth in relation to animals fed daily by increasing feed intake and efficiency, leading to the conclusion that the use of intermittent cycles of feeding can be employed to reduce production costs (Urbinati *et al.* 2014). In our experiment the animals were fed daily and the feed intake did not differ among the treatments.

In other feed tests of matrinxã that used similar initial weight of juveniles and evaluation periods, but only commercial extruded feed (Arbelaez-Rojas *et* al. 2011; Urbinati *et al*. 2014) the growth performance of fish was better than in our study. The same discrepancy in growth rates was observed for juvenile tambaqui (Chagas *et al.* 2013; Silva and Fujimoto 2015) in comparison with our results. These observations show clearly the necessity to adapt the experimental feed tested in our study to the nutrition performance of commercial extruded feed. The significantly lower feed convertion rate of the 30% sacha inchi feed in relation to the control feed in tambaqui suggests that higher concentrations of sacha inchi might have some inhibitory effect, that may be due the elevated crude fiber content of sacha inchi (6.29%). The amount of feces siphoned from tanks housing tambaquis fed 30% sacha inchi diets was higher than in the other treatments.

Our results open the prospect for the use of sacha inchi in aquaculture fish nutrition, which is a good alternative given the high content of crude protein and oil rich in omega 3 PUFA – linolenic acid (see Table 1). Like other vertebrates, fish cannot produce linoleic (18:2 n-6) and linolenic acid (18:3 n-3) and therefore need to obtain these essential fatty acids from their diet, according to species-specific requirements (New 1987; Valenzuela *et al.* 2014). Fish can convert 18-carbon PUFA into highly unsaturated fatty acids (HUFA) of the same omega 3 series, namely eicosapentanoic acid (EPA; 20:5 n-3) and docosaexanoic acid (DHA; 22:6 n-3) (Vargas *et al.* 2008; Tanamati *et al.* 2009; Kamarudin *et al.* 2012). In human nutrition, HUFAs are associated with the prevention

**Table 2.** Growth performance and body composition indices of juvenile tambaqui and matrinxã fed diets containing 0, 15 and 30% sacha inchi levels. IW = initial weight, FW = final weight, FI = feed intake, WG = weight gain, FCR = feed conversion ratio, SGR = specific growth rate, TL = total length, HSI = hepatosomatic index, LSI = liposomatic index, VSI = viscerosomatic index and S = survival. Values are means followed by the standard deviation. Superscripts for FCR of tambaquis denote significant differences (Tukey's test, $p < 0.05$).

| Variable | Tambaqui feed | | | Matrinxã feed | | |
|---|---|---|---|---|---|---|
| | 0% | 15% | 30% | 0% | 15% | 30% |
| $IW_{lot}$ (g) | 352.0 ± 5.3 | 355.3 ± 13.3 | 364.7 ± 16.2 | 288.7 ± 3.1 | 269.0 ± 7.0 | 277.3 ± 9.4 |
| $IW_{unitary}$ (g) | 29.3 ± 0.4 | 29.6 ± 1.1 | 30.4 ± 1.3 | 36.1 ± 0.4 | 33.6 ± 0.9 | 34.7 ± 1.2 |
| $FW_{lot}$ (g) | 615.3 ± 12.7 | 577.3 ± 104.5 | 511.3 ± 27.1 | 460.0 ± 25.0 | 458.0 ± 20.8 | 484.0 ± 46.8 |
| $FW_{unitary}$ (g) | 51.3 ± 1.1 | 48.1 ± 8.7 | 42.6 ± 2.3 | 57.5 ± 3.1 | 57.3 ± 2.6 | 63.2 ± 5.5 |
| $FI_{lot}$ (g) | 386.3 ± 2.4 | 395.4 ± 91.4 | 359.7 ± 39.9 | 314.8 ± 28.2 | 355.4 ± 24.0 | 370.5 ± 22.7 |
| $FI_{unitary}$ (g) | 32.2 ± 0.2 | 33.0 ± 7.6 | 30.0 ± 3.3 | 39.4 ± 3.52 | 44.4 ± 3.0 | 48.4 ± 2.8 |
| $WG_{lot}$ (g) | 263.3 ± 7.6 | 222.0 ± 92.4 | 146.7 ± 42.7 | 171.3 ± 26.9 | 189.0 ± 15.1 | 206.7 ± 50.3 |
| $WG_{unitary}$ (g) | 21.9 ± 0.6 | 18.5 ± 7.7 | 12.2 ± 3.6 | 21.4 ± 3.4 | 23.6 ± 1.9 | 28.6 ± 4.9 |
| FCR | $1.5 \pm 0.1^{b}$ | $1.9 \pm 0.5^{ab}$ | $2.6 \pm 0.6^{a}$ | 1.8 ± 0.1 | 1.9 ± 0.1 | 1.8 ± 0.3 |
| SCR (%$day^{-1}$) | 1.2 ± 0.1 | 1.1 ± 0.3 | 0.8 ± 0.2 | 1.0 ± 0.1 | 1.2 ± 0.1 | 1.2 ± 0.2 |
| TL (cm) | 14.1 ± 1.1 | 14.1 ± 1.6 | 13.7 ± 1.3 | 16.2 ± 1.1 | 16.1 ± 1.6 | 16.4 ± 1.2 |
| HSI (%) | 2.6 ± 0.5 | 2.2 ± 0.3 | 1.6 ± 0.3 | 2.0 ± 0.6 | 1.4 ± 0.3 | 1.7 ± 0.3 |
| LSI (%) | 0.1 ± 0.2 | 0.3 ± 0.3 | 0.5 ± 0.6 | 0.1 ± 0.3 | 0.2 ± 0.3 | 0.2 ± 0.4 |
| VSI (%) | 7.4 ± 1.2 | 7.2 ± 1.1 | 7.6 ± 0.7 | 9.7 ± 2.5 | 8.6 ± 3.1 | 8.2 ± 1.6 |
| S (%) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 95.8 |

of a number of diseases and therefore the promotion of health benefits (Catalán *et al.* 2015). Cold-water marine fish exhibit adequate levels of HUFAs, which are mainly obtained from the diet (Garcia *et al.* 2012). The fatty acid composition of Atlantic cod (*Gadus morhua*) tissue can be regulated by mixing different plant or animal fats into their diets (Jobling *et al.* 2008). Rainbow trout (*Oncorhynchus mykiss*) fed flaxseed oil, which is also rich in omega 3, accumulate this fatty acid series primarily in the carcass (58.1%), whereas 29.5% of PUFA is oxidized and only 12.4% bioconverted into HUFA (Turchini and Francis 2009). Future studies will focus on testing the incorporation of fatty acids in tambaqui and matrinxã meat.

## CONCLUSIONS

The present study indicates that seed meal of sacha inchi can be included in fish feed at levels up to 15% for juvenile tambaqui and 30% for matrinxã without compromising growth performance and body composition. Our results indicate that locally produced sacha inchi seeds have the potential to substitute more expensive non-regional sources of PUFA in tambaqui and matrinxã diets in Amazonian aquaculture.

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