



EVALUATION OF THE POTENTIAL INCLUSION OF MALAYSIAN RUBBER SEED, *Hevea brasiliensis* Muell. Arg. (EUPHORBIACEAE) ON THE TAMBACU DIET

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ABSTRACT

In intensive production systems, fish are exposed to stressful conditions, becoming more susceptible to diseases, which may reflect on their growth parameters. Alternative growth promotion methods without environmental impacts have become the target of researchers and fish farmers. For this reason, the objective of this work was to evaluate the potential of including Malaysian rubber seed in the feeding of tambacu raised in cages. The seeds were collected on a farm in the municipality of Imperatriz, Maranhão, a sample was sent for physical-chemical analysis of the fixed oil. For inoculation in the diet, the husks were removed, dried at room temperature, crushed and manually mixed with the commercial ration feed following the experimental design. The quality of the water was measured throughout the experiment. Biometric measurements were performed to infer growth gains with the tested diet. The results show an influence on the inclusion of 40% of the Malaysian rubber seed in the tambacu diet during rearing. In order to reduce costs resulting from the use of alternative ration feeds, we suggest the use of Malaysian rubber seed in an attempt to minimize barriers due to the high prices of commercial ration feeds within the fish farming production chain.

Keywords: Fish farming. Animal nutrition. Nutritional requirement.

1 INTRODUCTION

Tambacu is a hybrid resulting from the breeding induced between the tambaqui female, *Colossoma macropomum*, and the pacu male, *Piractus mesopotamicus* (SOUZA, 1998). It has an omnivorous habit and its grayish color characteristics are similar to those of tambaqui. Its commercial creation has been growing due to the meat flavor, the low maintenance cost, and the high adaptability to cultivation in tanks and nurseries. However, in intensive production systems, fish are exposed to stressful conditions, decreasing their growth condition (ALVES *et al.*, 2016), increasing the mortality rate, which can result in economic losses for producers (ADORIAN *et al.*, 2018).

This is one of the concerns of this sector, since the most recurrent strategy in aquaculture for prophylactic or therapeutic treatment is the use of antibiotics, but their negative impacts related to human health (bioaccumulation in animal tissues with consequent selection of resistant strains) and with the environment (discharge of untreated effluents and deposition of waste) have aroused great concerns (COSTA and SILVA-JÚNIOR, 2017; BORTOLOTTI *et al.*, 2019).

One of the alternatives to its use is the inoculation of products of vegetal origin inoculated directly in the feed ration, where, despite the controversies, studies have demonstrated its efficiency to increase the mortality rate (SANTOS *et al.*, 2015), in addition to acting as growth promoters, pathogen control (antimicrobial activity), antioxidant activity and stimulation of enzymatic activity (SANTOS, LUDKE and LIMA, 2009).

Among these nutritional prophylactic strategies, we highlight the use of garlic, rosemary and sage as digestive stimulants and antioxidants (RODRIGUES, MEURES and BOSCOLO, 2015; INOUE *et al.*, 2016), in addition to green tea in improving health conditions of fish (CORRÊA *et al.*, 2018). Despite the rubber tree, *Hevea brasiliensis* Muell. Arg., is included among the species of fruits and seeds consumed by Tambaqui, *Colossoma macropomum*, in the natural environment (SILVA, PEREIRA-FILHO and OLIVEIRA-PEREIRA, 2003), this is the first work that uses this food source inoculated in commercial ration feed during the cultivation of

tambacu. Thus, the objective of this work is to evaluate the introduction of rubber tree seed in commercial ration feed as a growth promoter in tambacu.

2 METHODOLOGY

The rubber tree seeds were collected on a farm in Estrada do Arroz, Municipality of Imperatriz, Maranhão, Brazil, Northeast (5°19'58.6"S 47° 36'59.5"W). Then, a sample was sent for analysis at the Institute of Scientific and Technological Research of Amapá, at the Food Science and Technology Center, to evaluate the chemical characteristics, such as acidity, iodine rate, refraction, saponification and content of lipids according to the techniques described by the Adolfo Lutz Institute (ZENEBO, PASCUET and TIGLEA, 2008).

2.1 Chemical analysis

The lipid content was carried out in a Soxhlet extractor. The determination of acidity was performed by titration with a neutral ether-alcohol solution and phenolphthalein indicator and the result was expressed in % oleic acid (m/m). The saponification index was determined by 4% titrating potassium hydroxide and hydrochloric acid. The iodine index was determined using titration with the Wijs solution, in which the amount, in mg, of iodine absorbed per 100g of oil was obtained by the difference between the volumes spent on the titration of the blank and the sample. The peroxide index was determined by the sample's ability to oxidize potassium iodide, and the results were expressed in milliequivalents. The refractive index was performed using an Abbe refractometer.

2.2 Ration Feed production with implementation of the rubber tree seed

The seeds were taken to the property to remove the husks, and according to Lee and Wendy (2017) research, they were put to dry at room temperature for two days and then crushed in a forage machine. After the end of this process, they were added manually to the commercial ration feed (Table 1), according to the

experimental design. The ration was offered to the fish twice a day, in the morning (7am) and in the afternoon (5:30pm), paying attention to avoid the accumulation of ration in the bottom of the cages, as it is a pelleted diet.

Table1: Characteristics of the commercial ration feed used.

| PARAMETER | VALUE |
|----------------------|-------------|
| Humidity (max) | 100g/kg |
| Raw Protein(mín.) | 280g/kg |
| Extrato Etéreo (mín) | 40g/kg |
| Raw Fiber (máx.) | 85g/kg |
| Ashes (máx.) | 140g/kg |
| Calcium (máx.) | 360g/kg |
| Phosphor (mín.) | 8g/kg |
| Vitamin E | 100mg/kg |
| Vitamin K3 | 2,5mg/kg |
| Vitamin B1 | 12,5mg/kg |
| Vitamin B2 | 12,5mg/kg |
| Vitamin B6 | 12,5mg/kg |
| Vitamin B12 | 15mcg/kg |
| Vitamin A | 500 UI/kg |
| Vitamin D3 | 2.000 UI kg |
| Niacin | 50mg/kg |
| Folic Acid | 2,5mg/kg |
| Pantothenic Acid | 25mg/kg |
| Biotin | 4mg/kg |
| Choline | 750mg/kg |
| Inositol | 150 mg kg |
| Vitamin C | 200 mg kg |
| Manganese | 63 mg kg |
| Zinc | 94 mg kg |
| iron | 279 mg kg |
| Copper | 16 mg kg |
| Cobalt | 0,13 mg kg |
| Iodine | 0,57 mg kg |
| Selenium | 0,36 mg kg |

2.3 Experimental design

The experiment was conducted in a fish farm, located on a farm in the municipality of Montes Altos (Latitude: -5.83197, Longitude: -47.0678), Southwest of the State of Maranhão, Northeast, Brazil. The design consisted of 4 net tanks with a size of 1 m² for up to thirty fry.

In the first phase, 30 tambacu fish fry whose size varied between 25 to 30 cm in length were selected to compose random sampling and biometrics were monitored for a period of 60 days, alternating the measurement record every twenty days, this period was standardized in order not to cause stress in animals. The abiotic characteristics of the water were monitored such as dissolved oxygen, nitrite, hardness, toxic ammonia and turbidity of the water weekly and pH and temperature daily according to the kit (Labcon Test).

After acclimatizing for seven days, the fish started to be fed with food supplemented with rubber tree seed, and the experimental design consisted of a group (40% seed + 60% commercial ration feed) with three replicates and the control (Commercial ration feed) according to the studies by Lee and Wendy (2017) for eight weeks

2.4 Statistical analysis

The results of the experimental growth study were presented as mean \pm standard error through the Statistical Analysis Program (SPSS).

3 RESULTS AND DISCUSSION

3.1 Chemical characterization of the ration feed

The values of the chemical analyzes performed are shown in table 2. In this study, a value of 0.68 mg KHO.g⁻¹ of acidity was found in the rubber tree seed. It is worth mentioning that the grain maturation and the storage time of the raw material are factors directly proportional to the acidity index, being a factor related to the quality of the raw material (BORDIGNON, 2009; SANTOS *et al.*, 2017). The high values found are indicative of high hydrolytic breakdown in the seeds, suggesting that the sample was not well preserved, and the seeds did not preserve their chemical quality (MARTIN *et al.*, 2006; MORAIS *et al.*, 2017).

Table 2: Chemical evaluation of the rubber tree oil, *H. brasiliensis* used for inoculation in the feed.

| Chemical constants | Values found |
|--|--------------|
| Acidity (% oleic acid) | 0,68±0,02 |
| Iodine index (g100g ⁻¹) | 18,9±1,2 |
| Refractive index | 1,37±0,1 |
| Saponification index (mgKHOg ⁻¹) | 127,6±3,4 |
| Total lipids (%) | 20 |

The iodine index found was $18.9 \pm 1.2 \text{ g } 100 \text{ g}^{-1}$. In a study carried out by Eka, Tajul and Wan (2010), they found values of iodine in the rubber tree seeds of $28.07 \text{ g} \cdot 100 \text{ g}^{-1}$. The low iodine value of the rubber tree seed oil recorded in our study may be related to exposure to ambient light during storage, causing its oxidation, resulting, therefore, in a decrease in iodine levels.

The refractive index was 1.37 ± 0.1 . This fat index rises with the increase in the length of the glyceride chain and also with unsaturation and correlates with the iodine index that allows to know the degree of unsaturation of the molecules (FERRARI *et al.*, 2005). The refractive index is characteristic for each type of oil (BARROSO *et al.*, 2014).

As for the saponification index, the values recorded in this study were lower than those observed by Silva *et al.* (2018) ($182.57 \text{ mg KHO g}^{-1}$) for *Jatropha Curcas*. Lower saponification rates indicate the presence of esters of high molecular weight fatty acids in the sample. The higher the saponification rate, the more useful it is for food (SARMENTO *et al.*, 2015).

It is important to highlight that although the seed used in this study is considered a protein source, there is the presence of a toxic factor, the cyanogenetic glycoside. And that can be an impediment to making use of it as a food source. As an alternative to reduce the amount of cyanogenetic glycoside in these seeds, it is necessary to put them to dry at room temperature for a few days (EKA, TAJUL and WAN (2010), as was done in this work.

3.2 Evaluation of the inclusion of the seed in the tambacu feed ration

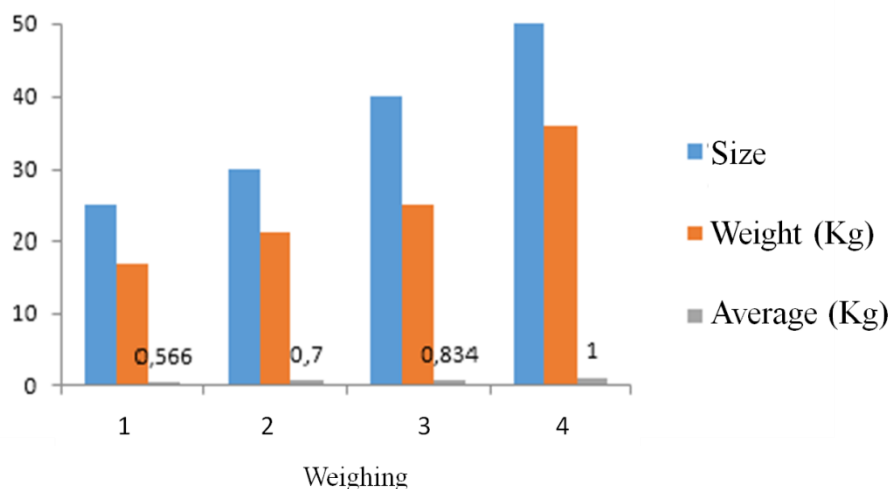
The physical-chemical variables did not change during the experimental time, being adequate to the well-being of the fish (ARIDE, ROUBACH and VAL, 2007). The average water temperature (29.9 ± 0.78 °C), dissolved oxygen (OD) (10.6 ± 0 ppm) and pH (7.4 ± 0.1) were within the range recommended by Faria et al. (2013) for the species, where the ideal temperature for a good growth of tropical fish species is 28 to 32 °C. Therefore, the data demonstrate that this parameter was presented in adequate conditions for the creation of tambacu.

As for the OD, it was above what the resolution Conama 357/2005 (BRASIL, 2005) recommends, which suggests values above 5 mg.L^{-1} , Faria *et al.* (2013) state that values between 1 to 5 mg.L^{-1} can decrease the survival rate in addition to affecting growth rates in the face of prolonged exposures. In the work of Hurtado *et al.* (2018), carried out in semi-intensive fish farming, the OD was in almost all tanks below 5 mg.L^{-1} .

As for transparency, values between 30-45 and 45-60 cm were verified. In the work of Cunha *et al.* (2017), the transparency of the water varied between 0-46 cm. It is important to note that high transparency indicates scarcity of phytoplankton. Regarding nitrite, values of 0.00 ppm were found, with Silva *et al.* (2019) in his research found results with values similar to those of this study, of $0.01 \pm 0.02 \text{ mg L}^{-1}$; and ammonia values were obtained of $1.6 \pm 1.1 \text{ mg L}^{-1}$.

After eight weeks of experiment, weight and growth gains corresponded to $0.509 \pm 0.44 \text{ g}$ and $10 \pm 6.92 \text{ cm}$, respectively, as shown in Figure 1.

Figure 1: Average length, weight and average weight gain of tambacu fish, Montes Altos, Maranhão, Brazil



According to data verified in the literature for fish production, the percentage of 40% inclusion is widely used for adding substrates (LEE *et al.*, 2016; LEE and WENDY, 2017; CORRÊA *et al.*, 2018). The values found in this research demonstrated that the growth in length and weight are proportional and positive. In the work of Araújo-Dairiki, Chaves and Dairiki (2018), in the inclusion of sachá inchi seeds (15 and 30%), *Plukenetia volubilis*, in tambaqui feeding, did not interfere with growth performance and body composition.

The inclusion of a new ingredient in a feed should be carefully evaluated. Braga *et al.* (2014) concluded that higher levels of cellulose inclusion affected negatively the performance of juveniles of tambacu. However, in this research there was a positive increase in weight and growth in the development of tambacu with the addition of the syringe seed in the feed. It is worth mentioning that even though the seed had significant lipid values, it did not interfere negatively, since, according to Pereira *et al.* (2011) and Azevedo, Tonini and Braga (2013), the inclusion of soy and palm oil did not improve the performance of tambacu and tilapia juveniles, respectively.

It is worth mentioning that the price of the ration feed, represents 60% to 70% of the aquaculture production cost (ROTTA, 2002), and the supply of nutritionally balanced food has a great impact on the fish growth rate (CHAGAS *et al.*, 2005). Hence the importance of alternative foods to supply the nutritional needs of each species, based on the idea that efficient and low-cost food can become the difference in productive success (RIBEIRO *et al.*, 2016).

The omnivorous eating habit of tambacu facilitates the exploration of alternative diets (MORAES-VIEIRA, 2018). Silva *et al.* (2017) state that several foods from family farming can be used in rations for Tambaqui, among them, açai (Euterpe oleracea E.), banana (Musa sp.), Cupuaçu (Theobroma gradiflorun L.), pupunha (Bactris gasipaes Kunth .) and feijão de corda (Vigna unguiculata). The present work brings an alternative source, expanding this range with the rubber tree seed.

4 CONCLUSION

The results show an influence on the inclusion of 40% of the rubber tree seed in the tambacu diet during rearing. In order to reduce costs resulting from the use of alternative ration feeds, we suggest the use of rubber tree seed in an attempt to minimize barriers due to the high prices of commercial ration feeds within the fish farming production chain. It is worth mentioning that it is necessary to continue the work with the evaluation of food digestibility in fish and attention to the anti-nutritional factors present in vegetables to meet the nutritional requirements of the species.

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