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# REPLACING CORNMEAL WITH MANGO MEAL IN DIETS FOR JUVENILE TAMBAQUI *Colossoma macropomum:* GROWTH AND METABOLIC PARAMETERS

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## ABSTRACT

The effect on tambaqui fingerlings performance caused by diets containing two different carbohydrate sources was evaluated. Four isoproteic diets with different mango meal concentrations (0, 33, 66 and 100%) replacing cornmeal were tested. The feeding trial lasted 45 days and the feeding rate was 8% of total biomass. 240 tambaqui fingerlings with an initial weight average of 3.66g were distributed in 12 500-liter tanks in a recirculating water system with biofilter. Performance, feed intake, carcass yield, survival, total cholesterol, plasmatic free amino acids, hepatic aspartate aminotransferase(AST), hepatic glycogen and plasma glucose values were evaluated. The results showed that weight gain, daily weight gain, specific growth rate, apparent food consumption, cholesterol, AST and glycogen showed higher values in the treatments with the highest levels of mango meal. Apparent feed conversion, carcass yield, survival and plasma glucose were not affected by the two sources of carbohydrates tested. In conclusion, mango meal used in a practical diet for tambaqui improved growth performance.

**Key words:** carbohydrate; performance; fish nutrition; metabolic profile.

## SUBSTITUIÇÃO DO FARELO DE MILHO POR FARINHA DE MANGA EM DIETAS PARA TAMBAQUI *Colossoma macropomum* JUVENIL: CRESCIMENTO E PARÂMETROS METABÓLICOS

## RESUMO

Avaliou-se o efeito sobre o desempenho de alevinos de tambaqui alimentados com dietas contendo duas fontes diferentes de carboidratos. Foram testadas quatro dietas isoprotéicas com diferentes concentrações de farinha de manga (0, 33, 66 e 100%) em substituição ao farelo de milho. O teste de alimentação foi de 45 dias e a taxa de alimentação foi de 8% da biomassa total. Foram utilizados 240 alevinos de tambaqui com peso inicial médio de 3,66g distribuídos em 12 caixas de 500L em sistema de recirculação de água com biofiltro. Foram avaliados os índices de desempenho, consumo de ração, rendimento de carcaça, sobrevivência, colesterol total, aminoácidos livres plasmáticos, aspartato aminotransferase hepática (AST), glicogênio hepático e glicose plasmática. Os resultados mostraram que o ganho ponderal de peso, a taxa de crescimento específico, o consumo aparente de dieta, colesterol, AST e glicogênio apresentaram valores maiores nos tratamentos com os maiores níveis de farinha de manga. A conversão alimentar aparente, o rendimento de carcaça, a sobrevivência e a glicose plasmática não foram afetados pelas duas fontes de hidratos de carbono testadas. Em conclusão, a farinha de manga utilizado na dieta prática para tambaqui melhorou o desempenho.

**Palavras-chave:** carboidrato; desempenho; nutrição de peixes; perfil metabólico.

## INTRODUCTION

The continuous demand for fish, not only due to demographic growth but also as a healthy food preference has made fish production viable, as have the development of new technologies related to it.

Nutrition has been a technology tool that helps to improve fish growth in a shorter time and contributes to the benefit of worldwide production. Numerous ingredients have been tested around the world in different fish species (XAVIER *et al*., 2012; PEREIRA JUNIOR *et al*., 2013; MUIN *et al*., 2013; NAQVI *et al*., 2015). There is a great variety of vegetable-source ingredients with the potential to be used in fish feed (GABER, 2006; SAMPAIO-OLIVEIRA and CYRINO, 2008; NYINA-WAMWIZA *et al*., 2010; PEREIRA JUNIOR *et al*., 2013), since many species consume vegetables as their natural feeding behavior (LUCAS, 2008). According to BATISTA *et al*. (2015), mango (*Mangífera índica* L.) is rich in carbohydrates such as starch, reducing sugars and pectic substances. This fruit is very important for Brazil and for exports, however there is a large surplus volume generating food waste, environmental contamination and economic loss. According to MARQUES *et al*. (2010) the mango has in its composition lipids (0.61% ± 0.03), proteins (0.44% ± 0.08), non-reducing sugars (4.13% ± 0.12), starch (0.15% ± 0.16), dietary fiber (3.28% ± 0.28), and total carbohydrates (16.5%). Studies have been done testing the fruit or its by-products for its use in fish feed and its effects on performance and metabolism (SILVA *et al*., 2003; GUIMARÃES and STORTI-FILHO, 2004; AZAZA *et al*., 2009; SOUZA *et al*., 2013). Therefore, it can be an important source of feed ingredients in the future and is already proving to be a successful ingredient (BEZERRA *et al*., 2014) since it has a carbohydrate profile that is used by omnivore fish (KANSCI *et al*., 2003).

Intermediary metabolism parameters in the plasma and liver have been used as important tools to evaluate the effects of carbohydrates in fish diets (SKIBA-CASSY *et al*., 2013). Plasma glucose is an indicator of the ingested saccharide effect on fish metabolism (BOOTH *et al*., 2013). Total free amino acids and growth performance parameters together may indicate if dietary protein has been mobilized as a key mechanism in metabolism adaptation (COSTAS *et al*., 2011). It has also been used to evaluate the effect of alternative ingredients for fish feed (ADAMIDOU *et al*., 2009; HANSEN *et al*., 2011; AZEVEDO *et al*., 2013).

Brazil produced 1.163 million tons of mangoes in 2013, most notably in the states of Bahia, São Paulo and Pernambuco, which together accounted for 77.25% of Brazilian production. With these results, Brazil is the seventh largest producer and the fourth largest exporter of mango in the world (AZERÊDO *et al*., 2016). This situation might be an opportunity for its use as an ingredient in animal feed, since mango and its by-products are a source of fiber and carbohydrates (RÊGO *et al*., 2010; PEREIRA JUNIOR *et al*., 2013). In *in natura* and dried mangos, total sugar can reach values of 52.49%, where 17% can be glucose, and fructose values can vary from 2.3 to 3.1% (BERNARDES-SILVA *et al*., 2003). Its known that fructose reaches the cell first, since it does not need to pass through an enzymatic pathway (ENES *et al*., 2009). This advantage can possibly make fish grow faster, with less energy costs.

This work aims to evaluate juvenile tambaqui growth performance and metabolic profile, when fed different inclusion levels of mango meal as a replacement for cornmeal.

## METHODS

The trial was carried out in the Aquiculture Laboratory from the Federal University of Vale do São Francisco in Petrolina-PE, Brazil, through the ethics committee protocol 0016/14415 on animal use.

### Experimental diets

Four experimental diets were formulated, replacing cornmeal with mango meal: 0; 33; 66 and 100%. Diets were prepared according to the species' nutritional requirements (RODRIGUES, 2016) at the age studied, while performing four experimental diets (Table 1).

Mango meal was made in the university laboratory with mango pulp, cut in small pieces and taken to a forced air circulation oven at 65 °C for 72 hours. When it came out it was crushed in a mill and stored frozen at -18 °C until the feeds were made. The other feed ingredients were also crushed in a mill and sifted on a 0.5 mm net. All ingredients were hand mixed to complete homogenization. Water at 50 °C was added to the mixture. Pellets were produced in a pellet mill and then dried in a forced air circulation oven at 65 °C for 24 hours. Pellets were ground in a manual mill and separated in different diameters with different-sized net until it had the specific diameter for fish size in accord with their development. Diet composition was analyzed as established by the Association of Official Analytical Chemists (AOAC, 2000).

**Table 1.** Formulation and chemical composition of the experimental diets.

| Ingredients (g kg$^{-1}$) | Experimental diets | | | |
|---|---|---|---|---|
| | 0% | 33% | 66% | 100% |
| Soybean meal | 621.2 | 621.2 | 621.2 | 621.2 |
| Cornmeal | 300.0 | 200.0 | 100.0 | 0 |
| Mango meal[1] | 0 | 99.9 | 199.9 | 300.0 |
| Bicalcic phosphate | 30.3 | 30.3 | 30.3 | 30.3 |
| Soybean oil | 22.3 | 22.3 | 22.3 | 22.3 |
| Premix[2] | 20.0 | 20.0 | 20.0 | 20.0 |
| NaCl | 5.0 | 5.0 | 5.0 | 5.0 |
| DL-methionine | 1.0 | 1.0 | 1.0 | 1.0 |
| Butylhydroxy-toluene | 00.1 | 00.1 | 00.1 | 00.1 |
| *Chemical composition* (g kg$^{-1}$)* | | | | |
| Dry matter | 940.0 | 952.4 | 936.6 | 933.8 |
| Crude protein | 348.0 | 336.3 | 334.1 | 329.9 |
| Lipid | 34.6 | 33.4 | 32.2 | 31.0 |
| Total Carbohydrates[3] | 270.7 | 265.0 | 259.3 | 253.6 |
| Mineral matter | 78.3 | 79.8 | 81.0 | 82.2 |
| Crude energy (Kcal kg$^{-1}$) | 3.261 | 3.290 | 3.319 | 3.339 |

[1]Composition in g kg$^{-1}$: 93.51 dry matter (%); 2.81 ash (%); 1.35 lipid (%); 3.99 crude protein (%) and gross energy is 4619.04 Kcal kg$^{-1}$. [2]A = 1,200,000 UI; D3 = 200,000 UI; E = 12,000 mg; K3 = 2400 mg; B1 = 4800 mg; B2 = 4800 mg; B6 = 4000 mg; B12 = 4800 mg; folic acid = 1200 mg; calcium pantothenate = 12,000 mg; vit. C = 48,000 mg; biotin = 48 mg; choline = 65,000 mg; nicotinic acid = 24,000 mg; minerals: Fe = 10,000 g; Cu = 600 mg; Mn = 4000 mg; Zn = 6000 mg; I = 20 mg; Co = 2 mg e Se = 20 mg. [3]*Calculated value based on dry matter (SUPERCRAC 2006).

### Experimental design

Fish were stocked in 12 500-liter plastic tanks. Tanks were organized in a closed recirculation system with a biofilter, with physical and biological treatments. Twenty juvenile tambaqui with an initial weight of 3.66 ± 0.84 g and initial length of 5.79 ± 0.50 cm were placed in each tank and acclimated for 15 days before the trial began and were fed the controlled diet.

Organic matter was daily removed from the tank's bottom by suction. Water parameters of dissolved oxygen, temperature and pH were daily monitored with automatic monitors. Water quality parameters were analyzed daily with a multiparameter probe. The average values were: 27.12 ± 1.31 temperature; 5.4 ± 1.08 mg $L^{-1}$ for dissolved oxygen; 0.13 ± 0.001 mS for electrical conductivity; 7.77 ± 1.24 for pH; and 0.01 ± 0.001 mg $L^{-1}$ ammonia ($NH_3$). During the experiment these parameters were within the recommendations for the culture of this species (SIPAÚBA-TAVARES, 1995).

Feed was offered at 8% of fish biomass, twice a day, at 9:00 a.m. and 5:00 p.m., with the experimental diets performing four treatments in triplicate. Every 10 days fish were weighed to adjust these values, but the last weighing was done within 5 days. The experiment was performed over a 45-day period.

Growth parameters in each treatment were calculated by analyzing: weight gain (WG. g) = final body weigh – initial body weight; length gain (LG, cm) = final body length – initial body length; daily weight gain (DWG, g) = f(final body weight – initial body weight)/days of trial; specific growth rate (SGR %) = 100 x (ln final weight – ln initial weight)/days of trial; carcass yield (CY, g) = final body weight – viscera weight; feed intake (FI, g) = feed offered related to body weight % x days of trial; apparent feed conversion (AFC) = feed intake/weight gain; and survival (%) [S = 100 x (initial number of fish – final number of fish). For performance evaluation, 240 fish dispersed in the experimental units were measured at the beginning and at the end of the trial.

### Metabolic analyses

At the end of the experimental period, 10 animals from each treatment were sampled for blood collection and were considered as repetition. Fish were anesthetized with benzocaine (1g $10L^{-1}$) and blood was withdrawn through the caudal vein, with heparinized syringes. Afterwards, fish were euthanized by medullar section for liver collection. Metabolite concentration analyses were carried out in plasma and tissue extracts.

Free amino acids (mg $dL^{-1}$) were determined in neutral extracts at 570 nm (COPLEY, 1941). Liver glycogen (µmol glucose mg/ hepatic protein) was assayed in alcoholic precipitates from alkaline tissue homogenates (BIDINOTTO *et al*., 1997; DUBOIS *et al*., 1960).

Plasma glucose was quantified by an enzymatic method using a commercial kit with the reading done at a 340 nm wavelength. 10 microliters of sample incubated for 5 minutes at 37 °C were used. Total plasmatic cholesterol (mg $dL^{-1}$) was determined by a colorimetric enzymatic method, using a commercial kit with the reading done at a 500 nm wavelength. Ten (10) microliters of plasma sample was incubated for 10 minutes at 37 °C.

Aspartate Amino Transferase (AST) enzymatic activity (U $mg^{-1}$ protein) in the homogenized liver samples was determined by a kinetic method where the absorbance reduction in 340 nm was registered every 2 minutes. 30 microliters of homogenized liver were used. Enzymes were determined by a kinetic method (KANEKO, 1989) using a commercial kit. Two readings were performed in two minutes taking the average of three readings with a 60 second gap, using a 340 nm filter. The total protein was analyzed in plasma and liver with a commercial kit. 10 microliters of plasma and 100 mg of homogenized liver in pH 7.0 buffer were used. The samples were read at 545 nm.

### Statistics

The experimental design was established as incomplete blocks formed by four treatments and ten repetitions per treatment. Metabolite and enzyme data were submitted to variance analysis. The Tukey test ($p < 0.05$) was applied to measure average difference in the parameters among different treatments.

## RESULTS

### Growth parameters

Weight gain (WG) and daily weight gain (DWG) showed significant differences among treatments (Table 2). It can be observed that juveniles fed a diet containing 100% of mango meal

**Table 2.** Growth performance of *Colossoma macropomum* juvenile fed diet containing increasing levels of mango meal replacing cornmeal.

| Performance | Diets | | | |
|---|---|---|---|---|
| | 0% | 33% | 66.% | 100% |
| Weight Gain (g $fish^{-1}$) | $11.97^{b}$±1.60 | $12.91^{b}$±1.77 | $15.12^{ab}$±2.08 | $17.59^{a}$±1.98 |
| Daily weight gain (g $fish^{-1}$) | $0.26^{b}$±0.01 | $0.29^{b}$± 0.01 | $0.33^{ab}$±0.01 | $0.39^{a}$±0.009 |
| Specific growth rate (%) | $26.59^{b}$±3.21 | $28.70^{b}$±2.54 | $33.59^{ab}$±4.12 | $39.10^{a}$±3.99 |
| Feed consumption (g $fish^{-1}$) | $16.14^{b}$±2.10 | $17.24^{ab}$±3.22 | $20.05^{a}$±2.30 | $20.31^{a}$±2.16 |
| Feed conversion rate | 1.36 | 1.34 | 1.33 | 1.16 |
| Carcass yield (%) | 80.78±3.77 | 85.22±3.90 | 83.89±4.18 | 79.98±3.61 |
| Survival (%) | 100 | 100 | 100 | 100 |

Metabolic profile.

**Table 3.** Metabolic intermediates and enzymatic activity in *Colossoma macropomum* juvenile fed diets containing increasing levels of mango meal replacing cornmeal.

| Tissue | Variable | Diets 0% | 33% | 66% | 100% |
|---|---|---|---|---|---|
| Plasma | Cholesterol (mg $dL^{-1}$) | 104.84$^{a}$±12.33 | 44.09$^{b}$±4.21 | 66.13$^{b}$± 5.62 | 52.42$^{b}$±5.00 |
| | Free amino acids (µmols $dL^{-1}$) | 10.61$^{c}$±0.98 | 37.55$^{a}$±2.12 | 16.03$^{bc}$±1.55 | 20.72$^{b}$±2.34 |
| | Glucose (mg $dL^{-1}$) | 68.5±3.70 | 75.8±4.09 | 71.0±3.86 | 63.8±3.43 |
| Liver | AST (U $mg^{-1}$ protein) | 6.39$^{b}$±0.95 | 10.65$^{ab}$±0,90 | 12.60$^{ab}$±1.03 | 16.36$^{a}$±0.88 |
| | Glycogen (µmol glucose $mg^{-1}$ g tissue) | 37.28$^{a}$±2.76 | 19.19$^{b}$±2.90 | 23.26$^{ab}$±3.16 | 22.00$^{ab}$±3.69 |

showed higher growth, with an average value of 17.59 g for WG and 0.39 $g.day^{-1}$ for DWG. The specific growth rate also showed a significant difference among evaluated treatments ($p < 0.05$) following the same patterns of the other evaluated parameters for fish fed a diet with 100% of mango meal. Feed consumption was also influenced by the mango meal percentage in the diet. The highest values were registered among treatments with higher inclusion of mango meal.

Feed conversion rate, carcass composition and survival were not influenced by levels of mango meal inclusion in the diets. There was no significant difference among treatments related to these parameters.

Increasing replacement of cornmeal by mango meal significantly reduced plasmatic cholesterol, increased free amino acids and increased AST activity in the liver (Table 3). Plasma glucose was kept constant in all treatments. There was a significant hepatic glycogen deposit in the treatment's control group.

## DISCUSSION

Weight gain in juvenile fish is indicative of feed's nutritional adequacy (CHO *et al*., 1986). In this trial, the diet containing a higher proportion of mango meal provided higher weight gain in the fish. This fact must be due to the high amounts fructose present in the mango meal as a simple carbohydrate source (KANSCI *et al*., 2003). Simple carbohydrate sources, such as fructose, demand less metabolic energy to provide energy to the animal body. Fruit and fruit by-products have been tested in fish feed with good performance results at different replacement levels (FALAYE and OLORUNTUYI, 1998; ULLOA ROJAS and VERRETH, 2003; LOCHMANN *et al*., 2009; CAMPECHE *et al*., 2014).

Cornmeal replacement by millet and mesquite for tambaqui juvenile diets, did not show significant differences among treatments for weight gain and feed consumption (MIRANDA *et al*., 2009; SILVA *et al*., 1997), which is contrary to this study. However, like millet and mesquite, mango meal in tambaqui diet did not affect feed conversion rate. Juvenile tambaqui growth performance was not affected when cornmeal was replaced by pupunha meal (*Bactris gasipaes)* in the diet (MORI-PINEDO *et al*., 1999). The same was observed when pupunha (*Bactris gasipaes*) replaced wheat bran in feeds for tambaqui (LOCHMANN *et al*., 2009). This fact must be due to the carbohydrate source found in the different diets evaluated as well due to the tambaqui feeding plasticity (DABROWSKI and PORTELLA, 2006). The effects of alternative carbohydrate sources on growth parameters might also depend on its inclusion percentage (NAGAE *et al*., 2002; SIGNOR *et al*., 2007). A significant variety of items can be part of an Amazon fish's natural diet, among them fruits and seeds that are natural sources of all kinds of nutrients (SILVA *et al*., 2003). According to LOVELL (1989) high water temperature promotes growth rate improvement as it maintains metabolic activities constant allowing energy ingested from the feed to be used for growth. Therefore, tropical species can show better feed conversion rates. Although tilapia and tambaqui have different food habits and may belong to different habitat climates, both accept very well a great diversity of feed ingredients.

Carcass yield can help to indicate how nutrients ingested were utilized by the animal. In the present study, although a difference in growth was observed, carcass yield did not show statistical differences, which is similar to what was observed for tilapia fed with cassava meal replacing cornmeal (BOSCOLO *et al*., 2002). In these cases, feed protein and energy efficiency analyses associated to metabolic parameters help to understand feed nutrients used by fish. Complex carbohydrates can be well used by fish when pre-treated to make its digestibility easier and better (DAVIES *et al*., 2011; RENUKDAS *et al*., 2014).

Plasmatic cholesterol reduction obtained in this study may be due to the carbohydrate source chosen, which was mango meal. There are two hypotheses related to the reduction effect mechanism in plasmatic cholesterol. The first is that soluble fiber found in fruits, vegetables and oats, for instance, increase bile acids leading the liver to remove cholesterol from the blood (DE ANGELIS, 2001). The other hypothesis shows that propionate, which is the product from soluble fiber fermentation, inhibits hepatic cholesterol synthesis. Although there are some controversies in the mechanism related to fibers, the plasmatic cholesterol reduction hypothesis is becoming strong (GALLAHER and SCHNEEMAN, 1997).

Glycaemia maintenance was efficient even with the change in the carbohydrate source. Glycaemia levels can indicate the utilization of carbohydrate source and inclusion percent by a fish species (BOOTH *et al*., 2013). Although a reduction in the hepatic glycogen seems to be related to glycaemia in order to keep its values; as was found in this study. The difference in glycogen levels was also observed in *Colossoma macropomum* and *Piaractus brachypomus* fed carbohydrate-rich alternative foodstuffs with 30% inclusion (LOCHMANN *et al*., 2009). The metabolic profile shown in the plasma and liver is an adaptation to keep energy maintenance. In fact, nutrient sources and concentrations can demonstrate different responses from what was registered in this study. European seabass (*Dicentrarchus labrax*), a carnivorous species fed diets with different legumes as an alternative carbohydrate source, did not show any difference in hepatic glycogen index (ADAMIDOU *et al*., 2009). This difference from results found in the present work may be related to the natural feeding habitat difference.

Since the increase in plasma free amino acids can be related to growth improvement or lack of nutrients (COSTAS *et al*., 2011), it is a very important variable to be observed. The difference in feed protein or energetic ingredients clearly affects plasma free amino acids (MACH and NORTVEDT, 2011; LARSEN *et al*., 2012). This fact is a consequence of the ingredient's amino acid profile and affects fish growth. Plasma free amino acid also leads to the better use of the carbohydrate source (mango meal) for the energetic process and, as a consequence, the use of available amino acids for fish growth. This, due to a possible sparing effect of dietary protein, is for maintaining observed glucose and increased amino acid levels in plasma. In this study, better growth was observed in the fish group with a higher inclusion of mango meal. Since the feed was isonitrogenous it was clearly noticed that plasma free amino acid differences were due to its different metabolic use as a consequence of the carbohydrate sources used in the feed. Available amino acids might be deaminated and excreted, or used as energy which might explain the AST activity increase. MOYANO *et al*. (1991) suggests that transaminases and deaminase quantification might be a metabolic indication of amino acid excess in fish. The increase of protein and the reduction of carbohydrate concentrations in *Leiarius marmoratus*'s diet led to an increase in transaminase activity, suggesting gluconeogenesis (SOUZA *et al*., 2014). Surplus amino acids required for protein biosynthesis are deaminated and carbon residues are converted in the Krebs cycle into fats or carbohydrates (DRIEDZIC and HOCHACHKA, 1978). This metabolic pathway of amino acid biosynthesis for energy production was verified in Jundia (*Rhamdia quelen*) fed high protein concentrations (MELO *et al*., 2016). The use of fruit for fish feed is still a recent practice. Further studies should be performed to verify the effects of mango from the fractions that compose this fruit, together with studies of digestibility to prove its efficiency.

In conclusion, data from this trial show that tambaqui growth parameters such as weight gain, and specific growth rate were improved. In addition, the replacement did not cause physiological injuries or affect feed conversion rates and carcass yield. Mango meal possesses an optimal metabolic profile for energy use that comes from its carbohydrate source as was tested in tambaqui.

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