# The Use of Ractopamine as a Fat-Reducing Feed Additive in Tambaqui Farming: Preliminary Results

Igo G. Guimarães, Cristielle N. Souto, Thiago M. Faria, Bruno S. M. Mazini, Graciela P. Martins, Silvio L. Oliveira and Denise S. Oliveira

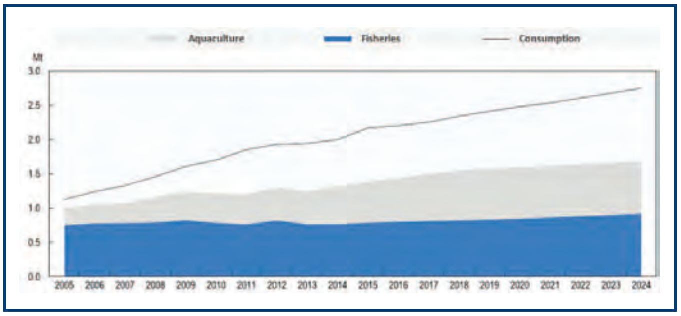


FIGURE 1. *Production and consumption of fish in Brazil (Source: FAO, OCDE 2015).*

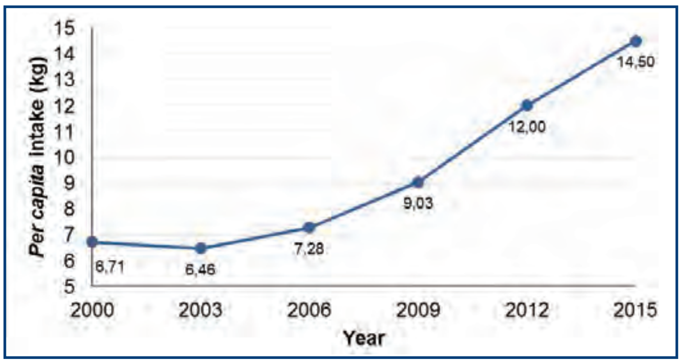


FIGURE 2. Per capita *fish intake in Brazil from 2000 to 2015 (Sources: MPA 2014, FAO 2016).*

FIGURE 3. *A tambaqui juvenile (~300g). Photo: C.N. Souto.*

FIGURE 4. *Fat content in abdominal cavity of tambaqui. Photo: C.N. Souto.*

Brazilian aquaculture has expanded tremendously in the last four years, with an annual growth rate of 15 percent and with an estimated future production increase of 1.972 million t by 2025 (Fig. 1). The increase in consumer purchasing power and the generally higher education level of the population have resulted in a greater demand for animal protein, including fish, and are the main drivers of increased aquaculture production. Per capita fish intake has almost tripled from 6 kg in 2000 to 14.5 kg per capita in 2015 (FAO, OCDE 2015) (Fig. 2). Brazil is the Latin American country that will make a major contribution to future fish supplies with an expected high rate of increase (104 percent higher), mainly due to significant investments in the sector (FAO 2016).

In the context of the high growth rate of Brazilian aquaculture, tambaqui *Colossoma macropomum* (Fig. 3) is a species that has been gaining space among the wide variety of cultured fish. Tambaqui is the second largest scaled freshwater fish and the second most farmed species in Brazil, just behind tilapia. Tambaqui has social and economic importance, mainly for some Amazonian communities, because it is the main protein source for this population. Specific cuts of tambaqui (such as the ribs) are now highly appreciated in the international market.

Tambaqui is fast growing, reaching 1.2 kg in eight months in net cages, and is well-adapted to different production systems. Although production of tambaqui on fish farms is increasing, some market problems associated with high fat content in the abdominal cavity — up to 70 percent of the total visceral content, depending on fish size (Fig. 4) — may cause decreased acceptance of tambaqui by consumers in some regions.

(CONTINUED ON PAGE 36)

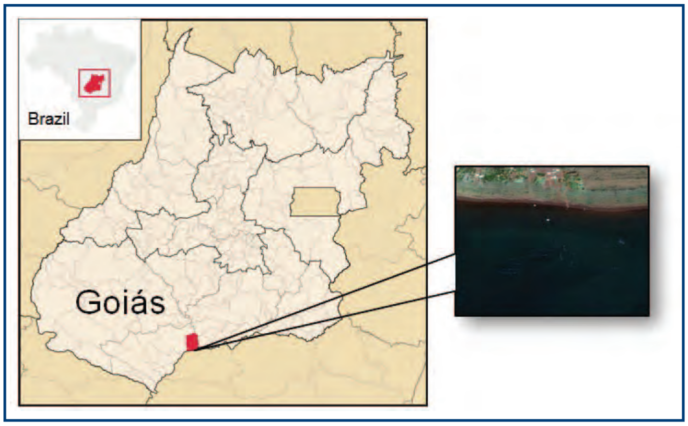


FIGURE 5. *Location of trial 1 (Pôr do Sol Farm). (Source: Adapted from Wikipedia and Google maps).*

## TABLE 1. Composition of basal diet[a] (as fed).

| *Ingredients* | *g/kg* |
|---|---|
| Soybean meal | 579.4 |
| Corn gluten meal | 42.0 |
| Fish meal | 16.4 |
| Corn | 90.0 |
| Wheat middlings | 151.0 |
| Broken rice | 50.0 |
| Cellulose | 5.9 |
| Soybean oil | 20.0 |
| Dicalcium phosphate | 25.7 |
| Limestone | 7.0 |
| Ascorbic acid | 2.5 |
| NaCl | 0.7 |
| Vitamin/mineral mix[b] | 5.0 |
| Antioxidant[c] | 0.2 |
| Total | 1000.0 |

[a] *Digestible protein: 286.9 g/kg, lipids: 45.7 g/kg, crude fiber: 57.0 g/kg; digestible energy: 13.81 Mj/kg.*
[b] *Vitamin and mineral mixture, provided the following per kg diet 1: vitamin A, 16060 IU; vitamin D3, 4510 IU; vitamin E, 250 IU; vitamin K, 30 mg; vitamin B1, 32 mg; vitamin B2, 32 mg; calcium pantothenate, 80 mg; niacin, 170 mg; biotin, 10 mg; folic acid, 10 mg; vitamin B12, 32 µg; vitamin B6, 32 mg.*
[c] *Antioxidant is butylhydroxytoluene (BHT).*

In terrestrial livestock, one way to reduce fat content is to incorporate beta-adrenergic agonists, such as ractopamine, in the feed. Beta-agonists are organic molecules that activate protein synthesis and decrease protein degradation at a cellular level, and ractopamine is efficient at reducing fat content by increasing lean mass gain in swine and cattle. Limited information is available on the use of ractopamine in fish, so we designed two trials to test the feasibility of using ractopamine as a lipolytic feed additive in tambaqui diets. The first trial determined the rate of tissue deposition during the production cycle of tambaqui in net cages and the timing for use of ractopamine. The second trial tested if dietary

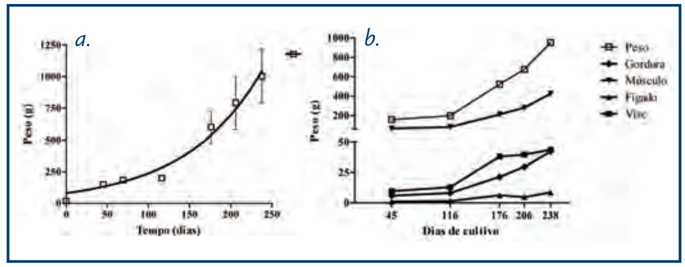


FIGURE 6. *Growth curve (a) and tissue deposition (visceral, liver, muscle and celomatic fat) (b) of tambaqui raised in net cages for eight months (n=10).*

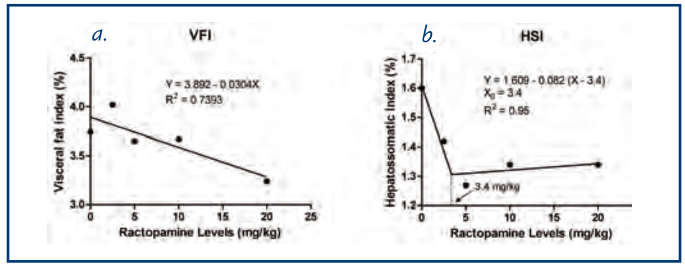


FIGURE 7. *The effect of dietary ractopamine supplementation on (a) visceral fat index (VFI) and (b) hepatosomatic index (HSI) of tambaqui (n=3).*

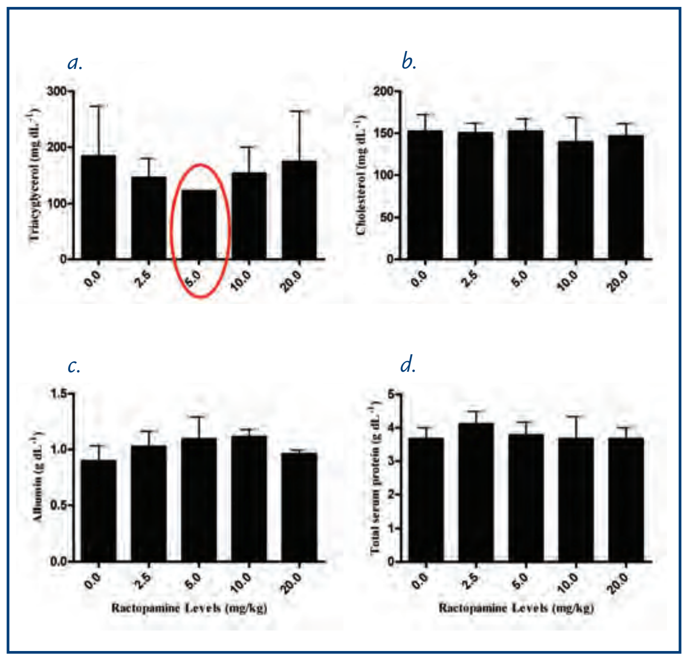


FIGURE 8. *The effect of dietary ractopamine supplementation on (a) visceral fat index (VFI) and (b) hepatosomatic index (HSI) of tambaqui (n=3).*

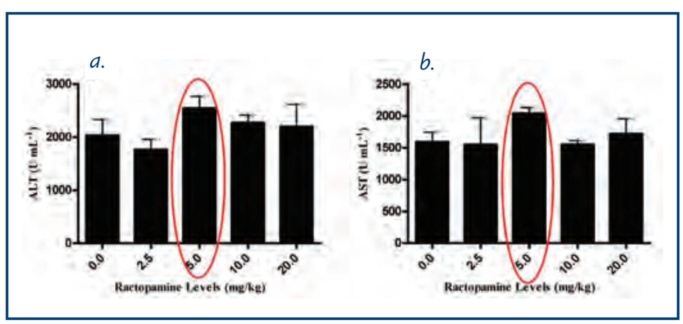


FIGURE 9. *Hepatic alanine (a) and aspartate transaminase (b) activity of tambaqui fed diets containing graded levels of ractopamine for 30 days (n=3).*

TABLE 2. Growth performance of tambaqui fed ractopamine-supplemented diets for 30 days (DWG=daily weight gain, FI=feed intake, FCR=feed conversion ratio, PER=protein efficiency ratio).

| Ractopamine (ppm) | DWG (g) | FI (g) | FCR | PER |
|---|---|---|---|---|
| 0.0 | 5.0±0.4[a] | 16.2±4.4[a] | 1.03±0.23[a] | 0.91±0.20[a] |
| 2.5 | 4.7±0.4[a] | 16.5±2.0[a] | 1.13±0.16[a] | 0.79±0.11[a] |
| 5.0 | 4.2±0.4[a] | 14.6±3.0[a] | 1.10±0.13[a] | 0.79±0.09[a] |
| 10.0 | 4.4±0.6[a] | 14.5±1.0[a] | 1.08±0.22[a] | 0.83±0.16[a] |
| 20.0 | 4.4±1.0[a] | 16.3±1.3[a] | 1.23±0.25[a] | 0.17±0.16[a] |

*Means within column followed by a different superscript were significantly different by SNK multiple range test (p < 0.05).*

supplementation with ractopamine can change metabolic responses and carcass traits of market-size tambaqui.

## Tissue Deposition in Tambaqui

The first trial determined the rate of tissue deposition in tambaqui (e.g. adipose, muscle and visceral tissues). Briefly, 1000 tambaqui of 19 g average weight were stocked into a 27-m$^3$ net cage on a commercial fish farm (Pôr do Sol farm, Gouvelândia-GO-Brazil, geographic coordinates 18º39'30.5"S and 50º10'58.8"W) (Fig. 5). Fish were fed a commercial feed (Guabi, Nutrição Animal) for eight months (from May to December). A sample (10 percent) of fish were weighed monthly, and feeding rate and pellet size were adjusted according to guidance provided by the feed manufacturer. At each sampling, ten fish were killed and carcass traits and chemical composition were measured. To determine visceral fat index, hepatosomatic index and visceral adiposity, viscera, liver and celomatic fat were removed and weighed.

At the end of the trial, data were plotted against fish weight and the growth curve was determined and the nutrient deposition trend line was fitted to determine the proper time to include ractopamine in fish diets (Fig. 6a). A Gompertz exponential model provided the best fit to body weight data. Tissue growth curves showed great variation within the population, however adipose and muscle tissues tended to show the same exponential growth as observed for wet weight (Fig. 6b). The best time to incorporate ractopamine occurred when the rate of increase in fat deposition was greater than that of muscle deposition. In this trial, the greatest change in visceral fat increase occurred between 116 and 176 days.

## Dietary Supplementation with Ractopamine

One hundred and thirty-five market-size tambaqui (1.00 ± 0.04 kg) were randomly assigned to 15 500-L aquaria connected to a recirculating system. This density was selected to maintain approximately the same stocking density as in the first trial. Treatments were randomly assigned to aquaria according to a completely randomized design with five treatments and three replicates. Treatments consisted of five dietary ractopamine levels (0, 2.5, 5, 10, and 20 mg/kg) reached by including commercially available ractopamine HCl (Ractosuin®, OuroFino Saúde Animal Ltda.) into diets. Proximate composition of the diets was similar for all nutrients (Table 1). Fish were fed *ad libitum* two times a day.

After 30 days, fish were bulk weighed and three fish per tank were anesthetized with eugenol, bled by caudal puncture and the liver removed for determination of serum metabolites (cholesterol, triacylglycerol, albumin, total serum protein and globulin), hepatic ALT (alanine aminotransferase) and AST (aspartate aminotransferase) activities. Carcasses were ground and used for determination of carcass traits. Growth parameters evaluated were daily weight gain (DWG), feed intake (FI), feed conversion rate (FCR) and protein efficiency ratio (PER) while for carcass traits, the dress-out percentage (DP), fillet percentage (F), hepatosomatic index (HSI) and visceral fat index (VFI) were evaluated. Data were analyzed with ANOVA and when differences between treatment means were significant ($p<0.05$), regression or SNK multiple range test were used to compare the results for growth performance and carcass traits, while Duncan's multiple range test was used to compare the results for serum metabolites and hepatic enzymes.

Dietary ractopamine supplementation did not affect growth parameters and protein efficiency ratio of tambaqui (Table 2). On the other hand, ractopamine supplementation at 20 mg/kg reduced the visceral fat index by 13.4 percent (Figure 7a, Table 3) compared to the control group. Similarly, the HIS was inversely related to dietary ractopamine supplementation, reaching a plateau at 3.4 mg/kg (Figure 7b). Other carcass traits were not affected by ractopamine supplementation (Table 3).

Generally, there was no pronounced effect of ractopamine supplementation on serum metabolites (Fig. 8). A significant decrease of plasma triacylglycerol concentration (Figure. 8a) was observed in fish fed diets containing 5 mg/kg ractopamine. There was a limited effect of ractopamine administration on hepatic ALT and AST activity (Fig. 9). A trend of increased ALT and AST activity was observed on fish fed 5 mg/kg ractopamine, however this was not significant.

## Modest Effects of Ractopamine Supplementation

As expected, ractopamine supplementation did not affect growth performance or feed utilization of tambaqui. A minor and not statistically significant effect on some carcass traits was observed, mainly on fish fed diets supplemented with ractopamine at the highest rate (20 mg/kg). Generally, most of the studies on the effect of ractopamine supplementation on fish growth performance showed inconsistent results. For instance, ractopamine supplementation does not affect growth and feed utilization of rainbow trout, blue catfish, Hungarian carp and pacu (Bicudo *et al.* 2012, Devens *et al.* 2012, Lortie *et al.* 2004, Salem *et al.* 2006, Vandenberg *et al.* 1998,

(CONTINUED ON PAGE 38)

TABLE 3. Carcass traits of tambaqui fed ractopamine-supplemented diets for 30 days (DP=dress-out percentage, F=fillet percentage, HIS=hepatosomatic index, VFI=visceral fat index).

| RAC | DP | F | HSI | VFI |
|---|---|---|---|---|
| 0.0 | 94.2±0.2 | 42.0±0.5 | 1.6±0.4 | 3.7±0.6 |
| 2.5 | 93.8±0.7 | 41.5±1.8 | 1.4±0.1 | 4.0±0.5 |
| 5.0 | 94.3±0.2 | 40.3±1.4 | 1.3±0.2 | 3.7±0.3 |
| 10.0 | 93.8±1.3 | 39.6±0.4 | 1.3±0.3 | 3.7±0.4 |
| 20.0 | 94.2±0.7 | 41.0±1.0 | 1.3±0.2 | 3.2±0.5 |

*Means within column followed by a different superscript were significantly different by SNK multiple range test ($p < 0.05$).*

Vandenberg and Moccia 1998, Webster *et al.* 1995), while positive effects were observed in channel catfish (Mustin and Lovell 1993). Ractopaminne supplementation seems to modulate protein synthesis and metabolic rate in some fish species, inducing a decrease in carcass and muscle fat, and an increase in protein deposition in fish muscle (Mustin and Lovell 1993, Vandenberg and Moccia 1998).

Ractopamine exposure induces a shift in muscle cell metabolism by changing the rate of protein turnover, consequently increasing nitrogen retention and diverting the use of non-protein energy to support protein accretion. The effect of these changes on intermediary metabolism has been sparsely studied in fish and even in higher vertebrates.

Our results suggest that ractopamine has limited effects on intermediary metabolism of tambaqui and for reducing fat content, which is consistent with the results of carcass traits. However, for a complete picture of the effects of ractopamine on muscle growth of tambaqui and associated metabolism, tissue-specific metabolites should be evaluated. Additionally, ractopamine has transient effects on swine and the response to ractopamine changes after long exposure periods (>14 days) (Adeola *et al.* 1990). This may have affected our results; however, no studies have been performed with fish to determine the timing of using dietary ractopamine.

Ractopamine supplementation at 5 mg/kg has limited effects on increasing protein metabolism and inducing lipolysis in tambaqui by using some indirect serum metabolites. However, the use of 20 mg/kg ractopamine for 30 days induces a slight decrease in visceral fat.

## Implications for Tambaqui Farming in Goias

The growth rate of tambaqui in net cages was high. Juvenile tambaqui had markedly lower growth rates during June and July, which was expected due to low water temperatures associated with the winter season in this region. However, tambaqui clearly showed great compensatory growth in the months that followed, reaching a growth rate of 13 g/day. These values are ten times greater than those of tambaqui subjected to feed deprivation for 28 days under laboratory conditions (Ituassú *et al.* 2004). The average daily weight gain observed in our study was 1.5 times greater than that reported for tambaqui grown for a similar duration (8 months) and stocking density, but without a prominent winter season (Gomes *et al.* 2006). The high growth rate observed in our trials may be an effect of differences in genetic groups of tambaqui used in the trials, feed quality, and differences in environmental conditions. Thus, comparing our results with previous studies should be made with care.

These results are promising for the farming of tambaqui in Goias, a species that has been traditionally cultured in regions with water temperature greater than 20 C. This was not the case of the water body (Paranaíba River) that we used for the trials. Adaptation of conventional management practices may be required for raising tambaqui in regions with cooler water temperatures.

## *Acknowledgments*

This research was financially supported by the Brazilian National Council of Technological and Scientific Development - CNPq (Project # 477920/2012-8). We are thankful to João M.C. Alves from Guabi Nutrição Animal for supplying the feed used in the first trial.

## *Notes*

Igo G. Guimarães*, Curso de Zootecnia, Universidade Federal de Goias, Jatai/GO, Brazil.

Thiago M. Faria, Bruno S. M. Mazini, Graciela P. Martins, Cristielle N. Souto. Laboratorio de Pesquisa em Aquicultura, Universidade Federal de Goias, Jatai, GO, Brazil

Silvio L. Oliveira, Denise S. Oliveira. Curso de Ciências Biológicas, Universidade Federal de Goias, Jatai/GO, Brazil.

* Corresponding author: Igo G. Guimarães, Laboratorio de Pesquisa em Aquicultura, Universidade Federal de Goias, Campus Jatai, PO Box 03,
75801615, Jatai, GO, Brazil. E-mail: igoguimaraes@ufg.br

## *References*

Adeola, O., E. A. Darko, P. He and L.G. Young. 1990. Manipulation of porcine carcass composition by ractopamine. Journal of Animal Science 68**:**3633-3641.

Bicudo, A., R.Y. Sado and J.E.P. Cyrino. 2012. Growth, body composition and hematology of juvenile pacu (*Piaractus mesopotamicus*) fed increasing levels of ractopamine. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 64**:**1335-1342.

de Carvalho Gomes, L., E.C. Chagas, H. Martins-Junior, R. Roubach, E.A. Ono and J. N. de Paula Lourenço. 2006. Cage culture of tambaqui (*Colossoma macropomum*) in a central Amazon floodplain lake. Aquaculture 253**:**374-384.

Devens, M., R. Lazzari, D. Rotilli, L. Pucci, C. Veiverberg and I. Coldebella. 012. Ractopamine in the diet of Hungarian Carp reared in net cages. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 64**:**1717-1722.

FAO. 2016. Food and Agriculture Organization, Fisheries and Aquaculture Departament. The Status of World Fisheries and Aquaculture (SOFIA) – 2016. Rome: FAO, 197.

Ituassú, D. R., G. D. Santos, R. Roubach and M. Pereira-Filho. 2004. Desenvolvimento de tambaqui submetido a períodos de privação alimentar. Pesquisa Agropecuária Brasileira 39**:**1199-1203.

Lortie, M., T. Arnason, S. Dugan, J. Nickerson and T. Moon. 2004. The impact of feeding β2-adrenergic agonists on rainbow trout muscle β2-adrenoceptors and protein synthesis. Journal of Fish Biology 65**:**769-787.

Mustin, W. and R. Lovell. 1993. Feeding the repartitioning agent, ractopamine, to channel catfish (*Ictalurus punctatus*) increases weight gain and reduces fat deposition. Aquaculture 109:145-152.

OCDE and FAO (Organisation for Economic Co-operation and Development and Food and Agriculture Organization of the United Nations). 2016. Perspectivas Agrícolas 2015-2024. available at: www.fao.org.br/download/PA20142015CB.pdf

Salem, M., H. Levesque, T.W. Moon, C.E. Rexroad and J. Yao. 2006. Anabolic effects of feeding β 2-adrenergic agonists on rainbow trout muscle proteases and proteins. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 144**:**145-154.

Vandenberg, G., J. Leatherland and R. Moccia. 1998. The effects of the beta-agonist ractopamine on growth hormone and intermediary metabolite concentrations in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research 29**:**79-87.

Vandenberg, G. and R. Moccia. 1998. Growth performance and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed the (β-agonist ractopamine. Aquaculture Research 29**:**469-479.

Webster, C.D., L.G. Tiu, J.H. Tidwell and E.B. Reed. 1995. Effects of feeding the repartitioning agent L644, 969 on growth and body composition of blue catfish, *Ictalurus furcatus,* fed diets containing two protein levels reared in cages. Aquaculture 134**:**247-256.

Ractopamine exposure induces a shift in muscle cell metabolism by changing the rate of protein turnover, consequently increasing nitrogen retention and diverting the use of non-protein energy to support protein accretion. Our results suggest that ractopamine has limited effects on intermediary metabolism of tambaqui and for reducing fat content. Ractopamine supplementation at 5 mg/kg has limited effects on increasing protein metabolism and inducing lipolysis in tambaqui.