ANDRÉ LUIZ SOUZA MODESTO

SOYBEAN AND LINSEED OILS AS AN ALTERNATIVE TO FISH OIL IN DIETS OF THE JUVENILE TAMBAQUI, *Colossoma macropomum*

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Animal Biology Graduate Program, to obtain the title of *Magister Scientiae.*

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APPROVED: February 7, 2019.

Graziela Domingues de Almeida Lima

Jener Alexandre Sampaio Zuanon

Pollyanna de Moraes França Ferreira

Ana Lúcia Salaro
(Adviser)

“Life is short, so make it what you wanna. Make it good, don't wait until mañana”.

Ricky Martin

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BIOGRAPHY

ANDRÉ LUIZ SOUZA MODESTO, son of Luiz Eugênio Modesto and Andréa do Nascimento Souza Modesto. He was born on January 3, 1993, in the city of Goiânia-GO, Brazil.

In December 2010, he finished high school at Colégio Santa Marcelina, Muriaé - MG, Brazil.

In December of 2015, he graduated in Veterinary Medicine at the Faculty of Sciences and Technology of Viçosa (UNIVIÇOSA / FACISA)

In March 2016, he joined the Postgraduate Program in Animal Biology, Department of Animal Biology, Federal University of Viçosa. He defended his dissertation on February 7, 2019.

SUMMARY

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ABSTRACT

MODESTO, André Luiz Souza, M.Sc., Universidade Federal de Viçosa, February, 2019. **Soybean and linseed oils as an alternative to fish oil in diets of the juvenile tambaqui**, ***Colossoma macropomum*****.** Adviser: Ana Lúcia Salaro. Co-Advisers: Daniel Abreu Vasconcelos Campelo and Martin Bessonart González.

In aquicola production, the use of fish oil as the main lipid ingredient of diets as a function of the energy supply and mainly of essential fatty acids is common. However, with the decrease in fish stocks, fish oil has become a scarce and consequently high value ingredient. Thus, it is necessary to search for new ingredients that can partially or totally replace fish oil in diets for aquatic organisms. In this aspect, the vegetable oils have been highlighting due to the high production and availability associated with the lowest cost. However, for replace fish oil with vegetable oils, research should evaluate the effect of such ingredients on the development and metabolism of various fish species. Therefore, with this research, the objective was to evaluate source and levels of oils in diets for tambaqui, *Colossoma macropomum*. We conducted a study to evaluate productive performance, muscle growth and energy metabolism. We used a randomized block design, with four treatments and four replicas arranged in a 2x2 factorial design. The sources were 100% fish oil and mixture containing 60% soybean oil and 40% linseed oil and inclusion levels of 5% and 10% oils. We selected fish in the following weight classes: B1: 6.9 ± 0.5 g; B2: 8.6 ± 0.5 g and B3: 10.3 ± 0.5 g. The fish were fed for 9 weeks. We evaluated the survival rate, weight gain, daily growth rate, body condition factor, carcass yield, feed conversion, viscerosomatic fat index, hepatosomatic and viscerosomatic index, glucose levels, total cholesterol, high (HDL) and low (LDL) density lipoproteins and triglycerides plasma, hepatic and muscular glycogen and muscle growth of fish. We observed that sources and levels of oil did not affect weight gain, daily growth rate, carcass yield and feed conversion. We noted that fish fed with 5% of fish oil had the lowest value for the condition factor. Fish fed with 10% oil, regardless of source, had the highest values for viscerosomatic index and viscerosomatic fat. However, the lowest values of the hepatosomatic index were observed in fish fed with 10% of oil. For HDL, triglycerides and hepatic glycogen were observed the highest values in the fish that received the diet containing the mixture of soybean and linseed oil, regardless of the level. The lowest values of LDL were found in these same fish. The lower frequency of muscle fibers of less than 20 µm was observed in fish fed with the diet containing 5% of soybean

oil and linseed oil. For muscle fibers with diameter greater than 20 µm and less than 50 µm the highest frequency was observed in the fish that received the mixture of oils, regardless of the level used. We repaired a higher value of moisture in the carcass of the fish fed with 5% of the mixture of soybean oils and linseed. Lipid and carcass energy were higher for fish that consumed 10% oil inclusion independent of the source and ash had lower values for the same level. We conclude that is possible to substitute the fish oil with the mixture containing 60% soybean oil and 40% linseed oil and inclusion level of 5%.

RESUMO

MODESTO, André Luiz Souza, M.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Óleo de soja e de linhaça como alternativa ao óleo de peixe em dietas para juvenis de tambaqui, *Colossoma macropomum*.** Orientador: Ana Lúcia Salaro. Coorientadores: Daniel Abreu Vasconcelos Campelo e Martin Bessonart González.

Na produção aquícola é comum o uso de óleo de peixe como o principal ingrediente energético das dietas em função do fornecimento de energia e principalmente de ácidos graxos essenciais. Porém com a diminuição dos estoques pesqueiro, o óleo de peixe vem se tornando um ingrediente escasso e consequentemente de alto valor. Assim, torna-se necessário pesquisas por novos ingredientes que possam substituir parcial ou totalmente o óleo de peixe em dietas para os organismos aquáticos. Neste aspecto, os óleos vegetais têm se destacado devido à alta produção e disponibilidade associada ao menor custo. No entanto, para substituir o óleo de peixe por óleos vegetais, as pesquisas devem avaliar o efeito de tais ingredientes no desenvolvimento e metabolismo de várias espécies de peixes. Portanto, com esta pesquisa, objetivou se avaliar fontes e níveis de óleos em dietas para o tambaqui, *Colossoma macropomum*. Conduzimos um estudo para avaliar desempenho produtivo, o crescimento muscular e metabolismo energético. O delineamento experimental foi blocos casualizados em esquema fatorial 2x2, duas fontes de óleo: 100% óleo de peixe e mistura contendo 60% de óleo de soja e 40% de óleo de linhaça e dois níveis de inclusão de óleo: 5% e 10%, com quatro repetições. Os blocos consistiam nas seguintes classes de peso: B1: 6,9 ± 0,5 g; B2: 8,6 ± 0,5 g e B3: 10,3 ± 0,5 g. Os peixes foram alimentados por nove semanas. Avaliamos a taxa de sobrevivência, ganho de peso, taxa de crescimento diário, fator de condição corporal, rendimento de carcaça, conversão alimentar, índices viscerossomático, hepatossomático e de gordura viscerossomático, níveis plasmáticos de glicose, colesterol total, lipoproteínas de alta (HDL) e baixa (LDL) densidade, triglicerídeos, glicogênios hepático e muscular e crescimento muscular dos peixes. Observamos que as fontes e os níveis de óleo não afetaram o ganho de peso, a taxa de crescimento diário, o rendimento de carcaça e a conversão alimentar. Observamos que os peixes alimentados com 5% de óleo de peixe tiveram o menor valor para o fator de condição. Peixes alimentados com 10% óleo, independentemente da fonte, apresentaram os maiores valores para índices viscerossomático e de gordura viscerossomático. No entanto, os menores valores do índice hepatossomático foram observados em peixes alimentados com 10% de óleo.

Foram observados os maiores valores para HDL, triglicérides plasmáticos e glicogênio hepático e menores valores de LDL nos peixes que receberam a dieta contendo a mistura dos óleos de soja e linhaça, independentemente do nível. A menor frequência de fibras musculares inferiores a 20 µm foi observada em peixes alimentados com a dieta contendo 5% da mistura de óleo de soja e óleo de linhaça. Para fibras musculares com diâmetro maior que 20 µm e menor que 50 µm, a maior frequência foi observada nos peixes que receberam a mistura de óleos, independente do nível utilizado. Observamos maior valor de matéria seca na carcaça dos peixes alimentados com 5% da mistura de óleos de soja e linhaça. O teor de lipídios e energia da carcaça foram maiores para os peixes que receberam a dieta com 10% de inclusão de óleo independente da fonte. Em contrapartida, as cinzas dos peixes que consumiram 10% foram menores. Conclui-se que para juvenis de tambaqui é possível substituir o óleo de peixe pela mistura contendo 60% de óleo de soja e 40% de óleo de linhaça no nível de inclusão de 5%.

ARTICLE

SOYBEAN AND LINSEED OILS AS AN ALTERNATIVE TO FISH OIL IN DIETS OF THE JUVENILE TAMBAQUI, *Colossoma macropomum*

Manuscript prepared according with the instructions of the journal Aquaculture

Soybean and linseed oils as an alternative to fish oil in diets of the juvenile tambaqui, *Colossoma macropomum*

Abstract

The present study aimed to demonstrate the effects of the replacement of fish oil by the mixture of soybean and linseed oils with two different inclusion levels of these oils in diets of *Colossoma macropomum*. We used a randomized block design, with four treatments and four replicas arranged in a 2x2 factorial design. The sources were 100% fish oil and mixture containing 60% soybean oil and 40% linseed oil and inclusion levels of 5% and 10% oils. We evaluated the survival rate, weight gain, daily growth rate, body condition factor, carcass yield, feed conversion, viscerosomatic fat index, hepatosomatic and viscerosomatic index, glucose levels, total cholesterol, high and low lipoproteins low density and triglycerides plasma, hepatic and muscular glycogen and muscle growth of fish. We observed that sources and levels of oil did not affect weight gain, daily growth rate, carcass yield and feed conversion. We observed that fish fed with 5% of fish oil had the lowest value for the condition factor. Fish fed with 10% oil, regardless of source, had the highest values for viscerosomatic index and viscerosomatic fat. However, the lowest values of the hepatosomatic index were observed in fish fed with 10 of oil. For HDL, triglycerides and hepatic glycogen were observed the highest values in the fish that received the diet containing the mixture of soybean and linseed oil, regardless of the level. The lowest values of LDL were found in these same fish. The lower frequency of muscle fibers of less than 20 µm was observed in fish fed with the diet containing 5% of soybean oil and linseed oil. For muscle fibers with diameter greater than 20 and less than 50 µm the highest frequency was observed in the fish that received the mixture of oils, regardless of the level used. We observed a higher value of moisture in the carcass of the fish fed with 5% of the mixture of soybean oils and linseed. Lipid and carcass energy were higher for fish that consumed 10% oil inclusion independent of the source and ash had lower values for the same level. We conclude that is possible to substitute the fish oil with the mixture containing 60% soybean oil and 40% linseed oil and inclusion level of 5%.

Keywords: energy metabolism, fish oil replacement, lipid nutrition, muscle growth, neotropical fish, vegetable oil.

1 INTRODUCTION

Among the ingredients that make up the diet of fish, lipids are noted for being the main source of energy and fatty acids (PEREZ *et al*., 1997; SARGENT *et al*., 2002; TOCHER, 2010) and are therefore essential for growth, reproduction (GLENCROSS, 2009), neural development (FELLER 2008; WASSAL & STILLWELL 2008) and visual development (BALFRY & HIGGS, 2001) and animal health (TURCHINI *et al*. 2009). A deficiency or excess fat in the diet of fish can lead to a slower growth or even the accumulation of body fat, which certainly compromises the animal health.

In fish diets fish oil is the main lipid component (BEEL *et al*., 2005), due to the balanced fatty acids composition (NG & WANG, 2011). However, with the advance of extractive fishery and a consequent reduction of fish stocks, fish oil is increasingly scarce and expensive, which has been impair the feed processing industries throughout the world (SARGENT *et al.*,2002; SPRAGUE *et al*., 2016). Due to the high production and good availability of vegetable oils, their use in fish diets becomes more accessible, stimulating researchers to study different sources of vegetable oils, as well as different proportions between them (NASOPOULOU & ZABETAKIS, 2012).

In experimental diets for freshwater tropical fish, they have been used vegetable oils such as soybean, canola, olive, corn, palm, sunflower and linseed because they are excellent sources of essential fatty acids. Among vegetable oils with potential to feed aquatic organisms, soybean oil is one of the most produced in the world (OIL WORLD, 2018), making it economically viable (NUNES, 2007). Although soybean oil is rich in linoleic acid (NG & WANG, 2011), it has an imbalance in the composition of fatty acids, being deficient in n3 fatty acids (CABALLERO *et al*., 2002). Compared to other sources of vegetable oil, linseed oil is still poorly produced and therefore with a high price, however, its use in fish diets is justified by the large percentage of linolenic acid (18:3-n3) (BELL *et al*., 2003; NG & WANG, 2011). Therefore, the mixture of linseed oil and soybean oil could provide the fatty acids necessary for the proper development of fish, replacing fish oil without damage to fish health. However, there must be a balance between the proportions of these oils, since the affinity of the enzymes involved in essential fatty acid biosynthesis is greater for n-3 fatty acids than for n-6 fatty acids (TOCHER, 2010).

Several studies have shown the possibility of partial or total replacement of fish oil by vegetable oils. In this aspect the total substitution cod liver oil by soybean, oil or linseed oil did not influence the productive performance of sharpsnout seabream, *Diplodus puntazzo* (PIEDECAUSA *et al*., 2007). In silvery-black porgy, *Sparidentex hasta*, the partial or total replacement of fish, oil by sunflower or canola oils did not cause differences in the productive performance of the fish (MOZANEZADEH *et al*., 2016). Similar results were found for murray cod, *Macullochella peelii peelii*, total oil replacement fish oil by linseed oil, olive oil, sunflower oil or palm oil also did not affect the productive performance of fish (TURCHINI *et al*., 2011).

Oil sources can also act on muscle growth, since they directly influence the growth of fish. Unlike other vertebrates, fish exhibit hyperplastic growth throughout life (VALENTE *et al*., 1999). Studies show that carnivorous fish have less muscle growth potential than omnivorous fish (PÁRRIZAS *et al*., 1994a). Moreover, they showed that muscle growth is closely related to diet due to the increase of receptors that seem to regulate hypertrophic growth (PÁRRIZAS *et al*., 1994b). Thus, the substitution of fish oil by vegetable oils in the fish diet can influence hypertrophic muscle growth.

The tambaqui, *Colossoma macropomum* (Cuvier, 1818), native to the Amazon and Orinoco basins is an omnivore freshwater fish (BUCKUP, 2007) with a tendency to frugivore (GOULDING and CARVALHO, 1982). Tambaqui presents high growth rates, being the second largest fish of scales in the Amazon basin reaching weights of 30 kg in nature (MEROLA & CANTELMO *et al*., 1987), being adapted to high density (CAMPOS-BACA *et al*., 2005), showing a great potential to be used in intensive aquaculture. Therefore, the aim of this research was evaluating the productive performance, muscle growth and energy metabolism of juveniles tambaqui fed with fish oil and mixture of soybean and linseed oils in diets with two oil levels.

2 MATERIAL AND METHODS

Animal experimentation in the present study was conducted according to the ethical principles of animal experimentation established by the Conselho Nacional de Controle da Experimentação Animal (CONCEA) and with the current legislation, being approved by the Committee of Ethics in the Use of Production Animals of the Federal University of Viçosa (CEUAP / UFV), and protocol 44/2017. Before handling experimental fish, they were euthanized with 400 mg L^{-1} of clove oil.

2.1 Experimental design and Test diets

The experimental design was randomized blocks in a factorial scheme 2x2, two sources of oil: 100% fish oil and a mixture containing 60% soybean oil and 40% linseed oil and two oil inclusion levels: 5% and 10% , with four replicates

It was used three different weight classes (B1 6.9 ± 0.5; B2 8.6 ± 0.5; B3 10.3 ± 0.5) The sources were fish oil and mixture containing 60% soybean oil and 40% linseed oil and inclusion levels of 5% and 10% oils.

Four isoproteic (327.05 g kg - 1) and isoenergetic (4404.25 MJ kg - 1) diets were formulated with the sources and the levels of oils. All dietary ingredients were finely ground, well mixed manually, and pelleted in an electric meat grinder (Filizola, P-22, São Paulo, SP, Brazil) dried in a forced air oven at 50 °C for 24 h (Marconi, Laboraty Equipment, MA035, SP, Brazil). Afterwards, they were ground in a manual mill (Botimental, Brazil) and sieve in granulometric sieves (Tecnal, Piracicaba, SP, Brazil) to obtain pellets of 1.0 to 4.0 mm. Moisture, crude protein, total lipid, mineral matter and gross energy were analyzed according DETMANN *et al.*, 2012, (Table 1). The diets was previously lyophilized (Labconco, Kansas City, MO, EUA) and ground in a ball mill (Marconi MA923, Piracicaba, SP, Brazil). Crude protein (%N x 6.25) was determined by the Kjeldahl method (Quimis, Diadema, SP, Brazil), total lipids was determinedby petroleum ether extraction in Ankom XT15 (ANKOM), and mineral matter was determined by combusting dry samples in a muffle furnace (TE-1100-1P, Tecnal, Piracicaba, SP, Brazil) at 550 °C for 6 h, according to Association of Official Analytical Chemists (AOAC, 2016). Gross energy content was determined using an adiabatic calorimetry bomb (Parr 1266, Parr Instruments Co., Moline, IL, USA). All analyses were performed in triplicate.

2.2 Growth trial

Juveniles tambaqui, Colossoma macropomum, with three different weight classes (B1 6.9 ± 0.5; B2 8.6 ± 0.5; B3 10.3 ± 0.5) were weighed on a precision scale (model MB45 Toledo 0.01 g, Brazil), and randomly distributed into 48 (70 L) at eight fish per aquária. The tank were maintained in a recirculation system (2 to 3 L min−1) with a mechanical, biological, ultraviolet filter, central thermoregulation and constant aeration and covered with nylon nets to prevent fish from escaping. Each aquarium was considered an experimental unit. The laboratory was maintained in photoperiod of 12hrs through fluorescent lamps (60W) and analog timer.

Table 1 Composition and proximate analyses of the experimental diets used to feed juvenile tambaqui, *Colossoma macropomum*, for 9 weeks

Ingredients (g kg^{-1})	5% S/L[1]	10% S/L[1]	5% FO[2]	10% FO[2]
Soybean meal	520.00	536.30	520.00	536.30
Corn Gluten	100.00	100.00	100.00	100.00
Wheat bran	100.00	100.00	100.00	100.00
Rice bran	162.30	45.60	162.30	45.60
Inert (Caulin)	10.00	50.00	10.00	50.00
Cellulose	00.00	10.00	00.00	10.00
L-Lysine	04.00	04.40	04.00	04.40
DL-Methionine	04.40	04.40	04.40	04.40
Fishoil	00.00	00.00	50.00	100.00
Soybeanoil	30.00	60.00	00.00	00.00
Linseed oil	20.00	40.00	00.00	00.00
Dicalcium phosphate	39.00	39.00	39.00	39.00
Vitamin C	00.10	00.10	00.10	00.10
Common salt	05.00	05.00	05.00	05.00
Mineral and vitamin mix[3]	05.00	05.00	05.00	05.00
Antioxidant BHT	00.20	00.20	00.20	00.20
*Proximate Composition g kg $^{-1}$				
Moisture (%)	945.80	937.30	938.90	951.30
Crude protein (%)	330.90	330.70	321.50	325.10
Crude lipid	97.50	104.60	93.70	114.80
Ash	109.00	142.50	109.00	140.40
Energy (MJ/Kg)	4396	4423	4359	4439

[1]S/L –Soybean and linseed oils in the proportion of 60% and 40%, respectively.
[2]FO – Fish oil.
[3]Levels of guarantee per kilogram of product: Vit. A, 1,200,000 IU; Vit. D3, 200,000 IU; Vit. E, 12,000mg; Vit. K3, 2,400mg; Vit. B1, 4,800mg; Vit. B2, 4,800mg; Vit. B6, 4,000mg; Vit. B12, 4,800mg; B.C. Folic acid, 1,200mg; Pantothenate Ca, 12,000mg; Vit. C, 48,000mg; Biotin, 48mg; choline, 65,000mg; Niacin, 24,000mg; Iron, 10,000mg; Copper, 6,000mg; Manganese, 4,000mg; Zinc, 6,000mg; Iodine, 20mg; Cobalt, 2mg; Selenium, 20mg.
* Values determined in the Food Analysis Laboratory of Animal Science Department of Federal University of Viçosa - MG, Brazil.

The water temperature (27 ± 1.5 ºC) was measured daily before each feeding, and weekly the dissolved oxygen (3.7 ± 0.5 mg/L) through digital oximeter (automatic oxygen analyzer YSI-550[a], EUA), pH (6.6 ± 0.1) and toxic ammonia (0.006 ± 0.002 mg / L) by means of Kit LabconTest (Industry and Commerce of Dehydrated Foods Alcon Ltda, Brazil). We considered the values obtained within the range of comfort for the species (ARIDE *et al*., 2007; ARAUJO-LIMA *et al*., 2013). Fish were hand-fed to apparent satiety three times a day, for nine weeks.

At the end of the experimental period, fish were fasted for 24 hours, euthanized with super dosage of clove oil (400 mg L^{-1}), counted, weighed, measured and eviscerated. We consider viscera the stomach, intestine, pyloric cecum, gonads, heart, liver, gallbladder, swim bladder and visceral fat. The survival rate, weight gain, daily growth rate, condition factor, carcass yield, feed conversion, and the viscerosomatic, hepatosomatic and viscerosomatic fat index were calculated.

2.3 Energy Metabolism

Samples of blood from one fish of each experimental unit (n = 6 per treatment) were obtained by means of a scalpel cut of caudal peduncle and collected in pre-heparinized eppendorf (2ml). Blood was centrifuged for 15 minutes at 5,000 rpm and plasma levels of glucose, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides were determined using the Bioclin® colorimetric kit (Quibasa - Basic Chemistry, Belo Horizonte, MG, Brazil). We performed readings on the BS 200 (Clinical Chemistry Analyzer Mindray®) at the wavelength indicated by the kit's manufacturer. Values determined in the Clinical Analysis Laboratory of Nutrition and Health Department of Federal University of Viçosa - MG, Brazil.

For extractions of hepatic and muscular glycogen, 200 mg of liver and 200 mg of muscle fragments were collected from six fish from each treatment, according to the methodology proposed by CARROLL *et al*. (1956). We dissolved the tissues in two ml of potassium hydroxide solution (30% KOH) and hydrolyzed in a water bath for one hour, with saturated Na_2SO_4 and absolute alcohol added after bathing. Then we centrifuged the tubes at 2000 rpm (Fanem, São Paulo, Brazil, model 206-R) for ten minutes and the supernatant discarded. For the glycogen quantification, the method described by CARROLL *et al*., (1956) with modifications for reading in ELISA reader. We diluted the precipitate in distilled water and there after added anthrone, this procedure being carried out in an ice bath. The solution, we placed in a water bath for 10 minutes and then pipetted into microplates. We done the reading in ELISA reader at 620nm. We obtain the standards with known concentrations of glucose by obtaining linear regression equations from which the sample concentrations were calculated. The concentration obtained was expressed as g of glycogen per 100 g of tissue (%). Values determined at the Chiroptera

Ecophysiology Laboratory of Animal Biology Department of Federal University of Viçosa - MG, Brazil.

2.4 Muscular growth

For muscle fiber frequency analysis, white muscle samples were collected from the epaxial region of 12 fish from each treatment, fixed in 10% buffered formaldehyde solution for 24 h at room temperature and transferred to 70% alcohol. Subsequently, the muscles were dehydrated in increasing series of ethyl alcohol and included in glycol methacrylate (Historesin®, Leica, São Paulo, SP, Brazil). Semi seriate transverse sections of three-μm thickness were obtained with the aid of a microtome with glass cutters. Semi seriate transverse sections of three-μm thickness were obtained with the aid of a microtome with glass cutters, mounted on glass slides and stained with toluidine blue.

The histological slides were photo-documented in a photomicroscope (Olympus® BX53, São Paulo, SP, Brazil) with a coupled camera (Olympus® DP73, São Paulo, SP, Brazil). The fibers were analyzed by the Image Pro-Plus® image analysis package, where the smallest diameter of 200 muscle fibers per animal was determined, which were grouped into classes of diameters (<20 μm, 20-50 μm,> 50 μm) (ALMEIDA *et al*., 2008). The frequency of the fibers within each class was determined in relation to the total number of fibers. Muscle fiber frequency determined in the Laboratory of Experimental Pathology of the Department of Animal Biology of the Federal University of Viçosa - MG, Brazil.

2.5 Chemical analysis

The whole body was previously lyophilized (Labconco, Kansas City, MO, EUA) and ground in a ball mill (Marconi MA923, Piracicaba, SP, Brazil) to analysis of moisture, crude protein, total lipid, mineral matter and gross energy content. Crude protein (%N x 6.25) was determined by the Kjeldahl method (Quimis, Diadema, SP, Brazil), total lipids was determined by petroleum ether extraction in Ankom XT15 (ANKOM), and mineral matter was determined by combusting dry samples in a muffle furnace (TE-1100-1P, Tecnal, Piracicaba, SP, Brazil) at 550 °C for 6 h, according to Association of Official Analytical Chemists (AOAC, 2016). Gross energy content was determined using an adiabatic calorimetry bomb (Parr 1266, Parr Instruments Co., Moline, IL, USA). All analyses were performed in triplicate.

2.6 Statistical analysis

Statistical analyses of data were done by one-way analysis of variance (ANOVA) after testing for normality and homogeneity of variances with Shapiro-Wilk and Barlett tests, respectively. A probability level of 0.05 was used for rejection of the null hypothesis. Statistical analyses were done using software R, version 3.3, package ExpDes.pt.

3 RESULTS

3.1 Growth trial

In all treatments 100% survival of the fish was observed. We observed interaction between levels and sources of oil only for body condition factor, where fish fed diets containing 5% of fish oil had the lowest values (Table 2).

There was an effect of the source of oil only for the hepatosomatic index and for the viscerosomatic fat index. The mixture of soybean oil and linseed oil provided the highest values for both indices. For the oil level, it observed effect only for the hepatosomatic and viscerosomatic index and viscerosomatic fat index. Except for the hepatosomatic index, fish fed diets containing 10% oil, regardless of source, had the highest values for these variables (Table 2).

3.2 Energy metabolism

We did not observe interaction effect between levels and sources of oil for the glucose, total cholesterol, HDL, LDL, triglycerides plasma, hepatic and muscle glycogen. There was effect of the oil source for HDL, LDL, triglycerides plasma and hepatic glycogen. Except for LDL, the highest values were found in fish fed with the mixture of soybean and linseed oil. We not observed effect of the oil level for the variables analyzed (Table 3).

3.3 Muscular growth

Interaction between levels and sources of oil was noted only for the frequency of muscle fibers of diameter (D) <20 μm. Fish consuming diets containing 5% of soybean and linseed oil showed the lowest fiber frequency with D <20 μm (Table 4).

Table 2. Survival rate, weight gain, daily growth rate, condition factor, carcass yield, feed conversion, and the viscerosomatic, hepatosomatic and viscerosomatic fat index of juvenile tambaqui, *Colossoma macropomum*, fed with different oil sources and levels

	Effect of source-level interaction	Source effect	Sources		Level effect	Levels (%)	
			S/L[1]	FO[2]		5	10
SR (%)[3]	p=1.00	p=1.00	100.00 ± 0.0	100.00 ± 0.0	p=1.00	100.00 ± 0.0	100.00 ± 0.0
WG (g)[4]	p = 0.57	p = 0.40	58.03 ± 17.37	55.00 ± 18.28	p = 0.15	59.13 ± 14.55	53.90 ± 20.36
DGI (% day^{-1})[5]	p = 0.95	p = 0.21	6.49 ± 0.47	6.35 ± 0.62	p = 0.06	6.53 ± 0.36	6.31 ± 0.67
K[6]	p = 0.00	p = 0.49	3.70 ± 0.12	3.68 ± 0.10	p = 0.41	3.68 ± 0.08	3.70 ± 0.13
CY (%)[7]	p = 0.74	p = 0.17	88.28 ± 0.70	88.55 ± 0.66	p = 0.27	88.52 ± 0.59	88.30 ± 0.77
FC[8]	p = 0.43	p = 0.37	1.57 ± 0.14	1.54 ± 0.13	p = 0.58	1.56 ± 0.11	1.54 ± 0.16
VSI (%)[9]	p = 0.33	p = 0.11	8.66 ± 0.45	8.48 ± 0.51	p < 0.01	8.40 ± 0.43	8.73 ± 0.49
HSI (%)[10]	p = 0.74	p < 0.01	1.12 ± 0.11	1.03 ± 0.10	p = 0.01	1.12 ± 0.11	1.04 ± 0.12
VSFI (%)[11]	p = 0.13	p < 0.01	1.57 ± 0.40	1.31 ± 0.35	p = 0.02	1.33 ± 0.34	1.55 ± 0.42

Condition factor interaction

	Sources x Levels				Splitting Sources x Levels interaction			
	S/L[1] 5%	S/L[1] 10%	FO[2] 5%	FO[2] 10%	S/L[1]	FO[2]	5%	10%
K[6]	3.37 ± 0.05	3.68 ± 0.16	3.64 ± 0.08	3.73 ± 0.10	p=0.16	p=0.01	p=0.01	p=0.13

[1]Soybean and linseed oils in the proportion of 60% and 40%, respectively. [2]FO: Fish oil. [3]Survival Rate (SR) = (fish final number / fish initial number) x 100; [4]Weight gain (WG) = final weight – initial weight; [5]Daily growth index (DGI) = (final weight – initial weight / experiment time) x 100; [6]Condition factor (K) = (final weight /final length^3) x 100; [7]Carcass yield (CY) = (carcass weight / final weight) x100; [8]Feed conversion (FC) = feed intake / WG; [9]Viscerosomatic index (VSI) = (viscera weight/ final weight) x100; [10]Hepatosomatic index (HSI) = (liver weight/ final weight) x100; [11]Viscerosomatic fat index (VSFI) = (visceral fat weight/ final weight) x100.

Table 3 – Hepatic and muscular glycogen and glucose, total cholesterol, HDL, LDL triglycerides plasma levels of tambaqui juveniles, *Colossoma macropomum*, fed experimental diets for 9 weeks (Values are mean ± standard deviation)

	Effect of source-level interaction	Source effect	Source		Level effect	Level (%)	
			S/L[1]	FO[2]		5	10
Glucose (mg/dl)	p=0.18	p=0.86	54.33 ± 7.75	54.91 ± 10.44	p=0.60	53.75 ± 7.09	55.50 ± 10.83
Total Cholesterol (mg/dl)	p=0.88	p=0.10	86.92 ± 18.74	101.00 ± 24.94	p=0.08	86.33 ±24.23	101.58 ± 19.18
HDL(mg/dl)	p=0.20	p=0.03	14.92 ± 5.11	10.17 ± 4.90	p=0.41	11.67 ± 4.31	13.42 ± 6.49
LDL(mg/dl)	p=0.96	p=0.01	11.00 ± 4.22	16.42 ± 6.67	p=0.06	11.75 ± 6.06	15.67 ± 5.76
Triglycerides (mg/dl)	p=0.84	p<0.01	171.58 ± 21.19	127.83 ± 19.48	p=0.53	146.92 ± 32.05	152.50 ± 28.78
Hepatic Glycogen (%)	p=0.68	p=0.02	24.59±14.85	15.97±7.38	p=0.12	22.76 ± 15.47	16.98±4.57
Muscular Glycogen (%)	p=0.20	p=0.18	0.66± 0.19	0.75±0.26	p=0.12	0.76±0.27	0.65± 0.16

[1]Soybean and linseed oils in the proportion of 60% and 40%, respectively. [2]FO: Fish oil.

Table 4. Frequency of muscle fiber diameter* of *Colossoma macropomum* juveniles, fed with different oil sources and levels

	Effect of source-level interaction	Source effect	Source		Level effect	Level	
			S/L[1]	FO[2]		5%	10%
D<20 μm	p=0.04	p=0.26	26.00 ± 6.44	28.17 ± 7.27	p=0.26	26.00 ± 6.33	28.16 ± 7.38
20<D<50 μm	p=0.46	p=0.01	50.35 ± 6.03	46.25 ± 6.03	p=0.12	49.59 ± 5.49	47.01 ± 5.84
D>50 μm	p=0.15	p=0.29	23.65 ± 6.78	25.58 ± 5.58	p=0.81	24.40 ± 6.65	24.83 ± 5.89
D mean	p=0.10	p=0.79	35.34 ± 3.76	35.62 ± 3.62	p=0.80	35.61 ± 3.63	35.35 ± 3.74

Frequency of muscle fiber diameter <20 μm interaction

	Sources x Levels				Splitting Sources x Levels interaction			
	S/L[1] 5%	S/L[1] 10%	FO[2] 5%	FO[2] 10%	S/L[1]	FO[2]	5%	10%
D<20 μm	22.98 ± 3.64	29.01 ± 7.32	29.03 ±7.11	27.31 ± 7.65	p=0.03	p=0.52	p=0.03	p=0.53

[1]S/L: mixture of 60% soybean oil and 40% linseed oil; [2]FO: fish oil. *Muscle fiber frequency was determined in the Laboratory of Experimental Pathology of the Department of Animal Biology of the Federal University of Viçosa - MG, Brazil. Values presented as means ± SD (n = 12).

The effect of oil source was observed only in the frequency of muscle fibers with diameter of 20-50 µm.

Fish fed diets containing soybean and linseed oil presented the highest frequencies for this class of fibers. No level oil effect was observed for the frequencies of D <20 µm, 20 <D <50 µm, D> 50 µm and D media (Table 4).

3.4 Chemical analysis

We did not observe interaction effect between levels and sources of oil for the moisture, total lipid, crude protein, mineral matter and gross energy. Effect from the source only observed to the moisture. Fish that consumed diets contain fish oil present highest values for moisture. Effect of the oil level for lipid, ashes and energy was observed. With the exception of the ashes, fish that consumed diets with the level of 10% of oil had higher values (Table 5).

Table 5. Whole body (mg g^{-1} of dry weight) composition of juvenile tambaqui, *Colossoma macropomum*, fed with different oil sources and levels

	Effect of source-level interaction	Source effect	Source		Level effect	Level	
			S/L[1]	FO[2]		5%	10%
Moisture (%)	p=0.45	p=0.02	71.19 ± 0.85	71.91 ± 1.24	p=0.18	71.34 ± 0.92	71.76 ± 1.26
Total lipid	p=0.21	p=0.76	28.31 ± 2.64	28.13 ± 1.66	p<0.01	26.59 ± 1.01	29.86 ± 1.70
Crude protein (%)	p=0.97	p=0.58	48.80 ± 2.98	49.39 ± 2.77	p=0.40	49.46 ± 2.92	48.70 ± 2.81
Mineral matter	p=0.51	p=0.48	12.83 ± 1.79	12.94 ± 1.40	p<0.01	13.51 ± 1.63	12.30 ± 1.35
Gross energy (MJ kg^{-1})	p=0.63	p=0.50	5633.84 ± 161.21	5594.00 ± 188.40	p<0.01	5517.78 ± 161.66	5715.92 ± 122.35

[1]S/L: mixture of 60% soybean oil and 40% linseed oil; [2]FO: Fish oil. Values presented as means ± SD (n = 3).

4 DISCUSSION

The results obtained for the productive performance of tambaquis indicate that it is possible to feed this species with diets containing 60% soybean oil and 40% linseed oil, replacing the diet containing 100% fish oil, at the 5% level. The present study clearly indicates that supplying energy and essential fatty acids requirements of tambaqui can be satisfied by C18 PUFA (linoleic and linolenic) of the mixture of soybean and linseed oils. Both sources were efficient, not leading to damage in fish performance. Several studies have shown the possibility of partial or total replacement of fish oil by vegetable oils. In this aspect the total substitution cod liver oil by soybean, oil or linseed oil did not influence the productive performance of sharpsnout seabream, *Diplodus puntazzo* (PIEDECAUSA *et al*., 2007). Similarly, different sources of vegetable oils did not affect the growth performance of the red hybrid tilapia, *Oreochromis* sp, (NG *et al*., 2013), panga, *Pangasius hypophthalmus*, (ASDARI *et al*., 2011), murray cod, *Maccullochella peelii peelii*, (TURCHINI *et al*., 2011) and florida pompano, T*rachinotus carolinus*, (ROMBENSO *et al*., 2016). In silvery-black porgy, *Sparidentex hasta*, the partial or total replacement of fish, oil by sunflower or canola oils did not cause differences in the productive performance of the fish (MOZANEZADEH *et al*., 2016). Similar results were found for murray cod, *Macullochella peelii peelii*, total oil replacement fish oil by linseed oil, olive oil, sunflower oil or palm oil also did not affect the productive performance of fish (TURCHINI *et al*., 2011). Tambaquis fed with corn oil, linseed oil and fish oil (PEREIRA, *et al*., 2018). The mixture of soybean oil with fish oil also did not influence the performance of tambaquis (VAN DER MEER, *et al*., 1997).

The results found to HSI may associated with high energy reserves and metabolic activity (COLLIER *et al*., 2012), indicating that the fish fed with these diets presented good nutritional status. These results show that there was accumulation of glycogen. Glucose can be stored in the liver mainly in the form of glycogen (ENES *et al*., 2009). This storage represents a greater endogenous energy reserve, which can be used to deal with adverse conditions (DA SILVEIRA *et al*., 2009). It may mean that fish fed diets containing the mixture of soybean and linseed oil, were more prepared to face adversity.

In this study, fish fed diets containing the mixture of soybean and linseed oils presented higher values for viscerosomatic fat index. However, these fish did not accumulate fat in the carcass. The level of lipid synthesis or catabolism or both can influence the deposition of body fat. Some genes appear to be closely linked to the activity of enzymatic mechanisms of lipolysis and lipogenesis. Probably in these fish there may have been an increase in lipolytic metabolism.The expression of the lipolysis genes is influenced by the dietary level of DHA / EPA being inversely proportional and the expression of the genes of the lipogenesis increase with the increase of the ratio (JIN *et al*. al., 2017). The fish fed with 10% of oil presented greater accumulation of fat in the carcass. Probably due to the lower lipolytic metabolism and higher lipogenic metabolism. Other studies with different levels of oils presented similar results for the viscerosomatic fat index for giant croaker, *Nibea japonica*, (HAN *et al*., 2014), red spotted grouper and *Epinephelus akaara*, (JIANG *et al*. 2015).

The lower value of plasma LDL and higher HDL observed in fish that receved diets containing soybean and linseed oil mixture might have occurred due to the phytosterols present in vegetable oils, which may induce a decrease in total cholesterol and LDL by reducing intestinal absorption (GILMAN *et al*., 2003). Besides that, the major protein constituents of HDL are hepatic LPL and ApoA1 apolipoproteins, and diet with vegetable oils induces the gene expression of these constituents, which may explain the increase in HDL (GEAY *et al*., 2011).

The frequency distribution of white muscle fibers with <20 μm is higher in juveniles than in adults, indicating muscle hyperplasia, in addition, the frequencies of fibers with diameter> 20 to <50 μm and> 50 μm are greater in adults than in juveniles, indicating muscle hypertrophy (Almeida *et al*., 2008). In the present study, the fish that consumed a diet containing 5% of soybean and linseed oil showed a lower frequency of muscle fiber diameter <20μm. This result suggests that these fish will present less growth potential when compared to the fish that consumed diets containing fish oil. However, among the fish that consumed the mixture of soybean and linseed oil at the 10% level presented a higher frequency of diameter <20 μm, indicating that the oil level of 10% leads to greater hyperplasia. Another interesting finding of this study was that fish consuming diets containing mixture of soybean and linseed oil presented a higher frequency of diameters >20μme <50μm, indicating initial hypertrophy faster than fish that consumed diets containing fish oil. In addition, the absence of significant findings for the frequencies of diameter >50 μms suggest that although the different sources and levels of oil promoted less muscle growth of hyperplastic cells (soybean oil + linseed - 5%) and intermediary (fish oil), muscle growth in hypertrophy was similar between the different diets.

In the present study, fish that consumed diets containing 5% the mixture of soybean and linseed oil showed higher values of moisture. Disagreeing with results found by PEREIRA *et al*. (2018), that replaced fish oil by corn oil or linseed oil in diets for tambaquis without changes in moisture, while ASDARI *et al*. (2011) substituted for soybean oil, crude palm oil and linseed oil in diets for panga *Pangasius hypophthalmuse* did not verify variations in the moisture values of the carcass of these fish. In relation to oil levels, results similar to those of the present study were verified in tambaquis, where diets with a level of 4% of corn oil showed higher values for moisture than fish that consumed diets containing 8% of oil inclusion (SANDRE *et al*., 2017). In contrast, VAN DER MEER *et al*. (1997) showed that the moisture content was directly proportional to the oil level of the diet. Moreover, in the present study, ash levels were positively associated with moisture content, as expected.

As expected, the lipid and body energy concentrations were higher when the oil level of the diets was higher, corroborating with results found in other studies (SANDRE *et al*., 2017). Carcass with high level of fat undergo lipid oxidation which can cause off-flavor, decrease nutritional quality and modify muscle texture and color (LIE, 2001).

The exchange of source of vegetable oil by fish oil is considered sustainable, since the vegetable oil are renewable resource. In addition, the use of vegetable oils in diets for tambaqui may also be related to their food habit in nature, which is omnivorous with a tendency to fructiferous, which allows the feeding of this species with diets containing ingredients of plant origin (PAULINO *et al*., 2018; VAN DER MEER *et al*., 1997). We conclude that is possible to substitute the fish oil with the mixture containing 60% soybean oil and 40% linseed oil and inclusion level of 5%.

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