

## **Palm oil in diets for Nile tilapia (*Oreochromis niloticus*) post-larvae**

Óleo de palma em dietas para pós-larvas de tilápia do Nilo (*Oreochromis niloticus*)

Aceite de palma en las dietas postlarva de tilapia del Nilo (*Oreochromis niloticus*)

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### **Abstract**

The objectives of this research were to evaluate the effects of the substitution of soybean oil by palm oil in the diet of Nile tilapia (*Oreochromis niloticus*) post-larvae, to observe the performance and attractiveness of the feed, and to evaluate the fatty acid methyl esters profile of palm oil. Four hundred Nile tilapias were used after hatching (seven days old), distributed in 20 fiberglass tanks of 20 L each. The experiment was carried out in a completely randomized design with five treatments and four replicates. The treatments consisted of different levels of inclusion of palm oil, which were: 0.0, 1.5, 3.0, 4.5 and 6.0 %. The concentration of monounsaturated and saturated fatty acids in palm oil was measured, resulting in high amounts of palmitic, oleic and linoleic fatty acids. Significant differences were observed for the variables final weight, total length, weight gain, specific growth rate and condition factor. No significant differences were observed for palatability of the diets. It is concluded that the inclusion of 3% or more palm oil in substitution of soybean oil during the masculinization stage of tilapia larvae contributes to the improvement of productive parameters.

**Keywords:** Fish nutrition; Performance; Lipids; *Oreochromis niloticus*.

### **Resumo**

A pesquisa objetivou avaliar os efeitos da substituição do óleo de soja pelo óleo de palma na dieta de pós-larvas de tilápia do Nilo, sobre o desempenho zootécnico e atrato-palatabilidade das rações bem como determinar o perfil dos ésteres metílicos de ácidos graxos do óleo de palma. Foram utilizadas 400 pós-larvas de tilápia do Nilo com sete dias após eclosão, distribuídas em 20 tanques de fibra de vidro de 20 L cada. O experimento foi realizado em um delineamento inteiramente ao acaso, com cinco tratamentos e quatro repetições. Os tratamentos consistiram em incluir de forma parcial e total o óleo de palma, sendo a inclusão de 0,0; 1,5; 3,0; 4,5 e 6,0%. Foram observadas concentrações de ácidos graxos monoinsaturados e gorduras saturadas no óleo de palma, observando elevada quantidade de palmítico, oléico e linoléico. No desempenho zootécnico foram observadas diferenças significativas para as variáveis peso final, comprimento total, ganho em peso, taxa de crescimento específico e fator de condição. Não foram observadas diferenças significativas para atrato-palatabilidade das dietas. Conclui-se que a inclusão de no mínimo 3,0% ou mais de óleo de palma em substituição ao óleo de soja na fase de masculinização de pós-larvas de tilápia, contribui para a melhora dos parâmetros produtivos.

**Palavras-chave:** Nutrição de peixes; Desempenho; Lipídios, *Oreochromis niloticus*.

## Resumen

La investigación tuvo como objetivo evaluar los efectos de la sustitución del aceite de soja por aceite de palma en la dieta de postlarvas de tilapia del Nilo, sobre el rendimiento y la atraibilidad de los alimentos, así como determinar el perfil de ésteres metílicos de ácidos grasos del aceite de palma. Se utilizaron 400 postlarvas de tilapia del Nilo siete días después de la eclosión, distribuidas en 20 tanques de fibra de vidrio de 20 L cada uno. El experimento se realizó en un diseño completamente aleatorio, con cinco tratamientos y cuatro repeticiones. Los tratamientos consistieron en incluir parcial y totalmente aceite de palma, siendo la inclusión de 0.0; 1,5; 3,0; 4,5 y 6,0%. Se observaron concentraciones de ácidos grasos monoinsaturados y grasas saturadas en aceite de palma, observándose altas cantidades de palmítico, oleico y linoleico. En el desempeño zootécnico, se observaron diferencias significativas para las variables peso final, longitud total, ganancia de peso, tasa de crecimiento específico y factor de condición. No se observaron diferencias significativas para el atractivo y la palatabilidad de las dietas. Se concluye que la inclusión de al menos 3.0% o más de aceite de palma para reemplazar el aceite de soja en la etapa de masculinización postlarva de la tilapia contribuye a la mejora de los parámetros de producción.

**Palabras clave:** Nutrición de peces; Rendimiento; Lípidos, *Oreochromis niloticus*.

## 1. Introduction

Fish farming in Brazil has been growing steadily and, among freshwater species, tilapia is responsible for 60% of the national production, with more than 486 thousand tons produced in 2020 (Peixe BR, 2021).

Among the commercially grown tilapia species, Nile tilapia (*Oreochromis niloticus*) is the most widely used in intensive systems in Brazil. This is due to its rapid growth and good quality meat (Furuya et al., 2008). In addition, because of its omnivorous feeding habit, tilapia easily accepts feed from the initial period to the termination phase (Boscolo et al., 2008).

Feeding is considered the main cost within an aquaculture sector, mainly in the intensive production of fish. The use of lipids in fish diet has the special advantage of being a low-cost energy supply (Boscolo et al., 2008; Juancey, 2000). In addition to providing substrates for energy production, lipids are sources of essential fatty acids and are structural components of cell membranes acting as transporters for other nutrients such as fat-soluble vitamins (Garcia et al., 2013). Therefore, deficiency of fatty acids can impair production, reducing growth and increasing mortality (Garcia et al., 2013; Glencross, 2009).

The use of fish oil in feed is considered an important source of essential fatty acids of the n-3 and n-6 family (Ng & Wang, 2011). However, its availability is becoming limited due to the decline of fish stocks (Péron et al., 2010; Silva Júnior et al., 2011), which brings economic implications, making necessary the use of alternative sources such as vegetable oil for animal nutrition (Figueiredo- Silva et al., 2005).

The production of vegetable oil has been growing steadily, is a significant portion of food production. In addition, the inclusion of vegetable oils can bring benefits to the animal's performance (Pereira et al., 2011), improving the use of the protein that is consumed, providing essential fatty acids for the development of the fish (Martino et al., 2002). Soy oil is the vegetable lipid most used in animal nutrition, which has essential fatty acids for the development of fish. This oil can be compared with palm oil, which has been playing a significant role in animal nutrition, being used as a source of energy and essential fatty acids, and contributing to the absorption of fat-soluble vitamins (Tomkins & Drackley, 2010). There are several benefits of palm oil, being a rich source of beta-carotene that gives it its characteristic reddish-orange color, being a great precursor of vitamin A for the body. Palm oil is also rich in vitamin E, antioxidants such as tocopherol and tocotrienol, as well as a variety of saturated and unsaturated fatty acids, with emphasis on palmitic (C16: 0), oleic (C18: 1) and linoleic (C18: 2n - 6) fatty acids (NG, 2002; Grimaldi et al., 2005).

Some authors have studied the effects of palm oil on the diet of fish. Bahurmiz et al. (2007) found that the replacement of up to 100% of palm oil by fish oil in the diet of red hybrid tilapia (*Oreochromis* sp.) Has no negative effects on performance and also does not influence the deposition of fatty acids in muscle tissue and digestibility of nutrients. Macedo-Viegas and Contrera-Guzman (1998) observed that diets containing more than 3% palm oil for tambaqui (*Colossoma*

*macropomum*) improved weight and length gain and had better protein efficiency and nitrogen retention efficiency. Legendre et al. (1995) and Ng et al. (2000) used palm oil at various levels in catfish (*Clarias gariepinus*) and Tortensen et al. (2000) for Atlantic salmon (*Salmo salar*), finding positive results for the growth and efficiency of using the diet when compared to fish fed diets containing fish oil.

Lipids play an important role in the growth phase of fish, being essential for their development, in which the use of vegetable oils becomes the main source of nutrients for this phase (Wilson, 1995; Hayashi et al., 2002; Boscolo et al., 2008;). It is observed that soy oil can be replaced by other vegetable oils without causing zootechnical damage, allowing compensatory animal gain (Nwanna & Bolarinwa, 2000i).

Studies evaluating the use of palm oil in the diet of tropical fish in the initial phase are still scarce, and studies involving its use are necessary to establish viability. Therefore, the aim of this work was to evaluate the effects of replacing soybean oil with palm oil in post-larval diets of Nile tilapia to evaluate the effects on zootechnical performance and attractiveness-palatability.

## 2. Methodology

This research can be characterized as an experimental study (Pereira, et al., 2018). The experiment was carried out at the Aquaculture Laboratory of the Aquaculture Management Study Group - GEMAg, at the State University of West Parana - UNIOESTE - Toledo Campus. Four hundred post-larvae Nile tilapias were used, 7 days after hatching with initial mean weight of  $28 \pm 5$  mg. The post-larvae were distributed in 20 cylindrical-tapered glass fiber tanks (20 L each), equipped with a water recirculation system and thermostat for temperature control. At the end of the day, the tanks were siphoned to remove any feed leftovers.

Water quality control was performed using the YSI Professional Plus Multiparameter Water Quality Meter (YSI, Pro Plus, Yellow Springs-Ohio, USA), where water temperature ( $26.3 \pm 1.66$  ° C), dissolved oxygen ( $7.5 \pm 0.06$  mg L<sup>-1</sup>) and pH ( $7.4 \pm 0.04$ ) were monitored. The values of the variables remained within the acceptable range for tropical fish farming (Tavares, 1995).

Five isoproteic (42% crude protein) and isoenergetic rations (3500.00 Kcal digestible energy) were prepared, with inclusion of palm oil, replacing soybean oil at the same inclusion rates of 0.0, 1.5, 3.0, 4.5 and 6.0% (Table 1) The inclusion of palm oil took into account the nutritional recommendations proposed by Furuya (2010) for Nile tilapia.

**Table 1.** Ingredients, chemical characteristics and inclusion levels of the experimental diets.

Ingredients (%)	Level of inclusion %				
	0.0	1.5	3.0	4.5	6.0
Soybean meal 45%	40.00	40.00	40.00	40.00	40.00
Fish meal (60%)	20.00	20.00	20.00	20.00	20.00
Soybean protein concentrate (60%)	11.89	11.89	11.89	11.89	11.89
Sour Rice	7.22	7.22	7.22	7.22	7.22
Corn gluten	6.00	6.00	6.00	6.00	6.00
Corn grain	3.88	3.88	3.88	3.88	3.88
<b>Soy oil</b>	<b>6.00</b>	<b>4.50</b>	<b>3.00</b>	<b>1.50</b>	<b>0.00</b>
<b>Palm oil</b>	<b>0.00</b>	<b>1.50</b>	<b>3.00</b>	<b>4.50</b>	<b>6.00</b>
Dicalcium phosphate	3.51	3.51	3.51	3.51	3.51
Premix <sup>1</sup>	1.00	1.00	1.00	1.00	1.00
L-Threonine 98%	0.31	0.31	0.31	0.31	0.31
DL-Methionine 99%	0.04	0.04	0.04	0.04	0.04
Regular salt	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.10	0.10	0.10	0.10	0.10
Antifungal 2	0.10	0.10	0.10	0.10	0.10
Vitamin C3	0.10	0.10	0.10	0.10	0.10
Antioxidant 4	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100
Chemical composition analyzed %					
	0.0	1.5	3.0	4.5	6.0
Dry matter	93.31	93.31	93.40	91.31	93.47
Protein	39.91	43.39	41.74	42.16	42.98
Ether extract	9.66	9.52	9.43	9.63	9.07
Mineral matter.	11.16	10.82	12.28	12.22	12.31
Crude fiber	6.86	7.70	8.97	7.05	6.55
Gross Energy (Kcal)	4.448	4.495	4.299	4.209	4.213

<sup>1</sup> Warranty Levels per kg of product - Premix (DSM-Roche®): Vit. A, 24,000 IU; Vit. D3, 6,000 IU; Vit. E, 300 mg; Vit. K 3, 30 mg; Vit. B1, 40 mg; Vit. B2, 40 mg; Vit. B6, 35 mg; Vit. B12, 80 mg; B.C. Folic acid, 12 mg; Pantothenate Ca, 100 mg; Vit. C, 600 mg; Biotin, 2 mg; Hill, 1000 mg; Niacin; Iron, 200 mg; Copper, 35 mg; Manganese, 100 mg; Zinc, 240 mg; Iodine, 1.6 mg; Cobalt, 0.8 mg.

<sup>2</sup> Calcium Propionate.

"Vitamin C - Rovimix" Stay-35.

4BHT - Butyl Hydroxy Toluene.

Source: Authors (2021).

The ingredients used to make the experimental diets were ground in a hammer mill (Vieira, MCS 280, Tatuí-SP, Brazil), with a 0.3 mm sieve and mixed with the oils. The diet was offered to the animals as extruded pellet, and hormone was added (17-alpha-methyltestosterone) for masculinization of the animals. The fish underwent a five-day adaptation period for experimental diets and conditions and soon after this period, the animals were fed four times a day (8h00, 11h00, 14h00 and 17h00) according to the adaptive period, for apparent satiety for 40 days.

The fatty acid methylation of the total lipids of the ingredients was performed according to the method of Hartman

and Lago (1973) and carried out at the laboratory of water and food of the department of chemistry of the State University of Maringá - Brazil. For analysis, approximately 0.030 g of total lipids (palm oil) were added in an esterification tube. The esterified sample was kept in the refrigerator until phase separation. The supernatant was collected and transferred to a vial for further chromatographic analysis.

The fatty acid methyl esters (FAME) were separated on a Trace Ultra 3300 Thermo brand gas chromatograph equipped with a flame ionization detector and CP-7420 fused silica capillary column (Select FAME, 100 m long, 0.25 mm internal diameter and 0.25 µm cyanopropyl). Retention times and peak areas of the FAME were determined using the ChromQuest™ 5.0 software. Fatty acids were identified by comparing their retention times with standard 189-19 of known composition (Sigma - USA). The absolute quantification of FAME was performed by internal standardization using tricosanoic acid methyl ester (23: 0), Sigma - USA, and calculations performed according to the method of Joseph & Ackman (1992). Theoretical correction factors (Visentainer, 2012) were used to determine the concentration values. The amount of fatty acid in the samples was calculated in mg compared to total lipids (mg g<sup>-1</sup>LT AG) using Equation 1.

$$M_x = \frac{A_x \cdot M_p \cdot F_{ct}}{A_p \cdot M_a \cdot F_{cea}}$$

Equation 1

Where:  $M_x$  is the mass of fatty acid X in mg g<sup>-1</sup> of total lipids,  $A_x$  is the peak area of the fatty acid X,  $A_p$  is the peak area of the internal standard (23: 0),  $M_p$  is the mass of internal standard added to the sample in mg,  $M_a$  is the mass of the sample in g,  $F_{ct}$  is the theoretical correction factor of the flame ionization detector (DIC) and  $F_{cea}$  is the conversion factor of methyl ester to fatty acid.

After the experimental period, the fish were food deprived for 24 hours to empty the gastrointestinal tract, then anesthetized with benzocaine at a dose of 75 mg.L<sup>-1</sup> (Gomes et al., 2001) and individual measurements of weight (g) and total length (cm) were performed.

The performance data evaluated: initial weight (g); final weight (g); weight gain (final body weight - initial body weight); condition factor ((body weight, g/total length<sup>3</sup>, cm) × 100); specific growth rate (% day<sup>-1</sup>) ((ln final weight) - (ln initial weight) / time X 100); survival (%) (100 X (final fish number / initial fish number)).

To evaluate the attractiveness of the diets, 12 Nile tilapia fry weighing 1 g were used, where they were allocated to aquariums with a capacity of 30 liters, one individual per aquarium, where they were separated by a barrier, isolating them from any movement in the laboratory. The animals stayed two days in the aquarium for acclimation before the test of feed choice. For this test, three diets were used, one control (without addition of oil) and two tests (soybean oil and palm oil). For this experiment, a commercial microextruded feed containing 380 g/ kg of crude protein and without addition of oil in its composition was used, then 6% of each oil tested was pulverized on this food for the test.

In each aquarium were attached three feeders made with silicone hoses, remaining in one of the three corners of the aquarium. In the morning and afternoon (8:00 a.m. and 2:00 p.m.), 20 grains of feed of each treatment were offered in each feeder at random. The frequency of visits to each feeder and the number of granules ingested were observed, where each aquarium was timed for a period of 10 minutes.

The experimental design was completely randomized with five treatments and four replicates for the zootechnical performance test and three treatments and four replicates for palatability. The data was analyzed with ANOVA and when significant differences were observed, the Tukey averages comparison test was applied, with 5% significance. The analyzes were performed using the Statistic 7.1 software (2005).

### 3. Results

Concentrations of monounsaturated, polyunsaturated and saturated fatty acids in palm oil were observed, and the most prominent fatty acids were palmitic (C16: 0), oleic (C18: 1n-7) and linoleic acid (C18: 2n-6 ) (Table 2).

**Table 2.** Profile of fatty acids (%) in palm and soybean oil.

Fatty Acids	Name	Palm Oil*	Soybean Oil**
C12:0	Lauric	0.34	-
C14:0	Myristic	0.65	0.06
C15:0	Pentadecyl	0.06	-
C16:0	Palmitic	33.51	9.90
C16:1	Palmitoleic	0.19	0.04
C17:0	Margaric	0.09	0.10
C18:0	Stearic	3.90	3.94
C18:1n-9c	Oleic	48.63	23.44
C18:1n-7	-	0.50	-
C18:2n-6	Linoleic	11.28	52.92
C18:3n-3	Alpha-linoleic	0.38	7.60
C20:0	Arachidonic	0.28	0.48
C20:1n-9	Cis-11-Eicosanoic	0.21	0.12
AGPI	Polyunsaturated fat	11.65	60.64
AGMI	Monounsaturated fat	49.53	23.92
AGS	Saturated Fat	38.82	4.01

\*Analyzed values.

\*\*Literature values (Zambiasi et al., 2007).

Source: Authors (2021).

Significant differences ( $P < 0.05$ ) were observed for the variables final weight (FW), total length (TL), weight gain (WG), specific growth rate (SGR) and condition factor (CF) (Table 3). Except for TL and FC, the inclusion of 3 and 6% of palm oil in the diet resulted in better results compared to the diet containing only soybean oil. Diets containing 3; 4.5 and 6% of palm oil increased fish total length, while for CF, higher results were observed compared to the control diet for fish fed with diet containing 100% palm oil.

**Table 3.** Mean and standard deviation of the mean zootechnical performance of post-larvae of Nile tilapia fed diets containing palm oil.

Variables*	0.0%	1.5%	3.0%	4.5%	6.0%
IW(mg)	28	28	28	28	28
FW(mg)	789±0.06	869±0.09	956±0.04	929±0.04	982±0.06
TL (cm)	35.99±1.39	37.45±1.90	38.98±0.55	38.69±1.20	38.96±0.98
WG (mg)	760±0.06	840±0.09	928±0.04	900±0.04	953±0.06
SGR(%)	9.44±0.23	9.71±0.33	9.99±0.13	9.91±0.15	10.06±0.19
CF	2.19±0.17	2.31±0.15	2.45±0.10	2.42±0.06	2.51±0.10
SUR. (%)	91.25±10.30	95.00±4.08	96.25±4.78	95.00±5.77	97.5±5.0

Mean ± SD. IW = Initial Weight; FW = Final Weight; TL = Total Length; WG = Weight Gain; SGR = Specific Growth Rate; CF = Condition Factor; SUR = Survival. Source: Authors (2021).

No significant differences were observed ( $P > 0.05$ ) for palatability of the diets tested (Table 4).

**Table 4.** Palatability of diets containing soybean oil and palm oil. Means followed by the same letter do not differ statistically by the Tukey test ( $P > 0.05$ ).

Variables (%)	Treatments			Effect (P)
	T1	T2	T3	
Visit Frequency	3.29±2.14a	3.87±2.57a	3.04±2.13a	0.1481
Granules Ingested	8.05±5.92a	9.02±6.23a	6.40±5.60a	0.0668

T1 = diet containing 6% soybean oil; T2 = diet containing 6% palm oil; T3 = oil-free diet. Source: Authors (2021).

#### 4. Discussion

The fatty acid composition (FA) obtained for palm oil used in this study was similar to values obtained by NRC (1993) and Ng et al. (2003), which reported high values for palmitic, oleic and linoleic acid. These fatty acids influence the productive performance of Nile tilapia (Furuya, 2010), being metabolized in large quantities, generating energy for fish growth (Henderson et al., 1984; Henderson & Altamar, 1989).

There are several studies on the requirement of polyunsaturated fatty acids for fish, varying according to species (Ribeiro et al., 2012). Nile tilapia has a requirement of 0.5% of linoleic fatty acid (C18: 2n-6) (Martino, 2003), an essential fatty acid for Nile tilapia, responsible for improving productive parameters (Twibell et al., 2000; Santos et al., 2007). In the ingredient of the present study, an amount of 11.28% of C18: 2n-6 was observed, which meets the needs of Nile tilapia and can be a positive factor for performance variables. The inclusion of palm oil in the experimental diets positively influenced the variables of final weight (FW), total length (TL), weight gain (WG), specific growth rate (SGR), and condition factor of Nile tilapia. In a study conducted by Ayise et al., 2014, using levels of palm oil in the Nile tilapia diet, they observed a greater weight gain and specific growth rate, in the animals that received bedding with the inclusion of 6% oil of palm. Uliana et al. (2001), significant results were found for WG, SGR, TL, FW of *Rhamdia quelen* larvae submitted to a diet of 5% canola oil. Similar results by Ferreira et al. (2014), verified the influence of oregano oil to *Astyanax altiparanae*, obtaining positive results for weigh gain and specific growth rate.

The use of coconut oil in diets for common carp larvae (*Cyprinus carpio* L.), obtained positive results for survival and SGR, justifying the high levels of oleic acid present (Fontagné et al., 1999). However, Ferraz (2015) reported a reduction in the survival rate of yellow-tailed lambari (*Astyanax altiparanae*) fingerlings fed palm and coconut oil, which, according to the author, is due to the low levels of polyunsaturated fatty acids found in these oils, reflecting in the immunological fitness of the fish.

The results of this study can also be justified by the low stocking density of post-larvae in the experimental period, where the fish appropriately used the lipids as energy source, taking advantage of the essential fatty acids for their development. Tolussi et al. (2010) working with *Brycon insignis* larvae observed that the increase in storage density causes changes in lipid metabolism and fatty acid profile. This parameter, besides compromising lipid metabolism causing low growth, can promote high stress (Portella, et al., 2013). In diets deficient in essential fatty acids, a decrease in growth may occur (Glencross, 2009), a fact that did not occur in the present study due to the high concentration of these fatty acids in palm oil.

When working with diets containing alternative sources, a great difficulty is observed in the acceptability of fish feed, which may be related to palatability (Rodríguez-Serna, 1996). The attractiveness of the diets did not show significant differences between the treatments, but it can be observed that through the average values there was a greater consumption of the feed containing palm oil, being the most attractive among the treatments tested. Therefore, the results found for the zootechnical performance of Nile tilapia can also be justified by the attractiveness/palatability of the diets containing palm oil, allowing a high consumption, positively influencing the evaluated variables. In agreement, Azevedo (2013), working with

palm pie in diets for tilapia juveniles, observed an increase in the daily intake values of the feed, which may have been influenced by the palatability of the diet. According to NRC (1994), the use of lipids in feed improves palatability and reduces nutrient loss.

Ng et al. (2000) agrees that palm oil can be used in tropical fish feed without compromising growth, making feeding more efficient. However, the inclusion of palm oil in the diet of Nile tilapia post-larvae proved to be effective for zootechnical performance, presenting positive effects in all treatments.

## 5. Conclusion

Based on the information obtained in the present study, it was concluded that the inclusion of at least 3% palm oil to replace soybean oil in the sexual determination phase of tilapia post-larvae contributes to the improvement of productive parameters, allowing the use of palm oil as an alternative source of energy in the feeding of this species.

New studies are important to evaluate the use of palm oil for Nile tilapia, especially in the other stages of cultivation, in addition to evaluating the fatty acid composition of the carcass.

## Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice in which the studies were conducted. All applicable international, national, and/or institutional guidelines for care and use of animals were followed by the authors.

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