

EVALUATION OF FISH OIL REPLACEMENT WITH JATROPHA CURCAS OIL IN DIETS FOR JUVENILE NILE TILAPIA

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ABSTRACT

The increasing worldwide demand for Nile tilapia heightens the need to develop novel ingredients suitable for balanced diets. Successful partial replacement of fish oils with vegetable oils is well-documented where adequate growth has been obtained and commercial size reached within a reasonable time period. Our trials on tilapia (*Oreochromis niloticus*) produced no changes in feeding behavior when *Jatropha curcas* oil (JcO) and fish oil (FO) were included in their diets. Survival rate with FO was lower than all JcO inclusions. Replacing 25% FO for JcO gave significantly lower growth than in any of the other treatments. And JcO-75, JcO-100 present the best growth (weight, size and gain levels) similar to Jc-0 treatment. The tilapia accepted all diet inclusions and presented normal behavior. Since JcO contains higher levels of linoleic and linolenic acid than conventional aquaculture ingredients such as fishmeal or soybean meal, its use as a lipid source was found to be feasible to complement Nile tilapia (*O. niloticus*) diets.

Keywords: *Oreochromis niloticus*, Nile tilapia, fish oil, *Jatropha curcas* oil, growth, survival.

1. INTRODUCTION

Fish oil (FO) is the main lipid source used in formulated diets for aquaculture, it contains high amounts of n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which promote survival and health, as well as make balanced diets palatable and attractive (Pike and Jackson, 2010; Teves and Razaga, 2016). The extraction and use of plant lipids as a substitute for FO in the diets of many fish species is well documented as it has been found to provide adequate fish growth and achieve commercial size within a reasonable time (Suttili *et al.*, 2018; Turchini *et al.*, 2009). Lim and Webster (2006) reported that Nile tilapia (*O. niloticus*) achieved better growth when fed diets containing vegetable sourced oils such as soybean or maize oil and they attribute this to high levels of linoleic acid (18:2 n-6). The same authors did not achieve good results with diets containing FO or beef fat despite them being rich in eicosapentaenoic acid (20:4 n-6) or oleic acid (18:1 n-9). Their study also describes how female *O. niloticus* fed diets containing soybean, maize and coconut oil had a higher hatch

frequency and more fry per spawn. Whilst humans consume oils such as safflower and sunflower they are also used in tilapia diets, nevertheless, several factors need to be carefully considered due to the disadvantage of having to satisfy both markets. *Jatropha curcas* is a drought-resistant shrub pertaining to the Euphorbiaceae family, found in wild or semi-cultivated areas in Central and South America, Africa, India, China and South-East Asia (Kumar *et al.*, 2010). Non-toxic *J. curcas* kernels contain at least 55% oil and are of significant interest as a potential source of biofuel (Makkar *et al.*, 1997; 1998a; 1998b; Achten *et al.*, 2009). The toasted kernels of the Mexican variety when consumed by humans have no side effects (Makkar *et al.*, 1997; 1998a; 1998b; 2009; 2011; Goel *et al.*, 2007; Devappa *et al.*, 2012). In order to determine their feasibility, Lucas and Southgate (2012) and Lim *et al.* (2008) stated that thorough assessments are necessary to determine the correct amount of *J. curcas* oil (JcO) in tilapia feeds. FO was therefore substituted for different inclusion levels of JcO in the tilapia diets and considerably better growth and survival rates were achieved in our trials.

2. MATERIALS AND METHODS

Diet formulation

Five experimental diets each containing 35% crude protein and 10% lipids were formulated (MixitWin 6 Agricultural Software Consultants). Non-toxic genotype of *J. curcas* seeds were obtained from Dimas and/or San Ignacio, Sinaloa State, México. JcO was extracted from the kernels and used to replace graded levels of FO in percentages of 0, 25, 50, 75 and 100. The ingredients of the diets were homogenized and mixed in a Hobart® mixer (AND EK-4100, Korea) and the resulting paste was then pelleted in a meat grinder (Torrey® M12F8, Mexico), and passed through a 5 mm diameter plate disc before drying in a convection oven for 24 h at 60 °C. All diets were packaged and labelled in sealed plastic bags and stored refrigerated at 4 °C throughout the experiment.

Experimental set-up

Approximately 1000 male *O. niloticus* were obtained from Genetilapia S.A. de C.V. (Los Pozos, El Rosario, Sinaloa, Mexico) and transported to the installations of CIAD-Mazatlán where the trial would be conducted. Plastic bags containing Nile tilapia larvae were placed in 300 L acclimatisation tanks (grey fiberglass) with continuous aeration until a constant temperature was reached. The experiment was conducted in a recirculation system consisting of eighteen 56L black fiberglass tanks with white interior bottoms, three filters and a reservoir (150 L). Each biological filter container (70 x 37 x 34 cm) was divided into three separate compartments. Wadding (1m x 1.30m) was placed in the first compartment, the second compartment contained oyster shells and in the third, a pump (18w) was placed and used to release water into the reservoir, which was then redistributed with a 45w pump to all the tanks. Water was distributed from six tanks to each biological filter and water flow to each tank was maintained at 1 ± 0.20 L/min and supplied with aeration. The whole system was kept at a mean temperature of 29 ± 1 °C and kept under photoperiod controlled conditions (12h light /12h dark). Before implementing the feed treatments, the tilapia were starved for 24 h and subsequently, all tanks were cleaned by siphoning out the faeces. Ten organisms (1.5 ± 0.2 g, 4.4 ± 0.2 cm) were stocked in each tank and fed a commercial diet over a seven-day acclimatisation period. Afterwards, each of the five

treatments were carried out in triplicate. Feed was given three times a day until apparent satiety (8:00, 12:00 and 16:00h). Body weight and length of each individual were recorded every 15 days until the end of the experiment (45 days). To anesthetize the organisms 1.5 mL clove solution (1:1 clove essence: ethanol 70%) diluted in 5 L of freshwater was employed and the organisms were left for 10 seconds before being weighed (AND Ek-4100 balance) and measured (digital calliper). Afterwards they were kept in clean freshwater with high aeration until completely recovered and subsequently returned to their respective tanks. Water parameters (temperature, pH and dissolved oxygen) were determined daily by means of an YSI-multimeter 85. Ammonium, nitrate and nitrite were measured using commercial kits (API™) and verified twice weekly by the Chemistry and Aquatic Production Laboratory of CIAD-Mazatlán in order to provide optimal conditions for this trial study.

Biochemical analyses

Analysis for proximate composition of the diets and tilapia were carried out at the beginning and end of the experiment as follows:

- Protein: Measured with a LECO FP-528 nitrogen combustion analyser (AOAC 1990 method 990.03)
- Total Lipids: Conducted using standard petroleum-ether extraction procedures (Soxtec 2050, Foss Tecator, AOAC 1990 method 2003.06).
- Gross energy: Combustion measurements were assessed by bomb calorimetry (Parr 6725 semi micro calorimeter)
- Ash: Samples were calcined at 550 °C in a muffle furnace (ISO 5984, 1978)
- Dry matter: Determined by oven drying at 60 °C for 24 h Parameters to evaluate the performance of different inclusions of JcO in Nile tilapia

Diets were based on the following formulas:

- Survival (S, %) = final number of tilapia / initial number of tilapia (Li et al., 2011)
- Weight gain (WG, g) = final weight(g) - initial weight (g) (Li et al., 2011)
- Specific growth ratio (SGR, %/d) = $100 * (\ln[\text{final weight}] - \ln[\text{initial weight}] / \text{days})$ (Azaza et al., 2009; Stadtlander et al., 2013)
- Condition factor (K, %) = $100 * [\text{final weight} / (\text{final size})^3]$ (Morales, 2004)
- Feed conversion ratio (FCR) = quantity of food consumed [g] / weight gain [g] Ahmad and Abdel-Tawwab, 2011; Stadtlander et al., 2013).

3. STATISTICAL ANALYSIS

Results were presented as mean \pm SD. Data were tested for normality distribution using the Kolmogorov-Smirnoff test and Bartlett's homoscedasticity test. One-way analysis of variance (ANOVA) was used to determine the significant differences ($P < 0.05$) followed by a Duncan's multiple range test. All statistical tests were conducted using SigmaStat (version 3.0).

4. RESULTS

No significant difference ($P > 0.05$) was found in diets for crude protein and lipid content.

On the other hand, the concentration of ash was significantly higher ($P < 0.05$) with JcO administered at 25% and with gross energy at 25% and 100 % (Table 1). During the experimental period, no apparent difference was observed between the feed acceptability of diets with different inclusions of JcO. No uneaten feed was detected and behavior was normal in all tanks.

Significantly higher protein content ($P < 0.05$) was found in juveniles of tilapia fed on JcO-100, -75 and -50. The lowest lipid content was observed with tilapias fed diets containing JcO-50. Ash content was significantly lower with JcO-0 and gross energy values were significantly lower ($P < 0.05$) for JcO-50 treatment (Table 2). Better survival occurred with all JcO inclusion (Table 3). Evidently, tilapia fed on JcO-25 were significantly lighter and smaller ($P < 0.05$) with much lower specific growth ratio (SGR) than in all other treatments (Table 3). Best final weights were achieved with JcO-75 and -100.

5. DISCUSSION

Our findings indicate that diets of mixed FO and JcO can be considered as alternative lipid sources for tilapia. Even though those with JcO demonstrate better results than the control (JcO-0). Tilapia fed on diets containing the percentage of JcO-75 and -100, gained more weight and were significantly different ($P < 0.05$) than those fed on JcO-0, -25 and -50. The poor growth obtained with diets containing JcO-25 can be attributed to the higher ash contained in the diets rather than the other diets, however further studies need to be done for better explanation. Treatments JcO-75 and -100, both present the highest weight gain (Table 3). JcO-25 was the smallest sized tilapia at the end (45d) of the experiment. The best feed conversion ratio (FCR) was recorded with JcO-100 which was significantly better than all other treatments (Table 3). The water parameters reported in this experiment were acceptable: temperature $29 \text{ }^\circ\text{C} \pm 1$, 33% oxygen saturation, 0.1 PSU, nitrate 0.0, nitrite 1.7, pH 7, and hardness was between 153-204 mg L⁻¹ (Lim and Webster, 2006; Suresh and Bhujel, 2012).

Common plant lipids extracted from safflower, soybean, maize and sunflower are often used in the fish industry and are commonly consumed by humans (Lim *et al.*, 2008 and Suresh and Bhujel, 2012). In this study we found that tilapia were able to tolerate the effects of FO by replacing with JcO at 0, 25, 50, 75 and 100 %, thus confirming that many plant/vegetable oil sources are good for diets. According to Lim and Webster (2006), diets for Nile tilapia need no more than 12% lipids and a source of essential fatty acids (EFA).

Substantial evidence shows that when linoleic acid (18:2 n-6) is included in tilapia diets, the nutrition quality improves and as a result promotes a higher proportion of spawning females, more frequent spawns and larger numbers of fry per spawn. Vegetables are a good source of linoleic acid-rich oils (Lim and Webster, 2006). Puello-Cruz *et al.* (2018) confirms that *J. curcas* contain significantly higher linolenic acid than fishmeal and soybean meal, therefore using JcO as a lipid source in tilapia diets is recommended. Since day one of the experiment, normal consumption and acceptability was observed in all treatments. Nile tilapia are known to be resistant and can survive and grow on almost any diet, their omnivorous characteristics call for a combination of animal and vegetable ingredients in order to obtain optimal growth.

6. CONCLUSIONS

Nevertheless, further studies are needed to assess why the growth rate worsened when 25% FO was substituted with JcO. The results of this study conclude that when 75 and 100% levels of FO are replaced by JcO, growth rate (length and weight) is significantly better than in the other treatments. Survival gave even better results for all JcO inclusions than FO-100% diets.

7. Declaration of Competing Interest

The authors declare no conflicts of interest.

8. Acknowledgements

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Table 1. Composition and proximate content (g/kg dry matter) of the experimental diets with different percentages of Jatropha curcas oil (JcO -0, -25, -50, -75, -100 %) as a substitute for fish oil in diets for Nile tilapia (O. niloticus) juveniles.

Ingredients (g/kg feed)	JcO-0	JcO-25	JcO-50	JcO-75	JcO-100
Fishmeal	380	380	380	380	380
Soybean meal	301.5	301.5	301.5	301.5	301.5
Dextrin	158.5	158.5	158.5	158.5	158.5
Soybean protein concentrate	50	50	50	50	50
Fish oil	60	45	30	15	0
<u>Jatropha curcas</u> oil	0	15	30	45	60
Alginate	20	20	20	20	20
Mineral and vitamin premix	20	20	20	20	20
Calcium phosphate	10	10	10	10	10
Proximate content (g/kg dry matter)					
Crude protein (%)	35.9±0.2	35.7±0.1	35.2±0.3	35.7±0.2	35.3±0.1
Crude lipid (%)	10.1±0.1	10.8±0.1	10.5±0.1	10.5±0.1	10.2±0.1
Crude ash (%)	9.7±0.1b	10.8±0.3a	9.7±0.1b	9.8±0.2b	9.7±0.1b
Gross energy (kJ/g)	18.5±0.1b	19.7±0.1a	18.6±0.2b	18.7±0.1 b	19.1±0.1a

Values are means of all organisms from the three replicates ± SD. Different superscripts per row differ significantly (P<0.05). a>b

Table 2. Nile tilapia (*O. niloticus*) juveniles fed diets with different percentage of *Jatropha curcas* oil (JcO -0, -25, -50, -75, -100 %) as a substitute for fish oil.

	JcO-0	JcO-25	JcO-50	JcO-75	JcO-100
Crude protein (%)	58.2±0.4 ^c	59.1±1.1 ^b	59.7±0.3 ^a	59.5±0.8 ^a	63.0±0.5 ^a
Crude lipid (%)	15.5±0.4 ^b	15.9±0.3 ^b	13.8±0.2 ^c	17.1±0.2 ^a	15.5±0.7 ^b
Crude ash (%)	14.6±0.5 ^c	15.4±0.1 ^a	15.3±0.2 ^a	15.0±0.1 ^b	15.6±0.6 ^a
Gross energy (kJ/g)	19.5 ±0.1 ^{ab}	19.7±0.1 ^{ab}	19.2±0.1 ^b	20.3±0.1 ^a	19.8±0.1 ^a

Values are means of all organisms from the three replicates ± SD. Different superscripts per row differ significantly (P<0.05). a>b>c

Table 3. Growth performance and nutrient utilization of Nile tilapia (*O. niloticus*) juveniles fed different percentages (0, 25, 50, 75, 100%) of *Jatropha curcas* oil (JcO) as a substitute for fish oil.

	JcO-0	JcO-25	JcO-50	JcO-75	JcO-100
Initial length (cm)	4.4±0.2	4.4±0.2	4.4±0.2	4.4±0.2	4.4±0.2
Final length (cm)	9.5±1.1 ^a	8.9±0.9 ^b	9.5±1.3 ^a	9.5±0.7 ^a	9.5±0.8 ^a
Initial weight (g)	1.5±0.2	1.5±0.2	1.5±0.2	1.5±0.2	1.5±0.2
Final weight (g)	15.7±5.6 ^b	13.1±3.9 ^c	15.1±5.0 ^b	16.1±2.1 ^a	16.2±3.1 ^a
Survival (%)	90.0±4.7 ^b	97.0±28.3 ^a	97.0±4.7 ^a	97.0±17.0 ^a	97.0±12.5 ^a
Humidity (%)	75.1±0.1	75.6±0.1	76.0±0.1	75.5±0.1	76.0±0.1
Weight gain (g)	14.2±0.5 ^{ab}	11.6±0.7 ^c	13.6±0.2 ^b	14.6±0.5 ^a	14.7±0.3 ^a
SGR (%)	5.2±0.3 ^a	4.8±0.3 ^c	5.1±0.6 ^b	5.3±0.4 ^a	5.3±0.1 ^a
K	1.8±0.1	1.9±0.1	1.8±0.1	1.9±0.1	1.9±0.1
FCR	1.9±0.1 ^b	2.3±0.1 ^a	1.7±0.1 ^{bc}	1.8±0.1 ^b	1.5±0.2 ^c

SGR: specific growth rate; K: condition factor; FCR: feed conversion ratio. Values are means of all organisms and three replicates ± SD. Different superscripts per row differ significantly (P<0.05). a>b>c