BIOCHEMICAL COMPOSITION AND EVALUATION OF *Jatropha curcas* MEAL AS A REPLACEMENT FOR FISH MEAL IN DIETS OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*)†

[COMPOSICIÓN BIOQUÍMICA Y EVALUACIÓN DE HARINA DE *Jatropha curcas* EN SUSTITUCIÓN DE HARINA DE PESCADO EN DIETAS PARA JUVENILES DE TILAPIA NILÓTICA (*Oreochromis niloticus*)]

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SUMMARY

Fish meal is still the main protein in feed formulations, unsustainable development and overfishing practices reduce captures since last decade. An increasing demand for high quality feed for aquaculture force the evaluation of new protein sources. *Jatropha curcas* meal was used in a 45 day feeding trial at five different inclusion levels ranging from 0, 15, 30, 45 and 60% as a substitute for fish meal in the diets of Nile tilapia juveniles. Both growth and feed efficiency were evaluated. It was found that tilapia fed 0% *Jatropha curcas meal* inclusion were significantly heavier and larger while treatments containing 30% inclusion gave the best survival. However, digestibility decreased as inclusion levels increased. The juveniles accepted all the different diet inclusions and presented normal behavior. Gross energy and crude lipid contents were significantly higher for *Jatropha curcas* meal than for fish meal and soybean meal. Amino acids and fatty acids content of *Jatropha curcas* meal were acceptable, thus suggesting its feasibility as an alternative plant ingredient for Nile tilapia (*Oreochromis niloticus*) diets. Better results could be achieved by standardizing lipid extraction and treatments to increase the digestibility of *Jatropha curcas* meal. This study contains novel information on developing strategies that provide alternative protein sources in tilapia feed formulation.
Key words: Nile tilapia; *Jatropha curcas;* alternative protein.

RESUMEN

La harina de pescado es la principal proteína empleada en las dietas formuladas, el desarrollo no sustentable y la sobrepesca han reducido las capturas desde la década pasada. La demanda creciente de alimentos de alta calidad para acuicultura obliga a la evaluación de nuevas fuentes proteicas. Harina de *Jatropha curcas* se utilizó para sustituir harina de pescado en dietas de juveniles de tilapia Nilótica. Se tuvieron cinco niveles de inclusión diferentes, de 0, 15, 30, 45 y 60%. El bioensayo duró 45 días. Se evaluó el crecimiento y la eficiencia de alimentación. Se encontró que las tilapias alimentadas con 0% de inclusión de harina de *Jatropha curcas* fueron significativamente más pesadas y grandes, mientras que los tratamientos que contenían 30% de inclusión tuvieron la mejor sobrevivencia. Sin embargo, la digestibilidad disminuyó a medida que aumentaron los niveles de inclusión. Los juveniles aceptaron todas las dietas adecuadamente y presentaron un comportamiento normal. La energía bruta y el contenido de lípidos fueron significativamente más altos para la harina de *Jatropha curcas* que para la harina de pescado y harina de soya. El contenido de aminoácidos y ácidos grasos de la harina de *Jatropha curcas* es aceptable para la especie, lo que sugiere su viabilidad como ingrediente vegetal alternativo para las dietas de tilapia Nilótica (*Oreochromis niloticus*). Se

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podrían lograr mejores resultados mediante la estandarización de la extracción de lípidos, así como tratamientos para aumentar la digestibilidad de la harina de *Jatropha curcas*. Este estudio contiene información novedosa sobre el desarrollo de estrategias que proporcionan fuentes alternativas de proteínas en la formulación de dietas para tilapia.
Palabras clave: Tilapia Nilótica; *Jatropha curcas*; proteína alternativa.

INTRODUCTION

Global aquaculture plays a crucial role and provide over half of all fish productions for human consumption, amounting to 20 kg per capita in 2014. The culture of marine and freshwater fish requires balanced diets that include nutrients for optimal growth and commercial size within a reasonable time. Fish meal (FM) is the main protein source used in commercial diets, as it contains an adequate amount of amino acids and essential fatty acids, as well as being highly digestible. Oily fish, mainly anchovies are used in the production of FM with peak production levels reaching 30.1 million tons in 1994. However, by 2014 this production level decreased to 15.8 million tons due to unsustainable development and overfishing practices. Increasing demands for this ingredient in formulated diets demand for studies on developing alternative high quality ingredients. The recycling of by-products to be incorporated in diet formulation could also reduce the use of FM (Hardy 2008, FAO 2016). Several publications have reported the use of plant protein in the elaboration of Nile tilapia (*Oreochromis niloticus*) feed. In general, plant proteins and by-products do not compete with human consumption or the pet industry and are cheaper. Thorough assessment of new ingredients need to be examined in order to determine their feasibility for use in fish feed (Lim *et al*., 2008). *Jatropha curcas* is a drought-resistant shrub, belonging to the Euphobiaceae family; it adapts to poor soil conditions and is found in the tropical and sub-tropical regions of the world. This plant has raised significant interest as a potential source of biofuel (Makkar *et al*., 1998a, 1998b; Achten *et al*., 2010) due the high lipid content and the by-products such as meal for animal feed formulations. Some studies have shown that non-toxic *J. curcas* seeds are rich in nutrients and are high in protein (~60%) and oil (50 a 55%) content (Teves and Ragaza 2016). Vasconcelos *et al*. (1997) reported that its amino acid profile is similar to that of soybean. However, some plant sources that are used as ingredients to elaborate diets may contain anti-nutritional or toxic factors (Hardy 2008). *Jatropha curcas* plants, in some Mexican regions (Veracruz, Quintana Roo and Sinaloa states), are of the non-toxic genotype that contain low phorbol esters, which can be inactivated with microbial phytate (Makkar *et al*., 1998a, 1998b, 2009; Devappa *et al*., 2012). Trypsin inhibitors can be inactivated by thermal treatments (121°C for 25 min), helping to increase diet digestibility and produce meals that can be used for livestock and aquaculture (Martínez-Herrera *et al*., 2004). Also, synthetic phytases can be used to breakdown the high content of phytate. Similarly, saponins can be destroyed by treating with 90% ethanol +$NaHCO_3$ and heat-treated to 121°C for 25 minutes and seed´s lectin treated with $NaHCO_3$ and heated to 121°C for 25 minutes was less toxic (Martínez-Herrera. 2004). Humans who consume edible roasted seeds of *J. curcas* did not suffer side effects (Makkar *et al*., 1998b) and fish diets containing defatted kernel meal improved growth (Devappa *et al*., 2012). Good results have also been obtained with ingredients of vegetal and animal origin (Lim and Webster 2006). The chemical composition of raw FM, soybean meal (SM) and *Jatropha curcas* meal (JcM) were analyzed. Substituting FM for JcM at different inclusion levels were evaluated in this study and found to provide adequate protein levels for Nile tilapia diets.

MATERIALS AND METHODS

Diet formulation

Kernels of non-toxic *J. curcas* seeds (Escoto *et al*., 2013) were obtained from the Dimas and San Ignacio areas of Sinaloa state, Mexico. After pulverization the unshelled kernels into a powder, the samples were defatted by immersion in petroleum ether at room temperature (28 °C) for 2 h. A heating treatment was necessary to inactivate the lectins and trypsin inhibitors but it was modified in this experiment from 121 °C to 100 °C for 30 min (Kumar *et al*., 2012), this was mainly due to equipment limitations. The samples were then allowed to cool. Five experimental diets each containing 40% crude protein, 12% lipids and 18 kJ/g gross energy were formulated using Mixit Win 6 software (Agricultural Software Consultants). Graded levels of FM were replaced with JcM in percentages of 0, 15, 30, 45 and 60 (Table 2, JcM0, JcM15, JcM30, JcM45 and JcM60). Chromic oxide (0.5%) was added to all diets for further digestibility analyses. All the diet ingredients were homogenized and mixed in a Hobart® mixer and the resulting paste was then pelleted in a meat grinder (Torrey® M12F8, Mexico) and oven dried at 60 °C for 24 h. All five diets were packaged and labelled in sealed plastic bags and preserved in a refrigerator (4 °C) until further use.

Experimental set-up

Approximately a 1000 male *O. niloticus* were obtained from Genetilapia S.A. de C.V. (Los Pozos, Mazatlán, Sinaloa, Mexico) and transported to CIAD-Mazatlán installations where the trial would be conducted. The larvae were placed in acclimation tanks (300 L grey fiberglass) with constant aeration until the same temperature was reached. Tilapia were fed on a balanced diet (44% protein, 12% lipids and 18 kJ/g gross energy) administered three times a day (8:00, 12:00 and 16:00h) for seven days. Tilapia biomass from each tank was weighed every 15 days and the feed amount was adjusted according to this. When biomass reached 14g, they received 10% of their biomass as feed, and when this weight was surpassed, the administered food was reduced to 6% and maintained at this level until the end of the experiment (45d).

The experiment consisted of a recirculation system utilizing eighteen 56L black fiberglass tanks with black interior walls and white-bottoms, three biological filters and a reservoir (150 L). Each biological filter container (70 x 37 x 34 cm) was partitioned into three equal sized compartments. Wadding measuring 1m x 1.30m was rolled and doubled to accommodate it in the first compartment, the second compartment contained oyster shells and in the third, a pump (18w) was placed and used to regulate and release water into the reservoir, which was then redistributed with a 45w pump to all the tanks. Discharged water from six tanks was distributed to a biological filter; this procedure was repeated for the other two biological filters. Water flow to each tank was at a rate of 1 ± 0.20 L/min, and an aeration stone was provided. The whole recirculation system was maintained at a constant temperature of 22 ± 2 °C and the photoperiod controlled at 12h light /12h dark. Ten organisms (5 ± 0.5 g, 7 ± 0.5 cm) were stocked in each tank. The five treatments (JcM0, JcM15, JcM30, JcM45 and JcM60) were carried out in triplicate.

Before the experiment commenced, the tilapia were starved for 24 h and subsequently all tanks were cleaned by siphoning out the faeces. The larvae were fed three times a day, at 8:00, 12:00 and 16:00h until apparent satiety. Daily before first feeding, faeces and uneaten pellets were siphoned from the tank bottom and passed through a sieve, and spread on petri dishes for examination under a compound microscope (Leica M26-L2). Afterwards, the samples were left to dry. Weight and size measurements for each individual from all tanks were taken every 15 days until the end of the experiment. Tilapias were anesthetised in a 1.5 mL clove solution (1:1 clove essence: 70% ethanol) diluted in 5 L of freshwater where they remained for 10 seconds before being weighed (AND Ek-4100 balance) and their length measured with a digital calliper. Before reverting them to their respective tanks, they were kept in clean freshwater with high aeration until they completely recovered from the anaesthesia.

Water parameters (temperature, pH and dissolved oxygen) were determined on a daily basis using an YSI-multimeter 85. Commercial kits (API ™) were used to measure ammonium, nitrate and nitrite and these parameters were validated twice weekly by personnel at the Chemistry and Aquatic Production Laboratory (CIAD-Mazatlán) in accordance to the methods of Parsons *et al.* (1984). These analyses were essential in providing the optimum level of water quality in the recirculation system.

Chemical analyses

Analysis of proximate composition, amino and fatty acids for raw FM, SM and JcM were done before formulating the diets and proximate analyses of diets, faeces and tilapias were measured at the beginning and end of the experiment using the following standard procedures:

Crude protein (N x 6.25) by means of a LECO FP-528 nitrogen combustion analyser (AOAC 1990; method 990.03). Total Lipids were measured with a Soxtec 2050, Foss Tecator, using a standard petroleum-ether extraction technique (AOAC 1990; method 2003.06). Gross energy was determined using bomb calorimetry (Parr 6725 semi micro calorimeter). Ash by incineration in a muffle furnace (550 °C) (ISO 5984, 1978). Dry matter after oven-dried (60 °C) for 24 h. Amino acids were determined by liquid chromatography analysis (Lindroth and Mopper 1979). Fatty acids were determined by gas chromatography analysis (AOAC 1990; method 969.33). Digestibility was estimated by chromium oxide using the acid digestion method (Furukawa and Tsukahara 1966).

Response variables

Parameters to evaluate the performance of Nile tilapia using different inclusions of JcM were calculated as follows:

Survival (S, %) = final number of tilapias / initial number of tilapias (Li *et al*., 2011)

Weight gain (WG, g) = final weight (g) –initial weight (g) (Li *et al*., 2011)

Specific growth rate (SGR, %/d) = 100*(ln [final weight] – ln [initial weight] /days) (Azaza *et al*., 2009; Stadtlander *et al*., 2013)

Condition factor (K, %) = 100 * [final weight / (final size)^3] (Morales, 2004)

Feed conversion rate (FCR) = weight of consumed food [g] / weight gain [g] (Stadtlander *et al*., 2013)

Apparent metabolized energy (AME)= ((energy in feed - energy in faeces) / (energy in feed)) *100 (Lovell, 1998)

Apparent digestibility coefficient (ADC, %) =100-100* (% CrO in feed / % CrO in feces) (Azaza *et al*., 2009)

Apparent energy digestibility coefficient (ADCe, %) = 100 (1- % Cr_2O_3 in feed)(energy in faeces)/(%Cr_2O_3 in faeces)(energy in feed) (Azaza *et al*., 2009).

Statistical analysis

Results were presented by means ± standard deviation (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) and proceeded by a Duncan´s multiple range test. The software package Sigma-Stat 3 for Windows was used for the statistical analysis.

RESULTS

Proximate composition, gross energy (GE), amino acid (AA) and fatty acid (FA) profiles of raw ingredients (FM, SM and JcM) are shown in Table 1. Crude protein content from animal sources (FM) was significantly higher (P<0.05) than in diets of vegetal origin (JcM and SM). Gross energy and crude lipid were significantly higher (P<0.05) for JcM than for FM and SM. According to (Lim and Webster 2006) all meals have the necessary essential amino acids and fatty acids in order to raise *O. niloticus* (Table 1). Feed proximate analysis showed that all five formulated diets were isonitrogenous, isolipidic and isocaloric with no significant differences between them (P>0.05) (Table 2).

During the experimental period, acceptability to diets with different inclusions of JcM was good, however a slightly faster response was noted in those that included less amounts of JcM (0, 15 and 30%). Feed intake was significantly higher (P<0.05) in JcM0 diet (Table 4). In all treatments, the feeding and eating behavior of the tilapia was normal and all given feed was consumed. These conditions are important as non-consumed feed causes water pollution, therefore cost-productions increased (FAO).

Tilapia fed JcM45 had significantly higher protein and lipid content (P<0.05) than those in the JCM0 treatment and the initial stock. Ash was significantly higher (P<0.05) in tilapia fed on JcM30 than in all the other treatments. Gross energy values were significantly higher (P<0.05) for initial tilapias (Table 3). The ones fed on JcM0 were significantly heavier (P<0.05) (Table 4). Survival was significantly lower (P<0.05) for those fed on JcM45 while JcM30 gave better survival rates. The effects of different inclusion levels of JcM15, JcM30, JcM45 and JcM60 resulted in decreased values for AME, ADC and ADCe, possibly due to increased amounts of JcM (Table 4). The water parameters reported in this experiment are detailed in Table 5. High levels of ammonium and nitrite were detected (1.0 and 0.5 respectively) on day three of the experiment and a complete renewal of water was carried out immediately. No further problems were reported and all the other parameters were within optimum range.

DISCUSSION

Water quality parameters were within the acceptable limits for tilapia growth. Temperature was below the optimal level of 27-30 °C as recommended by García-Ortega and Calvario-Martínez (2008) (Table 5). When water and feed quality are optimum (Lim and Webster 2006), *O. niloticus* are able to adapt to a wide range of temperatures but this should never be below 18 °C and not above 34 °C. In Mazatlán, Sinaloa some aquaculturists have successfully set up culture systems using similarly sized tilapia with the same mentioned temperatures. We decided to use these conditions throughout the experimental period and the effects were recorded so that the information can be directly applied to producers.

Both FM and SM are commonly used in commercial diets such as feed for cattle, poultry and the pet industry, and sometimes for human consumption. An advantage of using *J. curcas* is that it is not used in the feed industry (Lim *et al*., 2008) however, in conventional diets for aquaculture its use requires special considerations. JcM had higher levels of lipid composition and gross energy than FM and SM (Table 1). One important factor is that the lipid content should not exceed 15%, and in the case of Nile tilapia, Lim and Webster (2006) established the maximum lipid levels to be 12 % and that essential fatty acids (EFA) must be included in the diet because of their incapability of synthesizing them.

The lipid content of the processed kernels of *J. curcas* was found to be slightly higher than 30%. As we were unable to process large amounts of meal at our facilities, we used large containers so that we could defat enough JcM and elaborate the different inclusion diets. As a result of the heat-treated JcM, its lipid content increased. The results indicate that the highest value in gross energy obtained for JcM is attributed to its high lipid levels.

Table 1. Proximate, gross energy, amino acid and fatty acid composition of feed ingredients (% dry matter) used in experimental diets for juveniles of Nile tilapia (*Oreochromis niloticus*).

	Fish meal (FM)	Soybean meal (SM)	*Jatropha curcas* meal (JcM)
Proximate composition (%)			
Dry matter	93.1±0.4	92.2±0.5	90.5±0.7
Crude protein	63.4±0.8[a]	43.9±0.3[b]	44.6±1.0[b]
Crude lipid	11.8±0.8[b]	1.8±0.4[c]	22.9±0.3[a]
Ash	13.3±0.1[a]	6.1±0.5[b]	8.3±0.1[b]
Gross energy (kJ/g)	18.5±0.03[b]	15.2±0.07 [b]	20.6±0.05[a]
Essential amino acid composition (g 100g/protein)			
Arginine	0.1 ± 0.0	5.8 ± 0.9	1.3 ± 0.0
Histidine	0.2 ± 0.0	1.9 ± 0.5	1.8 ± 0.5
Isoleucine	2.8 ± 0.3	3.2 ± 0.6	2.5 ± 0.3
Leucine	5.2 ± 0.1	5.4 ± 1.0	4.6 ± 1.0
Lysine	1.8 ± 0.1	2.0 ±0.4	2.5 ± 0.3
Methionine	1.4 ± 0.8	0.5 ± 0.3	0.1 ± 0.1
Phenylalanine	4.3 ± 1.4	10.4 ± 0.6	10.1 ± 1.2
Glycine-Threonine	8.7 ± 0.6	3.1 ± 0.6	0.7 ± 0.7
Valine	6.4 ± 0.4	6.5 ± 1.1	5.8 ± 1.0
Non-essential amino acid composition (g/100g protein)			
Aspartic acid	4.9 ± 0.3	8.2 ± 1.2	4.3 ±0.4
Glutamic acid	7.3 ± 0.5	13.3 ± 2.3	9.3 ± 1.0
Serine	0.7 ± 0.2	3.4 ± 0.7	3.1 ± 0.5
Alanine-Tyrosine	7.8 ± 0.4	7.4 ± 0.5	6.4 ± 0.5
Total	51.6[b]	71.1[a]	52.5[b]
Fatty acid composition (%)			
C14:0	0.9	0.0	0.0
C14:1 cis-9	0.1	ND	0.0
C16:0	3.6	0.3	3.1
C16:1 cis-9	0.8	0.0	0.1
C17:0	ND	ND	0.0
C18:0	0.8	0.1	0.0
C18:1 cis-9	2.0	0.3	1.3
C18:2 trans-9,12	0.2	0.0	9.0
C18:3 cis-6,9,12	0.2	0.9	11.0
C20:0	0.1	0.1	0.1
C20:1 cis-11	ND	0.1	0.1
C20:2 cis-11-14	0.3	ND	0.0
C20:4 cis-5,8,11,14 (ARA)	0.2	ND	ND
C20:5 n-3 (EPA)	17.6	ND	ND
C22:2 cis-13-16	1.1	ND	ND
C22:6 n-3 (DHA)	15.2	ND	ND
C24:1 cis-15	1.6	ND	ND
Total	11.8[b]	1.8[c]	24.8[a]

ND: not detected. Values are means of three replicates ± SD. Different superscripts per row differ significantly ($P<0.05$). a>b>c.

Total amino acids (AA) content obtained for JcM were similar to those of FM (Table 1). Lim and Webster (2006) concluded that the protein percentage for Nile tilapia is not as important as having a well-balanced essential and non-essential AA content. Amongst the most essential AA are arginine, histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan and valine. Similarities were noted in the composition of AA for JcM and SM, particularly the high level of phenylalanine recorded (10.1 and 10.4 respectively), this was also true for valine (5.8 and 6.5 respectively) (Vasconcelos *et al.*, 1997).

Total fatty acid composition for JcM was inevitably higher to FM and SM, as can be observed in table 1 (linoleic acid (18:2) and linolenic acid (18:3). The presence of linoleic acid improves the nutrition quality of diets. According to Lim and Webster (2006), tilapia diets that include vegetable oils produce a higher proportion of spawning females as well as numerous spawns and larger numbers of fry per spawn.

Balanced diets including different levels of *J. curcas* meal

Lim *et al*. (2008) recommended between 35 and 50 % of protein levels for tilapia diets. Based on the initial tilapias weight and experimental period, 40 % of protein and 12 % lipid were included in each of the five formulated diets. Gross energy content ranged from 18.4 to 18.7 kJ/g with no significant differences observed between diets (P>0.05). Results demonstrate the feasibility of using JcM as a substitute for protein in Nile tilapia diets. Devappa *et al*. (2012) proposed adding phosphorus when phytates are present as it improves nutritional efficiency, so we added phosphate calcium to the diets (Table 1). FM levels included in this study are unusually high (>10%) for typically modern tilapia feeds. This high level was used so that we could evaluate the substitution of animal protein for vegetable protein. Tilapia adapt easily to JcM diets.

Table 2. Composition and proximate content (g/kg dry matter) of the experimental diets with different *Jatropha curcas* meal inclusion levels (0, 15, 30, 45, 60 %) as a substitute in fish meal for Nile tilapia (*Oreochromis niloticus*) juveniles.

Ingredients (g/kg feed)	JcM0	JcM15	JcM30	JcM45	JcM60
Fish meal	493.4	419.4	345.4	271.4	197
Dextrin	215.4	197.9	178.6	161.9	141.7
Soybean meal	150	145.5	150	141.6	150
Jatropha curcas meal	0	108	207.3	319	415
Soybean protein concentrate	40	40	40	40	40
Fish oil	30	30	20	10	3.5
Canola oil	21.2	9.2	8.7	6.1	2.7
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 (%)	44.1±0.1	43.6±0.1	43.5±0.1	43.8±0.1	44.5±0.1
Crude lipid (%)	12.5±0.1	12.2±0.1	12.7±0.1	12.6±0.1	12.6±0.1
Crude ash (%)	10.0±0.1	10.8±0.3	10.8±0.1	10.3±0.2	10.2±0.1
Gross energy (kJ/g)	18.4±0.1	18.4±0.1	18.7±0.1	18.5±0.1	18.6±0.1

Table 3. Proximate content (% dry matter) of Nile tilapia (*Oreochromis niloticus*) juveniles fed different *Jatropha curcas* meal inclusions (0, 15, 30, 45, 60 %) as a substitute for fish meal in experimental diets over a period of 45 days.

	Initial fish	JcM0	JcM15	JcM30	JcM45	JcM60
Crude protein (%)	55.9±0.3^{b}	57.3±0.4^{a}	55.2±1.1^{b}	51.7±0.3^{c}	58.5±0.8^{a}	57.9±0.5^{a}
Crude lipid (%)	15.7±0.8^{c}	16.6±0.4^{b}	16.3±0.3^{b}	15.5±0.2^{c}	22.9±0.2^{a}	14.4±0.7^{c}
Crude ash (%)	16.4±0.1^{c}	17.6±0.5^{b}	18.3±0.1^{b}	21.0±0.2^{a}	17.9±0.1^{b}	18.1±0.6^{b}
Gross energy (kJ/g)	21.0±0.0^{a}	19.9 ±0.1^{ab}	19.1±0.1^{bc}	18.4±0.1^{c}	19.5±0.1^{b}	19.0±0.1^{bc}

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

Table 4. Growth performance and nutrient utilization of Nile tilapia (*Oreochromis niloticus*) juveniles fed on experimental diets with different *Jatropha curcas* meal inclusion levels as a substitute for fish meal and a commercial diet for 45 days.

	JcM0	JcM15	JcM30	JcM45	JcM60
Initial length (cm)	7.3±0.2	7.3±0.2	7.3±0.2	7.3±0.2	7.3±0.2
Final length (cm)	9.8±1.1[a]	9.3±0.9[ab]	8.7±1.3[b]	8.7±0.7[b]	9.0±0.8[ab]
Initial weight (g)	5.3±0.2	5.3±0.2	5.3±0.2	5.3±0.2	5.3±0.2
Final weight (g)	16.3±5.6[a]	13.6±3.9[b]	11.3±5.0[c]	11.0±2.1[c]	12.3±3.1[b]
Survival (%)	63.3±4.7[bc]	80.0±28.3[a]	83.3±4.7[a]	36.7±17.0[c]	73.3±12.5[b]
Moisture (%)	74.6±0.1	75.0±0.1	77.2±0.1	74.2±0.1	75.5±0.1
Weight gain (g)	10.9±0.5[a]	8.2±0.7[b]	5.9±0.2[c]	5.6±0.5[c]	7.0±0.3[b]
SGR (%)	2.5±0.3[a]	2.1±0.3[b]	1.7±0.6[c]	1.6±0.4[c]	1.9±0.1[b]
Feed intake (g/fish)	11.3 ± 0.4[a]	9.1 ± 0.4[b]	9.9 ± 0.5[b]	9.0 ± 0.3[b]	9.6 ± 0.4[b]
K	1.7±0.1	1.7±0.1	1.7±0.1	1.6±0.1	1.7±0.1
FCR	1.0±0.1	1.1±0.1	1.5±0.1	1.6±0.1	1.3±0.2
AME	21.6±1.4[a]	25.2±2.1 [a]	25.8±1.7[a]	15.5±1.6[b]	14.5±1.9[b]
ADC	79.4±4.9[a]	80.4±5.1[a]	78.4±4.2[a]	68.1±4.5[b]	61.1±4.7[b]
ADCe	83.8±6.1[a]	85.3±5.9[a]	84.0±5.3[a]	73.1±5.7[b]	66.7±5.4[b]

SGR:specific growth rate; K:condition fac
tor; FCR:food conversion ratio; AME: Apparent metabolized energy; ADC: Apparent digestibility coefficient; ADCe: Apparent energy digestibility coefficient; different percentages of JcM meal inclusions (0, 15, 30, 45, 60 %) in experimental diets. Values are means of all organisms and three replicates ± SD. Different superscripts per row differ significantly (P<0.05). a>b>c

Table 5. Water quality parameters in the recirculation system of Nile tilapia (*Oreochromis niloticus*), fed on experimental diets with different *Jatropha curcas* meal inclusion levels as a substitute for fish meal and a commercial diet over a 45 day period.

	Max	Min	Optimum*
Temperature (°C)	23.7	21.0	25-36
Dissolved oxygen (mg/L)	3.5	2.1	<2.0; <4.5
Oxygen saturation (%)	42.9	24.6	-
Salinity (psu)	0.1	0.1	5-10
Nitrite ($N\text{-}NO_2$)	1.0[a]	0.0	>0.1
Nitrate ($N\text{-}NO_3$)	9.4	0.1	>10
Ammonium ($N\text{-}NH_4$)	0.5[1]	0	0.01 - 0.1
pH	7	7	6.5-9.0
Hardness (mg/L)	153	204	20-350

Max-Maximun, Min-Minimun
*García-Ortega and Calvario-Martínez 2008

Effect of diets with JcM as a substitute for FM

The 45 day period of the experiment was considered enough time to obtain sufficient information on the different inclusions of JcM in Nile tilapia juveniles after their digestion structures were developed (Lim and Webster 2006). A slight reduction in feed consumption was observed in diets with JcM when compared to the control diet (JcM0). However, after day 5 feed consumption was similar in all treatments and comparable with other authors results (Kumar *et al*. 2012, Akinleye *et al*. 2012). Significant differences were observed in growth between treatments (P>0.05), the highest rate was attained for tilapia fed on JcM0 (9.8 cm and 16.3 g), and the lowest rate for those fed on JcM45 (8.7 cm and 11.0 g).

Survival was significantly higher (P>0.05) for tilapia fed on JcM30 (83%) and JcM60 (73 %), when compared to those fed on JcM45 (37%), suggesting that JcM inclusion is not considered an issue as higher inclusions gave good results. On day 38, dead tilapia appeared in all the tanks, some with slightly prominent eyes. Shoemaker *et al*. (2006) suggests that the presence of Sporozoa, *Edwardisella tarda* and/or *Streptococcus* in tilapia recirculating systems can produce alterations such as hemorrhage, exophthalmia or opacity in the eyes. As a precautionary measure, we immersed the tilapia in a formaldehyde solution (37%) (0.17 mL/L) with strong aeration for 30 min as recommended by Francis-Floyd (1996). No parasites, diseases or any other abnormalities were detected and no further mortalities were observed after this procedure. Weight gain (WG) for tilapia in the control treatment JcM0 (10.9 g) was almost twice that of those fed on JcM45 (5.6 g) (P<0.05), compared with similar studies our values are acceptable considering that initial tilapias were half weight. Also the specific growth rate (SGR) was significantly higher (P<0.05) for tilapia fed on JcM0 (2.5 g/d) and lower for those fed on JcM45 (1.6 g/d). The SGR reported in similar studies are between 2.78 g/d and 1.9 g/d (Azaza *et al.* 2009, Kumar *et al*. 2011, Akinleye *et al*. 2012, Kumar *et al*. 2012, Krome *et al.* 2014) which demonstrated that inclusions higher than 45% of JcM negative effect in growth. Even though the condition factor (K) was not significantly different between treatments (P>0.05), our results are similar to those reported by Ighwela *et al*. (2011). Feeding convertion rates (FCR) of between 0.6 and 1.0 were reported by Lim *et al.* (2008) for tilapia not exceeding 10 g (wet weight) with a total length of 0 to 1.3 cm. The values obtained in our experiment were between 1.0 and 1.6 and are similar to those reported in previous studies. A higher apparent metabolizable energy (AME) value was obtained for those fed on JcM30 while a much lower value was observed for those fed on JcM60. The omnivorous feeding strategy of Nile tilapia requires a combination of animal and vegetable meal sources if optimal growth is to be expected. Further studies are needed as 60% FM subtituted with JcM tends to give better results than diets elaborated with 30 and 45% FM. The apparent digestible coefficient (ADC) and apparent digestible coefficient related to gross energy content (ADCe) induced an inverse behavior when JcM was included in the diet. The highest digestibility (80.4 %) was obtained with the lowest inclusion (JcM15), whilst the lowest digestibility value (61.1 %) was achieved with JcM60. These results are similar to the findings of Lim *et al*. (2008) who also used alternative protein meal sources.

Our preliminary findings show that Nile tilapia fed JcM diets displayed normal behavior but further experiments are recommended so that improvements to digestibility can be achieved. Makkar *et al*. (1998b) stated that roasting *J. curcas* seeds may be the most suitable technique to inactivate trypsin inhibitors and reduce lectin activity.

CONCLUSION

JcM contains good protein content (32-45 %), adequate lipid content (19-35 %) and an adequate balance of AA and AG, for elaboration of Nile tilapia feeds. Promising results were obtained at JcM15 levels in terms of lowest feed intake with heaviest weight gain, and one of the higher survival when compared with highest JcM inclusion and Control JcM0.

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