Nutritional studies on partial replacement of soybean meal by Jatropha curcas meal in Nile tilapia (Oreochromis niloticus) fingerlings diets

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ABSTRACT
This study was undertaken to investigate the efficiency of partial replacement of soybean meal (SBM) by Jatropha curcas meal treated with lactobacillus acidophilus at rate of 1g/100kg. Four experimental diets were formulated, as iso nitrogenous (30.5 % crude protein) and isocaloric (4543.56Kcal/kg diet), containing 0, 25, 50 and 75% of treated Jatropha meal (TJM) T1, T2, T3 and T4, respectively and fed to monosex Nile tilapia, (Oreochromis niloticus) fingerlings with average weight (30.43±1.14g/fish). Total of 60 fingerlings were random distributed in four treatments, triplicate groups each with five fish/aquarium. The experiment period was lasted for 70 days. Growth performance, feed utilization and body compositions as well as the economic evaluation were monitored. The obtained data showed that, higher weight gain and the best feed conversion ratio (FCR), protein efficiency ratios (PER), protein productive value (PPV %) and energy utilization (EU%) recorded for fish fed diets (up to 50 %) of treated Jatropha meal were insignificantly different from those fed soybean meal. It could be concluded that the SBM can be partial replaced with TJM up to 50 % of SBM without any adverse effects on growth performance or feed utilization of Nile tilapia.

Keywords: Jatropha curcas meal, soybean meal, lactobacillus acidophilus, growth performance, economic evaluation, Oreochromis niloticus

INTRODUCTION
Protein generally is the most expensive component in feeds for aquatic species a result to its cultured. Soybean meal (SBM) is the most studied plant feedstuff in aquaculture as availability, consistent quality, high protein content with good amino acid profile and low-cost (Lim and Dominy, 1989).

In Egypt, the Jatropha meal remained after oil extraction contains high protein level approximately 45-50% yet it could be considered as feed supplement for livestock producers (Aslani et al., 2007). The major problem with using Jatropha cake is its high content of some antinutritional compounds of inhibitor activities like Trypsin, Phytate, Saponins and Lectins. Lactobacillus acidophilus are the best method to reduce the antinutritional compounds in Jatropha meal. As a result of expensive importation of soybean meal, there is a need to evaluate alternative protein sources that help to reduce the shortage problem of plant protein sources. Jatropha curcas is native to Central America and has become naturalized in many tropical and subtropical areas, including India, Africa (Egypt) and North America. Originating in the Caribbean, Jatropha was spread as a valuable hedge plant to Africa and Asia by Portuguese traders (Fairless, 2007).

The seeds of Jatropha curcas contained 60–66% crude lipid and 30–32% crude protein (Liberalino et al., 1988). Jatropha meal remained after oil extraction contain high protein level approximately
40-50% (Aslani et al., 2007) which is characterized as a well-balanced amino acid composition according to the FAO/WHO reference pattern, except for lysine (Martinez-Herrera et al., 2006).

The levels of essential amino acids (except lysine) are higher in Jatropha seed cake than in the FAO reference protein for a growing animal (Harinder et al., 2008). The jatropha seed meal can be a good protein source for humans as well as for livestock, however, the application of Jatropha seed meal in fish feeding is limited by the relatively high content of antinutritional factors such as trypsin inhibitor, lectin and phytate (Makkar et al., 2008).

Although numerous studies evaluated the efficacy of the different methods (physical, mechanical, chemical and biological) on detoxification of the antinutrients/toxicants in Jatropha curcas meal, a few studies have focused on the effects of fermented Jatropha seed cake on the growth and physiological status of farmed aquatic species (Shamna et al., 2015). Consequently, this study was carried out to determine the effect of replacement of soybean meal combined with Jatropha treated meal with lactobacillus acidophilus on growth performance, feed and nutrient utilization and carcass composition of monosex Nile tilapia, (O. niloticus) fingerlings.

MATERIALS AND METHODS

The present experiment was implemented at Fish laboratory, Utilization of by-Products Department, Animal Production Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Experimental fish:

Total number of 60 monosex Nile tilapia (O. niloticus) fingerlings averaging of an initial weight of 30.43±1.14g/fish were obtained from commercial fish hatchery at El-Hamol, Kafr El-Shiekh Governorate. Five fingerlings were randomly stocked in aquaria with dimensions of 60cm L × 40cm H x 30cm W and 63 liter water/aquarium with triplications per treatment (four treatments). Fish were adapted for 15 days before starting the experiment which lasted for the actual experimental period 70 days. Aquaria were cleaned daily and one third of the water was replaced before feeding. The daily ration was divided into two equal portions and was offered handling two times a day at 9.00 and 13.00 hrs at a level of 4% of body weight. Fish in each triplicate were weighed biweekly at the 14th day whereas, the feeding was stopped on that day and the amount of daily diet were adjusted accordingly. At the beginning and end of the experiment, a sub-sample of fish from each triplicate was sacrificed for the whole body analysis.

Lactic acid bacteria treatment:

Jatropha curcas seed meal was obtained from the Center Administration for Tree, Dokki, Egypt which was treated with Lactic acid bacteria (lactobacillus acidophilus) at a rate of 1g/100kg and stored in plastic sacks for 21 days at room temperature. The treated Jatropha meal was dried to reach about 6% moisture and was ground to pass a 2 mm screen.

Anti-nutritional compounds analysis:

Trypsin inhibitor activity was determined essentially in untreated and treated Jatropha meal samples, according to Smith et al. (1980). Analysis of Lectin content was conducted by haemagglutination assay protocol described by Gordon and Marquard (1974). The total saponin (triepennid and steroidal) content was determined using a spectrophotometric method described by Hiai et al. (1976). Phytate content was determined by a colorimetric procedure described by Vairtrash and Laptera (1988).
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Experimental diets:
All feed ingredients and the necessary additives were purchased from the local market. The proximate analysis of the feed ingredients used in formulating the experimental diets is shown in Table (1) and The concentration of anti-nutritional compounds untreated and treated Jatropha curcas meal compared with soybean meal (Table 2)

Table (1): Proximate analysis (DM %) of the feed ingredients used in formulating the Experimental diets fed to Nile tilapia, (O. niloticus) fingerlings.

| Ingredients       | Moist. | Crude protein | Ether extract | Crude fiber | Ash   | NFE* | GE** |
|-------------------|--------|---------------|---------------|-------------|-------|------|------|
| Fish meal         | 7.10   | 64.13         | 5.71          | 1.02        | 14.87 | 14.27| 4775 |
| Soybean meal      | 8.85   | 44.00         | 1.49          | 7.19        | 6.23  | 41.09| 4558 |
| Wheat bran        | 10.78  | 13.73         | 3.35          | 11.62       | 7.11  | 64.19| 4222 |
| Yellow corn       | 11.00  | 7.50          | 3.80          | 2.60        | 1.30  | 84.80| 4280 |
| Corn gluten       | 9.45   | 60.42         | 2.04          | 1.36        | 1.280 | 34.90| 5051 |
| Cora oil          |        |               |               |             |       |      | 8000 |
| Untreated Jatropha| 7.02   | 36.81         | 6.73          | 13.53       | 9.12  | 33.81| 4609 |
| Treated Jatropha meal** | 7.24 | 40.83         | 7.13          | 12.47       | 8.72  | 30.85| 4714 |

* Calculated by difference,
** Gross energy was calculated from their chemical composition using the factors 5.65, 9.45, 4.0 and 4.0 (Kcal GE/Kg DM) for crude protein, ether extract, crude fiber and nitrogen free extract, respectively (Jobling, 1983).
*** Treated Jatropha curcas meal with Lacto bacillus bacteria

Table (2): Concentration of anti-nutritional compounds in untreated and treated Jatropha curcas meal compared with soybean meal.

| Anti-nutritional compounds       | Untreated Jatropha | Treated Jatropha | Soybean Meal |
|---------------------------------|--------------------|-----------------|--------------|
| Trypsin inhibitor (mg/g)        | 23.3               | 4.2             | 23.9         |
| Lectin activity (mg/ml1)        | 51-102             | 51-102          | 90-108       |
| Phytate (g/100g)                | 6.50               | 2.75            | 1.5          |
| Saponnin %                      | 2.6                | 3.4             | 4.7          |

Four practical tilapia diets were formulated (Table 3). The control diet in which soybean meal was used at 35 % level (T1), the three tested diets in which 25, 50 and 75% of soybean meal protein was replaced by treated Jatropha meal (TJM) protein (T2, T3 and T4), respectively. The averages of all diets were maintained almost isonitrogenous (30.51% CP) and isocaloric (4568.46 Kcal GE/kg diet). The formulated diets were processed by blending the dry ingredients into a homogeneous mixture, added 10% warm water and then passing the mixed of diet through a laboratory pellet mill with diy 2mm. The pelleted diets were dried in oven at 65°C overnight. Diets were kept in black plastic bags then stored in a refrigerator at 1°C throughout the whole experimental period.
Table (3). Diets formulation and proximate analysis (%) of the experimental diets fed to Nile tilapia, (*O. niloticus*) fingerlings.

| Ingredients (%) | T1 (Control) | T2 (25% TJM) | T3 (50% TJM) | T4 (75% TJM) |
|-----------------|--------------|--------------|--------------|--------------|
| Fish meal (64.13%CP) | 10.00        | 10.00        | 10.00        | 10.00        |
| Soybean meal (44%CP) | 35.00        | 26.25        | 17.50        | 8.75         |
| Corn gluten meal | 8.00         | 8.00         | 8.00         | 8.00         |
| Wheat bran | 14.00        | 14.00        | 14.00        | 14.00        |
| Yellow corn | 27.00        | 26.32        | 25.64        | 24.96        |
| Treated Jatropha meal (40.83% CP) | - | 9.43 | 18.86 | 28.29 |
| Corn oil | 4.00         | 4.00         | 4.00         | 4.00         |
| Vit and Min. Mix| 2.00         | 2.00         | 2.00         | 2.00         |
| **Total** | 100.00       | 100.00       | 100.00       | 100.00       |

**Proximate analysis (%) on DM basis**

| Ingredients | T1 (Control) | T2 (25% TJM) | T3 (50% TJM) | T4 (75% TJM) |
|-------------|--------------|--------------|--------------|--------------|
| Dry mater | 91.30        | 92.40        | 92.10        | 92.86        |
| Crude Protein | 30.59        | 30.53        | 30.48        | 30.43        |
| Ether Extract | 6.64         | 7.11         | 6.45         | 6.32         |
| Ash | 7.72         | 7.88         | 8.30         | 9.10         |
| Crude Fiber | 11.34        | 6.59         | 9.13         | 10.15        |
| Nitrogen free extract | 43.71        | 47.89        | 45.80        | 44.00        |
| GE³ (kcal/kg) | 4543.56 | 4560.17 | 576.76 | 4593.37 |

³Vitamin and Mineral mixture/kg premix containing the following: 3300 IU vitamin A, vitamin D3, 410 IU vitamin E, 2660 mg vitamin B1, 133mg vitamin B2, 580 mg vitamin B6, 410 mg vitamin B12, 50 mg biotin , 9330 mg Coln chloride, 4000mg vitamin C, 2660 mg Inositol, 330 mg para -amino benzoic acid, 9330 mg niacin, 26.60 mg pantothenic acid. and 325 mg Manganese, 200mg Iron, 25 mg Copper, 5 mg Iodine, 5mg Cobalt.

³Calculated by difference.

³GE : gross energy calculated as 5.64, 9.44 and 4.12 Kcal/g of protein, lipid and carbohydrate, respectively (Jobling, 1983).

**Analytical methods:**

At the end of the experiment, fish in each aquarium were metered, weighed and frozen at -20°C for final body composition analysis. At the beginning, from the batch of collected fish, 30 fish were analyzed for initial carcass composition. Fish samples were minced homogenized with Ultra-Tunax. The homogenized samples were oven dried at 60 - 80°C for 48 hrs. Proximate analyses of whole body moisture, protein, fiber, lipid, and ash performed according to the methods of A.O.A.C. (2000), while nitrogen free extract (NFE %) was calculated by difference. Gross energy (Kcal GE/Kg) contents of all the samples were calculated according to Jobling (1983). Water quality parameters were analyzed according to APHA (1980).

**Measurements of growth and feed utilization:**

The total weight gain, average daily gain, and specific growth rate; feed conversion ratio protein and energy utilization were calculated as:

1- Total weight gain (g/fish) = (WF-WI)
   Where: WF, Average of final weight (g) and WI: Average of Initial weight (g)

2- ADG (Average daily gain, g/fish/day) = total gain/duration period

3- SGR (Specific growth rate, % / day) = 100 × (ln WF - ln WI) / n.
   Where: ln, Natural log and n is the duration period.

4- Feed conversion ratio (FCR) = dry matter intake (g) / total gain (g)

5- Protein productive value (PPV %) = (PT – PI) x100 / protein intake (g)
   Where: PT, Protein content in fish carcass at the end and PI, Protein content at the start.

6- Energy utilization (EU%) = (ET–EI) x100/Energy intake (kcal)
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Where: ET, Energy in fish carcass (kcal) at the end and EI, Energy in fish carcass (kcal) at the start.

**Water quality:** All values of the water quality parameters in the present experiment were in the normal range for rearing Nile tilapia; temperature (28±0.5°C), dissolved oxygen (6.3 ± 0.4 mg/l), total ammonia (0.089 ± 0.11 mg/l), nitrite (0.04± 0.01 mg l⁻¹), and pH (8.7 ± 0.12).

**Statistical methods:** The collected data were subjected to one-way analysis of variance (ANOVA) using SAS procedure (SAS, 1993). Duncan’s multiple range test (Duncan, 1955) was used to compare differences among individual means. Treatment effects considered significant at (P≤0.05).

**RESULTS**

**Chemical analysis of untreated and treated Jatropha meal:**

Feed ingredients, untreated and treated Jatropha meal and their proximate analysis presented in Table (1) showed that increasing in protein content in treated Jatropha meal (40.83%) comparable to untreated meal (36.81%). Whereas; treated Jatropha meal is similar to soybean meal in protein content; the ether extract, crude fiber and gross energy were higher in treated Jatropha meal. The proximate analysis of the tested diets fed to Nile tilapia, (O. niloticus) fingerlings (Table 3) should almost isonitrogenous, isocaloric values, 30.51% CP and 4568.46 Kcal GE/kg diet, respectively.

**Growth performance:**

The effects of replacing SBM with treated Jatropha meal (TJM) on growth performance of Nile tilapia were reported in Table (4). Growth performance measured as final weight (g/fish) and weight gain were difference significantly with T4 (P<0.05). Data in Table (3) indicated that the worst final weight, WG and SGR were recorded with fish fed (T4), which was significantly lower than the other group fed TJM, T2 and T3. However, the higher final weight, WG and SGR were recorded with fish fed T3 comparing with the other experimental groups.

**Table (4). Effect of partial replacement of SBM protein by treated Jatropha curcas meal protein on growth performance of Nile tilapia, O. niloticus.**

| Exp. Diets | Live weight (g/fish) | Weight gain (g/fish) | SGR (%/day) |
|------------|----------------------|----------------------|-------------|
|            | Initial | Final           |            |
| T1         | 31.27±0.96 | 56.75±2.66 | 25.49±1.91 | 0.850±0.04 |
| T2         | 30.43±0.87 | 56.87±2.11 | 26.43±2.48 | 0.890±0.01 |
| T3         | 30.43±0.87 | 57.65±2.39 | 27.22±1.60 | 0.911±0.03 |
| T4         | 31.57±1.73 | 51.43±2.33 | 19.86±1.27 | 0.699±0.04 |

a,b Mean bearing the same letters within each column do not differ significantly (P<0.05).

**Feed and nutrient utilization**

The effects of replacing soybean meal and corn with different levels of treated Jatropha meal on feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (%) and energy utilization (%) of O. niloticus were presented in Table (5). There were no significant differences among treatments (P>0.05) of FI respectively. The best-feed conversion ratio (FCR) was recorded with fish fed T3, T2 followed by the control diet. However, T4 group had the worth value of FCR with significant differences (P< 0.05) compared to the other treatment groups.
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Fish fed T2 and T3 diets had significant (P < 0.05) higher values of PER, PPV and EU than control diets. However, PER, PPV and EU for fish fed T4 diet recorded lower value (1.199, 19.79 and 8.79%) respectively, compared to the other treatment groups. These results indicate that the soybean meal could be replaced up to 50% by treated Jatropha meal and this has no harmful effects on growth performance and feed utilization of the Nile tilapia fingerlings.

Table (5). Effect of partial replacement of SBM protein by treated Jatropha curcas meal protein on feed and nutrient utilization parameters of Nile tilapia, O. niloticus.

| Exp. Diets | Feed utilization | Protein utilization | EU% |
|------------|------------------|---------------------|-----|
|            | Feed intake (g/fish) | FCR | PER | PPV% |       |
| T1         | 53.64 ±1.46       | 2.12b ±0.11       | 1.58a ±0.08 | 23.97±1.01 | 12.86±1.19 |
| T2         | 53.52 ±0.68       | 2.06b ±0.20       | 1.64a ±0.14 | 23.82±0.44  | 13.29±0.91  |
| T3         | 54.01±0.87        | 2.00b ±0.03       | 1.68a ±0.12 | 25.87±1.56  | 13.32±0.48  |
| T4         | 55.16 ±2.74       | 2.79a ±0.08       | 1.19b±0.04  | 19.79±0.29  | 8.79b±0.30  |

a, b Mean bearing the same letters within each column do not differ significantly (P<0.05).

Carcass composition of fish

The whole body composition of experimental fish was shown in Table (6). There were no significant differences in dry matter and crude protein of the fish. Body lipid content was significantly higher at the start of experimental. Meanwhile, the fish fed T4 diet was significantly lower in lipid contents than the other treatments. Also, energy content was significantly higher at the start of experimental than the end of experimental. Body ash content at the final fish was significantly higher than the initial fish. Fish fed T4 diet had higher ash content than all other treatments (P>0.05).

Table (6). Effect of partial replacement of SBM protein by treated Jatropha curcas meal protein on carcass composition of Nile tilapia, O. niloticus.

| Exp. Diets | Dry Matter% | CP% | EE% | Ash% | Gross energy (kcal/kg) |
|------------|-------------|-----|-----|------|------------------------|
| At the start | 23.55±2.12  | 61.66±0.85 | 19.40a±2.34 | 15.30b±1.11 | 5549.0±181.23 |
| At the end | T1          | 23.94±0.75  | 59.62±1.53 | 17.39a±0.61 | 17.03±0.08 | 5241.5±72.25 |
| T2          | 24.24±0.42  | 57.59±1.47 | 16.43a±0.62 | 16.85±0.94 | 5163.4±72.31 |
| T3          | 23.99±0.68  | 59.73±1.58 | 15.52ab±0.41 | 16.96±0.92 | 5145.5±13.37 |
| T4          | 24.58±0.37  | 59.63±0.58 | 13.49a±0.48 | 18.88±0.44 | 4956.8±30.56 |

a, b Mean bearing the same letters within each column do not differ significantly (p<0.05)

Discussion

In the present study water quality parameters were within the acceptable range for Nile tilapia growth (Stickney, 1979). Jatropha curcas seed meal fermentation has been employed to enhance the nutritional value of plant protein and reduce the ant nutritional factors Joshi et al. (2011) and Hassaan et al. (2017). The increase in crude protein content of fermented Jatropha seed meal (JSM) it may be due to the addition of amino acid bacteria during the fermentation process Kumar et al. (2010). Similar results were reported by (Belewu and Sam (2010); Jacqueline and Visser,
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(1996) when Jatropha seed meal was treated with *Aspergillus niger* and *T. longbrachitum*.

In the present study, the reduction in fibre content of treated Jatropha meal compared with untreated Jatropha meal (Table 1), the reduction was not remarkable, may be due to the secretion of various fiber enzymes during the fermentation process. These results are in agreement with Belewu *et al.* (2011) who reported a decrease in fiber content of fermented *Jatropha curcas* by fungi, which worked synergistically due to the secretion of various enzymes during the fermentation process, that degrade complex polysaccharides.

The results of the present study indicated that lactobacillus acidophilus enhanced the proximate chemical composition and the degradation of ant nutritional factor contents of Jatropha meal. These obtained results are in agreement with that maintained by (Jacqueline and Visser, 1996). They recorded the lowest phytate, trypsin inhibitor and saponin levels by the samples treated with *B. licheniformis* and *B. pumilus*, which may be due to the secretion of various enzymes such as:cellulase, xylanase, xylosidases, hemicellulase, amylases, beta glycosidase, proteinases, pectinases and alpha-galactosidase during the fermentation process by Bacillus cells. These enzymes could have contributed to the detoxification of all ant nutritional factor contents of Jatropha seed meal. The obtained data showed that Nile tilapia fed the diets with SBM replaced by treated Jatropha meal up to 50% exhibited no significant difference in the growth performance or feed utilization. However, a significant reduction in the growth performance and feed utilization was observed in Nile tilapia fed 75% of treated Jatropha meal (Table 3). In the same results obtained by, Hassan *et al.* (2017) showed that, the growth performance of Nile tilapia fed diets 75% of Jatropha meal was significantly lower than that for those fed other diets, which may be due to several factors including lower digestibility of dietary protein and/or energy, dietary ant nutritional factor contents (such as phytate). Furthermore, Gomes *et al.* (1993) reported that poor growth performance commonly obtained for fish fed plant protein-rich diets was related to the reduction in the voluntary feed intake, which consequently reduces the intake of dietary essential nutrients and digestible energy.

These results may suggest that the growth performance and feed utilization of Nile tilapia could be successful when using moderate inclusion level of fermented Jatropha meal.

**Economic evaluation:**
Calculation of economical efficiency of the tested diets based on the cost of feed and cost of one kg gain in weight of Nile tilapia, its ratio with the control group, are shown in Table (7). Feed costs and cost per kg gain (L.E) were the highest for the control diet (15.83L.E) and gradually decreased with the increasing levels of treated Jatropha meal protein instead of SBM protein until 50% replacement. At the 50 % level of treated Jatropha meal, Nile tilapia could be produced cheaper than fish fed on the control diet. The relative percentages of feed cost/ kg fish were 95.35, 90.70, and 86.07% for diets 2, 3, and 4, respectively compared to control. Moreover, feed cost/ kg gain was 14.66, 13.54, and 17.93 (L.E) for diets 2, 3, and 4, respectively. These results indicated that the effect of replacement different levels of treated Jatropha meal instead of SBM improved growth and feed utilization parameters of Nile tilapia. On the other hand, the incorporation of treated Jatropha meal in Nile tilapia diets seemed to be economic at incorporation level till 50% but increasing its level up to 75 %, increased percentage.
change in feed cost to produce one kg fish gain by 113.27% compared to the control. The feed cost/Kg weight gain decreased with the increasing incorporation levels of 50% Jatropha meal instead of SBM protein for Nile tilapia diets as cited by Gaber (2006) and Soltan (2005 a,b).

Table (7). Cost of feeds required for producing one Kg gain of *O. niloticus* fingerlings fed the experimental die.

| Item                              | T1   | T2   | T3   | T4   |
|-----------------------------------|------|------|------|------|
| Cost/ton feed (L.E)               | 7465 | 7118 | 6771 | 6425 |
| Change in feed cost               | 100  | 95.35| 90.70| 86.07|
| Feed intake per Kg gain (FCR)     | 2.12 | 2.06 | 2.00 | 2.79 |
| 1Kg fish gain (L.E)/Feed cost     | 15.83| 14.66| 13.54| 17.93|
| Percentage change in feed cost to produce one kg fish gain | 100  | 92.61| 85.53| 113.27|

Local market price (L.E/ton) for feed ingredients used for formulating the experimental diets at the year (2015); 5200 L.E, soybean meal = 2500 L.E; fish meal= 2500 L.E.; yellow corn= 3500 L.E; wheat bran = 3000 L.E; corn gluten= 11000 L.E; corn oil = 10000 L.E; Jatropha meal = 1400 L.E and vitamin and minerals mix = 2 5000 L.E, Di calcium phosphate = 10000 L.E.

Conclusion:

It could be concluded that *Jatropha curcas* meal treated with *lactobacillus acidophilus* could be useful approach to improve the chemical composition and degrade the anti nutritional factors of Jatropha meal. Therefore, it could be used as fish feed and replace up to 50% SBM protein in Nile tilapia diet, without any adverse effect on the growth performance, and economical efficiency. Consequently, we recommend using treated *Jatropha curcas* meal in formulation of aquafeeds.

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