

Original Article

Toasted *Jatropha curcas* seed meal in Nile tilapia (*Oreochromis niloticus*) diet: Effect on growth, economic performance, haematology, serum biochemistry and liver histology

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Abstract: The effect of dietary inclusion of differently timed dry heat-treated *Jatropha curcas* on the growth and economic performance of Nile tilapia, *Oreochromis niloticus*, was evaluated in a 56-day feeding trial. Five isonitrogenous and isolipidic dietary treatments (35% crude protein and 10% crude lipid) were made consisting of soybean meal (control) which was replaced by *J. curcas* seed meal toasted either 5 min or 10 min at 20 and 40% to make other four test diets. A total of 225 juveniles of *O. niloticus* were acclimatized for a week, weighed and allotted into five dietary treatments. Each treatment was replicated three times with fifteen fish per replicate. Fish were fed 5% body weight on two equal proportions per day for 56 days. Growth data were collected at two-week intervals. The results from the study indicated that there was significant difference ($P < 0.05$) in the growth and economic performance parameters among the fish exposed to different dietary treatments. However, there was no significant variations ($P > 0.05$) in the different growth and economic performance parameters of fish fed CTR and fish fed D520T (5 min toasted, 20%). There was significant reduction in haematological and biochemical parameters of the blood of *O. niloticus* fed the different dietary treatments containing *J. curcas* seed meal. Based on economic and physiologic performance, soybean meal in Nile tilapia diet could be replaced up to 40% by 5-minute toasted *J. curcas* seed meal.

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Introduction

Protein feedstuffs such as fish meal used in fish feed formulation in developing countries are scarce, competitive and their cost is usually beyond the reach of fish farmers and producers of aqua feed (Fasakin and Balogun, 1996). Legume seeds and oilseed cake are known to be good alternative dietary protein sources for fish feed and are available in sub-saharan Africa on a large scale (Fagbenro et al., 2003). Soybean meal is a conventional plant protein source in fish feeds that has the capability to replace up to 50% of the fish meal (Storebakken, 2000). However, its sustainable use as fishmeal replacer is threatened because of the various uses to which soybean is put as feed for livestock and also consumed by human. This consequently led to its hike in price (Azaza et al., 2009). There is, therefore, need to look for cheaper plant protein source that can replace soybean meal in

fish diets (Yue and Zhou, 2008) so that sustainable aquaculture development could be witnessed from constant supply of cheaper, lesser used and available plant protein feed ingredients with nutritional profile similar to or better than soybean meal especially at village farm level where farmers can harness natural gift to the maximal. More so that secondary metabolites in plant sources such as tannins, alkaloids, polypeptides, saponin, volatile oils have been reported to have antimicrobial, anti-stress and disease resistant generally initiating immunomodulatory activity in fish (Hoseinifar et al., 2020). The review of Hoseinifar et al. (2020) detailed mechanisms and strategies of boosting immunity in fish using plant derived natural products. The natural products derived from plants, are capable of stimulating or suppressing the components of both adaptive and innate immune systems in such a way to provide prophylactic

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Table 1. Proximate composition of some feed ingredients.

| Parameter | Fish meal | Soybean Meal | 5-Min Toasted JCM | 10-min Toasted JCM** | Maize |
|---------------|-----------|--------------|-------------------|----------------------|-------|
| Moisture | 9.75 | 10.70 | 3.80 | 3.80 | 10.48 |
| Crude Protein | 72.4 | 38.74 | 33.96 | 34.72 | 9.87 |
| Crude Lipid | 10.45 | 30.68 | 39.03 | 39.43 | 4.28 |
| Crude Fibre | - | 5.10 | 13.11 | 10.16 | 5.78 |
| Ash | 8.32 | 4.48 | 6.09 | 4.25 | 6.73 |
| *NFE | - | 10.30 | 4.01 | 7.64 | 62.35 |

*NFE = Nitrogen free extract; **JCM = *Jatropha curcas* meal

measures rather than therapeutic measures against diseases (Hoseinifar et al., 2018).

Jatropha curcas grows very well in the tropical regions and it is well-distributed in Nigeria. Its seed meal has nutrient density that is comparable to any other protein sources. It has a good amino acid profile with its essential amino acids level (except lysine) higher than soybean meal (Kumar et al., 2011). Studies on the use of *Jatropha* seed meal are found in Kumar et al. (2008) on rainbow trout diets; Kumar et al. (2011) on carp diet; Fakunle et al. (2013) and Alatise et al. (2014) on *Clarias gariepinus* diet. There is paucity of information on the use of the seed meal as dietary protein source in Nile Tilapia diets, especially reports on the use of dry heat-treatment to reduce anti-nutritional factor in the seed meal and economic analysis of feeding the diets containing *J. curcas* seed meal in Nile Tilapia diets are scarce. Thus, this study examined the effect of dietary inclusion of graded level of differently timed dry heat-treated *J. curcas* seed meal as protein source on the growth, haematology, serum biochemistry, liver histology and economic performance of Nile tilapia (*Oreochromis niloticus*) juveniles.

Materials and Methods

Collection and processing of *J. curcas* seed: Three kilograms of mature *J. curcas* seed were sourced from neighboring village within Ilora, Oyo town, Nigeria. Before the commencement of the experiment, the *J. curcas* were decoated to obtain the seeds. The seeds were divided into two parts, the first part was roasted for 5 min and the second part was roasted for 10 min to eliminate the possible effect of anti-nutritional factor that could be present in the kernel. These seed meal and some other basic feed ingredients were then taken to the laboratory for proximate composition

analyses following the procedure of (AOAC, 2010).

Diet formulation and preparation: Based on the proximate composition of the basic feed ingredients in Table 1, five experimental diets were formulated consisting of a control diet which is made up of soybean meal and 4 test diets replacing soybean meal at a rate of 20 and 40% by 5-min and 10-min toasted *J. curcas* seed meal. The ingredients were mixed together in the laboratory using hand after which vitamin premix and fish oil were added to the dry ingredient and mixed thoroughly. Warm water was added to these ingredients and mixed until a dough-like paste was formed. The dough was passed through an improvised pelleting machine to produce 2 mm pellet size. The pellets were sun-dried and preserved in air tight containers. The gross composition of the experimental diets is given in Table 2. Table 2 reveals the proximate composition, calculated amino acid and fatty acid profile of the experimental diets. The optimal dietary protein and lipid requirement of tilapia were met by the prepared diets (Luquet, 2017).

Experimental design: A completely randomized design was adopted with three replicates per treatment. 225 juveniles of *O. niloticus*, obtained from Masopa fish farm along Alakia road Ibadan Oyo state, Nigeria, were equally randomly distributed into fish tank and grouped fed. The fish were allowed to acclimatize for 15 days while being fed on commercial diet. Prior to the commencement of the feeding trial, all fish were starved for 24 hours. This practice was to prepare the gastrointestinal tract for the experimental diet while at the same time to increase the appetite of the fish.

Feeding trial: The feeding trial was conducted in the wet laboratory of Federal College of Animal Health and Production Technology, Ibadan. The experimental system contained a set of 15 rectangular

Table 2. Nutrient composition (g/100 g) of experimental diets containing - differently timed dry heat-treated *Jatropha curcas* seed meal fed to *Oreochromis niloticus*.

| Ingredients | CTR | D520T | D540T | D1020T | D1040T |
|---|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| Fishmeal | 20.83 | 20.83 | 20.83 | 20.83 | 20.83 |
| Soybean Meal | 42.10 | 33.68 | 25.62 | 33.68 | 25.62 |
| 5-Min Toasted | - | 9.42 | 18.85 | - | - |
| 10-Min Toasted | - | - | - | 9.21 | 18.43 |
| Corn | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Fish Premix | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Fish Oil | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Starch | 19.57 | 18.59 | 17.20 | 18.78 | 17.62 |
| <u>Proximate Composition</u> | | | | | |
| Moisture | 9.95±0.17 ^a | 10.06±0.12 ^a | 9.70±0.17 ^a | 10.16±0.16 ^a | 9.94±0.25 ^a |
| Crude Protein | 35.17±0.05 ^a | 35.18±0.08 ^a | 35.18±0.01 ^a | 35.17±0.01 ^a | 35.16±0.01 ^a |
| Crude Lipid | 10.49±0.24 ^a | 10.66±0.31 ^a | 10.38±0.06 ^a | 10.38±0.01 ^a | 10.38±0.01 ^a |
| Crude Fibre | 11.92±0.14 ^c | 12.40±0.49 ^{bc} | 12.60±0.11 ^b | 13.16±0.16 ^a | 13.27±0.40 ^a |
| Ash | 6.44±0.01 ^b | 6.50±0.03 ^{ab} | 6.52±0.03 ^a | 6.37±0.06 ^c | 6.19±0.02 ^d |
| NFE | 26.04±0.1 ^a | 25.20±0.39 ^{bc} | 25.62±0.25 ^{ab} | 24.75±0.35 ^c | 25.06±0.33 ^{bc} |
| Energy | 4.00±0.03 ^a | 4.02±0.03 ^{ab} | 4.01±0.01 ^{ab} | 3.97±0.02 ^b | 3.99±0.01 ^{bc} |
| <u>Calculated Amino Acid Profile</u> | | | | | |
| Arginine% | 2.5 | 2.3 | 2.1 | 2.3 | 2.1 |
| Histidine% | 0.8 | 0.8 | 0.7 | 0.8 | 0.7 |
| Isoleucine% | 1.6 | 1.4 | 1.3 | 1.4 | 1.3 |
| Leucine% | 2.6 | 2.3 | 2.1 | 2.3 | 2.1 |
| Lysine% | 2.4 | 2.2 | 2.0 | 2.2 | 2.0 |
| Methionine% | 0.7 | 0.7 | 0.6 | 0.7 | 0.6 |
| M+C% | 1.2 | 1.1 | 1.0 | 1.1 | 1.0 |
| Phenylalanine% | 1.5 | 1.3 | 1.2 | 1.3 | 1.2 |
| P+T% | 2.6 | 2.3 | 2.1 | 2.3 | 2.1 |
| Threonine% | 1.5 | 1.4 | 1.3 | 1.4 | 1.3 |
| Tryptophan% | 0.4 | 0.4 | 0.3 | 0.4 | 0.3 |
| Valine% | 1.7 | 1.5 | 1.4 | 1.5 | 1.4 |
| <u>Calculated Fatty Acid Profile</u> | | | | | |
| LOA (18:2n-6)% | 5.1 | 4.2 | 3.3 | 4.2 | 3.3 |
| LNA (18:3n-3)% | 0.8 | 0.6 | 0.5 | 0.6 | 0.5 |
| ARA (20:4n-6)% | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| EPA (20:5n-3)% | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| DHA (22:6n-3)% | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| Total n-3% | 1.8 | 1.7 | 1.6 | 1.7 | 1.6 |
| Total n-6% | 5.2 | 4.2 | 3.4 | 4.2 | 3.4 |
| n3:n6 | 0.4 | 0.4 | 0.5 | 0.4 | 0.5 |

LOA: Linoleic Acid; LNA: Linolenic Acid; ARA: Arachidonic Acid; EPA: Eicosapentanoic Acid; DHA Docosahexanoic Acid; Fatty acid and amino acid profiles of the experimental diets were calculated using a program (excel) developed by NACA (2008) Specification: each kg contains: Vitamin A , 4,000,000 IU; Vitamin B, 800,000 IU; Vitamin E, 40,000 IU, Vitamin K₃, 1,600 mg; Vitamin B₁, 4,000 mg; Vitamin B₂, 3,000 mg; Vitamin B₆, 3,800 mg, Vitamin B₁₂ 3 mcg; Nicotinic Acid 18000 mg. Pantothenic Acid 8,000 mg; Folic Acid 800 mg Biotin, 100 mcg Choline Chloride 120,000 mg; Iron 8,000 mg; Copper 800 mg; Manganese,6,000 mg; Zinc 8,000 mg; Iodine 400 mg; Selenium, 40 mcg, vit C Coated 60,000 mg, Inositol 10,000 mg; Cobalt,150 m, Lysine 10,000 mg; Methionine 10,000 mg, Antioxidant 25,000 mg manufactured by Bi-mix Brand, Corporate head office/factory: 1, Odo-Olowu Street, Ijesatedo, Lagos, Nigeria.

plastic tanks each with a capacity of 55 liters of water. 225 *O. niloticus* juveniles with initial weight of 15.19±0.02 g was used for the experiment. All fish were fed twice daily at a fixed feeding rate of 5% of their body weight per day. Feeding was generally done

in the morning at 09:00 a.m. and 05:00 p.m. in the evening. Periodic weighing was done at two weeks interval and the feed adjusted as required. The procedures of Jimoh and Aroyehun (2011) was used in calculating growth performance indices. A

Table 3. Growth performance of *Oreochromis niloticus* fed diets containing graded levels of differently timed dry heat-treated *Jatropha curcass* seed meal.

| Parameters | CTR | D520T | D540T | D1020T | D1040T |
|------------------------------|-------------------------|--------------------------|-------------------------|--------------------------|-------------------------|
| Initial Weight (g) | 15.19±0.02 ^a | 15.14±0.01 ^a | 15.17±0.01 ^a | 15.15±0.01 ^a | 15.19±0.01 ^a |
| Final Weight (g) | 21.71±0.45 ^a | 21.08±0.17 ^{ab} | 20.77±0.65 ^b | 20.39±0.54 ^{bc} | 19.75±0.37 ^c |
| ¹ Weight Gain (g) | 6.52±0.45 ^a | 5.94±0.16 ^{ab} | 5.60±0.65 ^b | 5.24±0.54 ^{bc} | 4.56±0.37 ^c |
| ² % WG | 42.92±2.97 ^a | 39.23±1.01 ^{ab} | 36.92±4.30 ^b | 34.59±3.44 ^{bc} | 30.02±2.44 ^c |
| ³ SGR (%/day) | 0.64±0.36 ^a | 0.59±0.02 ^{ab} | 0.56±0.06 ^b | 0.53±0.05 ^{bc} | 0.47±0.03 ^c |
| Food Fed | 7.71±0.54 | 7.05±0.13 | 6.77±0.67 | 6.52±0.02 | 5.87±0.53 |
| ⁴ FCR | 1.18±0.25 ^c | 1.19±0.02 ^c | 1.21±0.0 ^{bc} | 1.24±0.02 ^b | 1.29±0.02 ^a |
| Protein Fed | 2.70±0.19 | 1.21±0.02 | 2.37±0.24 | 2.28±0.26 | 2.05±0.19 |
| ⁵ PER | 2.41±0.05 ^a | 2.41±0.05 ^a | 2.36±0.04 ^{ab} | 2.30±0.04 ^b | 2.22±0.04 ^c |
| ⁶ Survival (%) | 88.00±1.73 | 91.00±10.15 | 93.33±6.51 | 82.33±4.04 | 83.33±15.28 |

Row means with different superscripts are significantly different ($P<0.05$) from each other. ¹Mean weight gain= final mean weight – initial mean weight; ²Percentage weight gain= [final weight-initial weight/initial weight] X 100; ³ Specific growth rate= [ln final weight- ln initial weight] X 100; ⁴ Feed conversion ratio=dry weight of feed fed /Weight gain (g); ⁵ Protein efficiency ratio=fish body weight (g)/ Protein fed; ⁶ Percentage survival = {(total number of fish- mortality)/ total number of fish] X 100.

Table 4. Cost of production (N) of the experimental diets containing differently timed dry heat-treated *Jatropha curcass* seed meal fed to *Oreochromis niloticus*.

| | Price/kg | CTR | D520T | D540T | D1020T | D1040T |
|----------------|----------|---------------|---------------|---------------|---------------|---------------|
| Fishmeal | 680 | 141.64 | 141.64 | 141.64 | 141.64 | 141.64 |
| Soybean | 155 | 65.26 | 52.20 | 39.71 | 52.20 | 39.71 |
| 5-min toasted | 68 | - | 6.41 | 12.82 | - | - |
| 10-min toasted | 68 | - | - | - | 6.26 | 12.53 |
| Cornmeal | 72 | 7.20 | 7.20 | 7.20 | 7.20 | 7.20 |
| Fish Premix | 700 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 |
| Fish oil | 600 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Starch | 50 | 9.78 | 9.29 | 8.60 | 9.39 | 8.81 |
| Total | | 273.88 | 266.74 | 259.97 | 266.70 | 259.90 |

combined digital dissolved oxygen meter (YSI Model 57, Yellow Spring Ohio) was used to measure water temperature and dissolved oxygen; pH meter (Mettler Toledo – 320, Jenway UK) was used to monitor pH weekly. Bioeconomic analysis was done following the methods explained in Faturoti (1989), Abu et al. (2010), Boateng et al. (2013) and Jimoh et al. (2015b); Straight line method of depreciation was used to evaluate the cost of Aquaria tanks with the following properties.

| | |
|-------------------------|-------------------|
| Cost of plastic tanks | ₦57,000 |
| No of years (life Span) | 5 yrs. |
| Savage value | 10% of Cost price |

Histology, haematological, and serum biochemistry analysis were done following the methods explained in Jimoh et al. (2015c), Fagbenro et al. (2013) and Jimoh et al. (2015a), respectively.

Statistical analysis: Data obtained from the experiment was expressed in mean±SD and it was subjected to one-way Analysis of Variance (ANOVA)

using SPSS 16.0 version. Where the ANOVA reveals significant difference ($P<0.05$). Duncan multiple range test was used to compare differences among individual treatment means.

Results

Growth Performance: Table 3 reveals the growth performance of *O. niloticus* fed diets containing graded levels of differently timed dry heat-treated *J. curcass* seed meal. The weight gain of fish fed CTR was the highest which was significantly different ($P<0.05$) from the weight gain of fish fed other dietary treatments except the fish fed D520T. The lowest weight gain was observed among fish fed D1040T which was not significantly different ($P>0.05$) from the weight gain of fish fed D1020T. No significant variation ($P>0.05$) existed among the weight gain of fish fed D520T, D540T and D1020T.

Similarly, trends of the results as observed for weight gain was also as observed for final weight,

Table 5. Economic analysis of producing *Oreochromis niloticus* with diets containing differently timed dry heat-treated *Jatropha curcas* Seed meal.

| Parameters | CTR | D520T | D540T | D1020T | D1040T |
|--|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| Output biomass (kg) | 21.71±0.45 ^a | 20.39±0.57 ^{ab} | 19.75±0.37 ^b | 21.08±0.17 ^{bc} | 20.77±0.65 ^c |
| Cost of feeding (x10 ³ ₦) | 2.11±0.15 ^a | 1.88±0.036 ^{ab} | 1.76±0.17 ^{bc} | 1.74±0.0.20 ^{bc} | 1.53±0.00 ^c |
| Incidence of cost (₦) | 324.10±6.89 ^{ab} | 316.53±4.08 ^b | 314.57±5.20 ^b | 331.60±5.55 ^a | 334.40±5.41 ^a |
| Value of fish (₦) | 3.91±0.27 ^a | 3.57±0.96 ^{ab} | 3.36±0.39 ^b | 3.14±3.15 ^{bc} | 2.73±0.22 ^c |
| Profit index (₦) | 1.85±0.40 ^{ab} | 1.89±0.25 ^a | 1.91±0.30 ^a | 1.81±0.26 ^{bc} | 1.79±0.31 ^c |
| Gross profit (₦) | 275.90±6.89 ^{ab} | 283.47±4.10 ^a | 285.43±5.20 ^a | 268.40±5.55 ^b | 265.60±5.41 ^b |
| Total variable cost (x10 ³ ₦) | 2.34±0.15 ^a | 1.96±0.20 ^{ab} | 1.75±0.14 ^b | 2.10±0.34 ^{bc} | 1.98±0.18 ^c |
| *Total fixed cost (x10 ³ ₦) | 1.71 | 1.71 | 1.71 | 1.71 | 1.71 |
| Total cost (x10 ³ ₦) | 4.05±0.15 ^a | 3.67±0.20 ^{ab} | 3.46±0.14 ^{bc} | 3.81±0.35 ^{bc} | 3.69±0.18 ^c |
| Total revenue (x10 ³ ₦) | 13.03±0.27 ^a | 12.23±0.32 ^{ab} | 11.85±0.22 ^b | 12.65±0.10 ^{bc} | 11.33±0.58 ^c |
| Gross margin (x10 ³ ₦) | 10.80±0.42 | 10.40±0.23 | 10.69±0.41 | 10.60±0.00 | 10.40±1.58 |
| Net return (x10 ³ ₦) | 8.98±0.13 ^a | 8.56±0.12 ^a | 8.39±0.09 ^{ab} | 8.83±0.07 ^{bc} | 8.77±0.21 ^c |
| Cost of Juveniles: ₦15 | 1kg Tilapia=₦600 | | 1 USD=₦365 | | |
| Cost of Aquaria tank | 57,000 | | | | |
| Less 10% Savage Value | 5,700 | | | | |
| Depreciation | 10,260 | | | | |
| Value of Aquaria Tank (2 months) | 1,710 | | | | |

Table 6. Profitability analysis of producing *Oreochromis niloticus* with diets containing differently timed dry heat-treated *Jatropha curcas* seed meal.

| Parameters | CTR | D520T | D540T | D1020T | D1040T |
|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| Benefit Cost Ratio | 3.22±0.60 ^b | 3.32±0.15 ^{ab} | 3.38±0.51 ^a | 3.33±0.95 ^{ab} | 3.43±0.70 ^c |
| Gross Ratio | 0.31±0.10 ^a | 0.30±0.00 ^{ab} | 0.29±0.06 ^b | 0.30±0.10 ^{ab} | 0.29±0.06 ^b |
| Expense Structure Ratio | 0.42±0.15 ^c | 0.45±0.00 ^{bc} | 0.46±0.21 ^{ab} | 0.46±0.25 ^{ab} | 0.50±0.25 ^a |
| Rate of Return | 2.22±0.60 ^b | 2.32±0.15 ^{ab} | 2.38±0.51 ^a | 2.33±0.95 ^{ab} | 2.43±0.70 ^a |

Row means with different superscripts are significantly different ($P<0.05$) from each other

%weight gain and SGR, and a reverse trend was also observed for FCR. The PER of fish exposed to diet CTR was the highest which was not significantly different ($P>0.05$) from the PER of fish fed D520T and D540T. The PER of fish exposed to diet D1040T was significantly the lowest among other treatments. There was no significant difference ($P>0.05$) in the PER of fish fed diet D540T and diet D1020T.

Economic Analysis

Cost of production of 1 kg diet: The cost (N) of producing 1 kg of each experimental diet containing differently timed dry heat-treated *J. curcas* fed to *O. niloticus* is presented in Table 4. There was reduction trend in the cost of producing the diet with increasing inclusion of *J. curcas* when compared with control.

Table 5 shows the economic analysis of producing *O. niloticus* with diets containing differently timed dry heat-treated *J. curcas* seed meal. The incidence of cost,

which is the cost of producing 1 kg Tilapia fish, was highest using diet D1040T and lowest using diet D520T. There existed significant variation ($P<0.05$) in the incidence of the cost of the fish produced using different dietary treatments. However, there was no significant difference ($P>0.05$) in the incidence of the cost of producing 1 kg tilapia fish with diet D1040T, D1020T, and CTR. The profit index and gross profit of the produced with D540T was the highest which is not significantly different ($P>0.05$) from profit index and gross profit of fish produced by using diet CTR and D520T. Table 6 shows the profitability analysis of producing *O. niloticus* with diets containing differently timed dry heat-treated *J. curcas* seed meal. Benefit cost ratio (BCR) of producing fish with D540T was the highest which is not significantly different ($P>0.05$) from the BCR of fish produced with other dietary treatments except D520T and D1020T. BCR of fish produced by using diet D1040T was the

Table 7. Haematological and biochemical response in *Oreochromis niloticus* juveniles fed diets containing differently timed dry-heat-treated *Jatropha curcas* seed meal.

| Parameters | CTR | D5020T | D5040T | D1020T | D1040T |
|----------------------------|--------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| PCV (%) | 17.33±0.58 ^a | 16.87±0.15 ^{ab} | 16.53±0.05 ^b | 15.73±0.44 ^c | 15.13±0.06 ^c |
| Hb (g/dl) | 9.60±0.26 ^a | 9.20±0.36 ^{ab} | 9.10±0.36 ^{ab} | 8.70±0.20 ^{bc} | 8.17±0.31 ^c |
| RBC (x10 ¹² mm) | 1.38±0.02 ^a | 1.36±0.04 ^a | 1.32±0.03 ^{ab} | 1.29±0.03 ^b | 1.26±0.06 ^b |
| WBC (x10 ⁶ mm) | 86.0±4.58 ^a | 83.67±2.51 ^a | 84.67±3.80 ^a | 87.00±6.60 ^a | 88.67±9.02 ^a |
| MCH(pg) | 69.40±1.31 ^a | 67.63±1.40 ^a | 68.90±1.30 ^a | 67.63±1.80 ^a | 64.70±0.60 ^b |
| MCHC (g/dl) | 5.53±0.15 ^a | 5.43±0.25 ^a | 5.50±0.30 ^a | 5.53±0.06 ^a | 5.40±0.20 ^a |
| MCV (fl) | 125.30±2.90 ^a | 124.10±4.40 ^a | 125.23±3.80 ^a | 122.23±3.82 ^a | 119.93±5.60 ^a |
| Total Cholesterol | 141.6±16.80 ^a | 125.67±7.77 ^{ab} | 113.00±3.00 ^{bc} | 108.33±8.51 ^{bc} | 100.00±9.85 ^c |
| Total Protein(g/dl) | 7.53±0.31 ^a | 6.70±0.20 ^b | 5.80±0.36 ^c | 5.60±0.20 ^c | 4.90±0.20 ^d |
| Albumin (mg/dl) | 5.13±0.21 ^a | 4.37±0.06 ^b | 3.73±0.21 ^c | 3.73±0.15 ^c | 3.50±0.27 ^c |
| Globulin (mg/dl) | 2.40±0.17 ^a | 2.33±0.25 ^{ab} | 2.07±0.15 ^{bc} | 1.87±0.06 ^c | 1.40±0.10 ^d |
| Glucose(mg/dl) | 84.33±2.52 ^d | 88.67±2.08 ^{cd} | 91.67±2.52 ^{bc} | 95.33±3.79 ^b | 102.00±3.00 ^a |

Row means with different superscripts are significantly different ($P<0.05$).

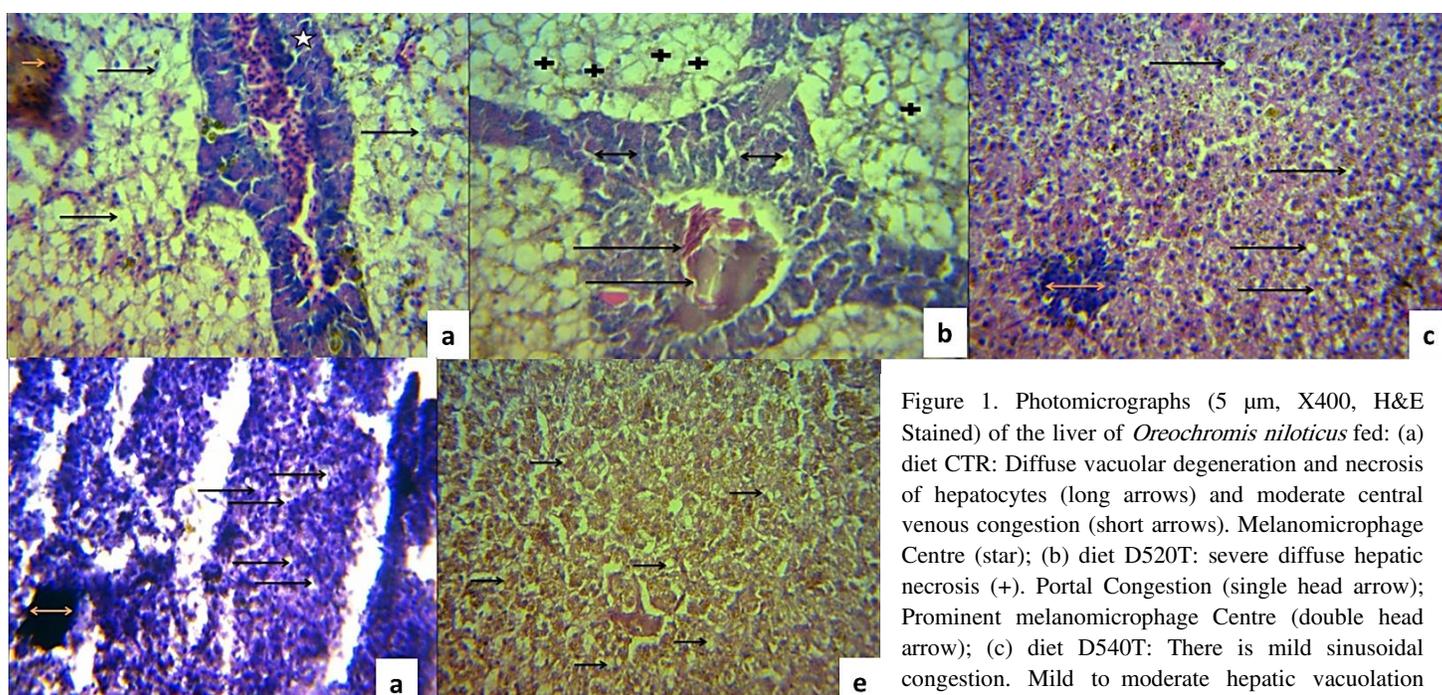


Figure 1. Photomicrographs (5 μ m, X400, H&E Stained) of the liver of *Oreochromis niloticus* fed: (a) diet CTR: Diffuse vacuolar degeneration and necrosis of hepatocytes (long arrows) and moderate central venous congestion (short arrows). Melanomicrophage Centre (star); (b) diet D520T: severe diffuse hepatic necrosis (+). Portal Congestion (single head arrow); Prominent melanomicrophage Centre (double head arrow); (c) diet D540T: There is mild sinusoidal congestion. Mild to moderate hepatic vacuolation

(single head arrow). The melanomicrophage centre is not very prominent(double head arrow); (d) diet D1020T: There is very mild vacuolation of hepatocyte (single head arrow) and the melanomicrophage centre is not very prominent(double head arrow); (e) diet D1040T: There is mild to moderate diffuse vacuolar degeneration and necrosis of hepatocytes (arrow).

lowest.

Rate of return of producing Nile tilapia diets with different dietary treatments follow the same trends of results as accounted for BCR. Gross ratio (GR) of fish with CTR was the highest but it was not significantly different from the GR of fish produced by using diets D520T and D1020T. The expense structure ratio (ESR) followed reverse trends of results.

Haematology and serum biochemistry: Table 7 shows the haematological and serum biochemical changes in the blood of *O. niloticus* fed diets containing

differently timed dry-heat-treated *J. curcas* seed meal. The haemoglobin content of the blood of fish fed CTR was the highest while the lowest haemoglobin content was found in D1040T. There was significant difference ($P<0.05$) in the haemoglobin content of the blood of fish fed various dietary treatments. However, there was no significant variation ($P>0.05$) in the haemoglobin content of the blood of fish fed diets CTR and D520T. There was significant difference ($P<0.05$) in the total cholesterol of the blood of fish exposed to different dietary treatments. Fish fed diet

CTR had the highest blood total cholesterol while fish fed diet D1040T had the lowest. However, there was no significant difference ($P>0.05$) in the blood total cholesterol of fish fed diets CTR and D520T.

Histology: Figure 1 shows the photomicrographs (5 μm , X400, H&E Stained) of the liver of *O. niloticus* fed diets containing differently timed dry-heat-treated *J. curcas* seed meal. Diffuse vacuolar degeneration, necrosis of hepatocytes and moderate central venous congestion were recorded in the liver of fish fed control diet. Severe diffuse hepatic necrosis cum prominent melanomicrophage centre were observed in the liver of fish fed D520T. Mild to moderate hepatic vacuolation were observed in the livers of fish fed diet D540T; D1020T and D1040T: There is mild to moderate diffuse vacuolar degeneration and necrosis of hepatocytes (arrow)

Discussions

Based on the results, processing time and levels of inclusion of *J. curcas* had impact on the growth performance of Nile tilapia. The growth performance of tilapia fed diets containing lower timed heat-treated *J. curcas* were comparable to that of control even at higher replacement level with soybean using FCR and PER as indices of assessment. These results are in consonance with the work of Workagegn et al. (2013) for Nile Tilapia juveniles fed the same seed meal replacing soybean meal that the growth performance of heat-treated *J. carcass* kernel meal was comparable to that of control at 10% replacement level. The results, however, differ from what Kumar et al. (2011), who reported that inclusion of cooked *Jatropha* seed meal in the diet of carp was possible at levels higher than 50% in the diet of fish without compromising their growth performance. Plausible reasons might be the different heat treatments applied more so that the seed meal was completely detoxified of the phorbol esters (PEs) which is a toxic compound in the seed meal (Kumar et al., 2011). Makkar et al. (2012) reported that detoxified *J. carcass* seed meal could replace more than 50% of fishmeal in the fish diets. Although Davies and Gouveia (2008) reported that thermal processing of raw pea seed meal, dry heat

treatment in particular (180°C:30 min), led to a greatly improved feed utilization and consequent better growth performances; various factors could imply the relatively poor growth performance recorded at higher processing time and inclusion level recorded in this study, one of which could be incomplete inactivation of the anti-nutrients in the seed meal, the principal of it being PEs by the heat treatment applied which consequently led to poor digestibility of protein (Kumar et al., 2008). Kumar et al. (2011) reported that antinutrients in *J. curcass* seed meal especially the major toxic components called phorbol esters restricts its use in fish feed. Lower growth observed among the group fed test dietary treatments might not be unconnected to reduced feed consumption recorded among the test dietary treatment group relative to control group. Similar observation to what was observed in this study was made by Paray et al. (2020) when *Cyprinus carpio* was fed oak (*Quercus castaneifolia*) leaf extract.

This incidence of cost analysis of feeding *J. curcas* seed meal to *O. niloticus* showed the impact of the cost of feed on the variable cost of fish production since maintenance and sustenance of aquaculture depends on its economic viability and relative profitability (Adeparusi and Balogun, 1999). A profit index above 1 showed that it is profitable to feed the fish with the diet (Jimoh et al., 2013). Gross profit remains the primary interest on most capital investment, the gross profit margin and loan repayments where applicable form the basis after operational evaluation (Faturoti, 1989). Gross margin was reported to be a good measure of profitability (Olagunju et al., 2007). The experiment showed that it is profitable to replace soybean meal with differently timed dry heat-treated *J. curcas* seedmeal using gross profit as an index of assessment. This result agrees with the finding of Fagbenro et al. (2001), Abu et al. (2010) and Jimoh et al. (2012) that feeding fish with cheaper and lesser known feed ingredients left some profit margin. Although the economic implication of using the different dietary treatments might not be well appreciated since the margin might be too small, it will be much explicit when the magnitude of total cost and

expected revenue of its large-scale operation is critically and objectively considered (Faturoti, 1989). Adeparusi and Balogun (1999) reported profit margin as increasing when fish meal was replaced by roasted pigeon pea meal in a diet fed to *C. gariepinus*. Jimoh (2004) also reported an increase in profit margin in the production of Tilapia up to 30% of soybean meal with jackbean meal. The reduced gross profit on tilapia produced by diet D1040T was due to lower growth rate of growth of fish. This result is in agreement with the report of Jimoh (2004) that profit margin reduced with jackbean replacement level beyond 30%. This implies that feeding fish with differently timed dry heat-treated *J. curcas* seed meal had minimum impact on variable cost of production hence the profit recorded.

The results showed that the average total variable and total cost of producing *O. niloticus* with the diets was met or covered by the total revenue realized from the sale tilapia and left a positive gross margin and net returns in all the dietary treatment groups. Positive gross margin and net returns indicate that it is profitable to feed *O. niloticus* differently timed dry heat-treated *J. curcas* seed meal. A result similar to what was reported in Jimoh et al. (2015b) when soybean meal was replaced by watermelon seed meal. (Boateng et al., 2013) reported a positive operating profit when all variable cost of production of all male tilapia was covered by the gross revenue as an indicator of profitability of all male tilapia aquaculture enterprise in Ghana. Jimoh et al. (2014) reported similar trends of results on incidence of cost analysis when *C. gariepinus* was fed diets containing *Chrysophyllum albidum* seed meal. The ESR value reported in this study is lower than the value reported by Adebayo and Daramola (2013). The rate of return recorded in this study is higher than what Boateng et al. (2013) reported on return on investment for all male tilapia farming in Ghana. Adebayo and Daramola (2013) reported ROR value of 0.62 for catfish production.

The values recorded for some haematological parameters of the blood of Nile tilapia fed diets containing differently timed dry-heat-treated *J. curcas*

seed meal fell within the range of normal haematology of a healthy fish (Fagbenro et al., 1993). The observed reduction in haematological parameters of the blood of Nile tilapia fed diets containing differently timed dry-heat-treated *J. curcas* seed meal in this study might not be unconnected to the presence of anti-nutrients present in the seed meal. Increase in white blood cell as observed in the fish fed differently timed dry-heat-treated *J. curcas* seed-meal based diets is attributed to increase in the production of leucocyte in the haematopoietic tissue of the kidney and perhaps the spleen (Akinwande et al., 2016). The increase in white blood cell among the test dietary treatment groups may plausibly be induced by the anti-oxidant effect of bioactive compounds in *J. curcas* seed-meal based diets thereby improving the immune system of fish (Hoseinifar et al., 2020). Decrease in the blood total protein levels with higher levels of plant protein based diets may be attributed to the destruction of cells and consequent impairment in protein synthesis (Singh and Singh, 2002) and may be due to mobilization of protein to meet energy requirements and to sustain increased physiological activity (Arellano-Martinez et al., 2004). A reduction in blood cholesterol may be related to its utilization in the manufacture of cortisol arising from stress created by consumption of diets containing higher concentration of anti-nutrients similar to the observation of Kumar et al. (2011). Cortisol elevation due to stress always aggravate blood glucose (Paray et al., 2020) consistent with the observation recorded in this study; blood glucose increased among the test dietary treatment groups relative to control group. The trend of increase in blood glucose levels recorded in this study is in line with the results of previous studies on common carp during stress (Hoseini et al., 2019; Hosseini and Hoseini, 2012; Yousefi et al., 2019, 2020) which are plausibly due to adaptive responses to provide energy required by fish during the stress condition (Barton, 2002). Reduction in albumin and globulin among the test dietary treatment groups when compared with control group is consistent with the result obtained in liver histology of this study; from control to test dietary treatment groups liver condition improved

from degraded liver (graded 3 for control and D520T fed group) to intermediate liver (grade 2 for D540T, D1020T and D1040T fed groups) condition, respectively using the assessment of Martínez-Llorens et al. (2012), indicating some forms of hepatoprotection. Significantly higher concentration of serum total protein in the control group might have been as a result of liver damage (Coz-Rakovac et al., 2005; Francesco et al., 2012) which consequently results into reduced deamination capacity of the liver by reducing its aminotransferase ability (Kavadias et al., 2003). The higher serum protein in the control might be due to protein mobilization as a substrate for hepatic glycconeogenesis (Barcellos et al., 2003). Some forms of hepatoprotection was also conferred to *Jatropha* fed fish.

Conclusion and recommendation

It is evident from the present study that soybean meal could be replaced up to 40% by incompletely detoxified *J. curcass* seed meal at lower dry heat processing time without compromising the growth performance of tilapia fish but with a mild negative impact on the haematological and biochemical profiles of the blood of the fish.

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