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Effects of Replacement of Vegetable oil with Detoxified *Jatropha curcas* oil in the Diet of *Clarias gariepinus* (Burchell, 1822) fingerlings

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ABSTRACT
High cost of fish oil and other lipid sources for fish feed production had prompted the research investigation into the use of detoxified *Jatropha curcas* oil (DJCO) as an alternative dietary lipid source in the diet of *Clarias gariepinus* fingerlings (3.87±0.03g). A Complete randomized block design was adopted for the experiment where 240 *Clarias gariepinus* fingerlings and treatments were randomly allotted in 12 tanks in triplicate. Four diets were formulated to contain 45% crude protein at 0% lipid inclusion (diet 1-control), 12% detoxified *Jatropha curcas* oil (diet 2), 12% raw *Jatropha curcas* oil (diet 3) and 12% vegetable oil (diet 4) respectively. At the end of the experiment the results obtained indicated significant differences ($P<0.05$) for fishes fed diet 2 in terms of growth parameters evaluated, body compositions and survival. Fishes fed with detoxified *Jatropha curcas* oil-based diet had the best mean weight (3.38g) specific growth rate (2.06%/day), feed conversion ratio (1.59), protein efficiency ratio (93.19), survival (88.33%) and body nutrient compositions while those fed raw *Jatroval curcas* oil gave poor growth, low body nutrients composition and survival rate. This study established that detoxified *Jatroval curcas* oil can be adopted as alternative dietary lipid in the diet of *Clarias gariepinus* fingerlings.

Key words: Detoxification, *Jatropha curcas*, dietary lipid, growth, *Clarias gariepinus,*

INTRODUCTION

Feed is the largest single cost item in fish production that accounted for 60%-80% of the production cost (Aduku, 1993) due to competition between man and animal (Robinson and Singh, 2001). The use of unconventional and lesser known feed ingredients could be a way out (Hassan *et al*., 2015). The genus *Jatropha* belongs to the tribe Joannesieae of Crotonoideae in the Euphorbiaceae family (well known for its toxicity) and contains approximately 175 known species. It is considered to have originated in Central America, most probably Mexico. Jatropha species for which the toxicity has been widely studied are *Jatropha curcas*, *J. elliptica, J. glauca, J. gossypifolea, J. aceroides, J. tanoresisi, J. macarantha, J. integerrima, J. glandulifera, J. podagrica* and *J. multifida* (Makkar and Becker, 2009; Devappa *et al*., 2010a; 2011). Jatropha seeds have been extensively investigated as a source of oil. *J. curcas* seeds contain 25 – 35 percent crude oil (Makkar and Becker, 2009; King *et al.*, 2009). The oil contains 21percent saturated fatty acids and 79 percent unsaturated fatty acids (Gübitz and Trabi, 1999; Makkar and Becker, 2009). Jatropha oil fatty acid composition includes 14–16 percent palmitate (16:0), 5–8 percent stearate (18:0), 34 – 46 percent oleic acid (18:1), 29 – 44 percent linoleic acid (18:2) and a trace of longer-chain saturated fatty acids (Foidl *et al.*, 1996; Gübitz and Trabi, 1999; King *et al.*, 2009). *Jatropha curcas* oil has good feed stock qualities (Makkar and Becker, 2009; King *et al.*, 2009). Some countries, including India, Pakistan, China, Mexico, Brazil, Nigeria, Indonesia, Madagascar, Mali, Thailand, Ghana, Bangladesh, Kenya, Zimbabwe and Cape Verde, have initiated programmes for planting of *Jatroval curcas* (*Harinder et al.*, 2012). *Jatroval curcas* contains extremely toxic substances like toxalbumin curcin a lectin and carcinogenic phorbol (Makkar and Becker (1997). Seeds are often roasted before being eaten to reduce its toxicity (Harinder *et al*., 2012). However, some of the toxins are not heat tolerant, especially the phorbol esters present in the oil (Makkar and Becker, 1997, 2009;

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Devappa *et al*., 2010a, b, 2011). Aquaculture production is primarily dependent on the nutritional quality of feed as its affect both growth efficiency and feed utilization (FAO, 2005). Catfish is a species of the family Clariidae, an air breathing fish, and a fast-growing fish that feeds on a variety of agricultural by-products (Osibona, 2005). The fish species is noted for good metabolization of fat as an energy source than carbohydrate and for protein sparing effect (Orire *et al*., 2011; 2013; Mourente *et al*., 2005; Storebakken, 2002; Rodriguez *et al*., 2004). African catfish has been reported to utilize lipid of different sources; *Canarium schweinfurtii* oil Yisa *et al*. (2014), differently combined lipids of fish oil, palm oil and groundnut oil (Orire *et al*., 2013). However, the decline in supply of of fish oil has been identified as one of the constraints that could hinder the realization of the world fish output hitting a record high of 52% by 2025 (FAO, 2016). Alternative to fish oil are palm oil, groundnut oil and coconut oil (Ng et al., 2003; Ng *et al*., 2004), sunflower oil (Brandson *et al*., 2003) and milk fat (Orire and Fawole, 2012). Other dietary lipids sources found to enhance growth in fish include Omega 3 fatty acid in hybrid red tilapia (Chou and Shiau, 1999); cod liver oil in hybrid tilapia diet (Lim *et al*., 2001; Chou *et al.*, 2000) and efficient utilization of palm oil by tropical catfish (Lim *et al*., 2001). This study thus, focus its aim on the effects of replacement of vegetable oil with detoxified *Jatropha curcas* oil on the growth, carcass compositions and survival of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

Study area

This study was conducted in the fish nutrition laboratory, of the Department of Water Resources, Aquaculture and Fisheries Technology, Gidan Kwano campus, Federal University of Technology, Minna, Niger State. Gidan Kwano lies between Latitudes 9°31'15"Nand 9°32'30"N and Longitudes 6°26'15"E and 6°28'00"E (Onuigbo *et al*., 2015).

Experimental fish

The fish used for this study was *Clarias gariepinus* fingerlings (mean weight 3.85±0.09g) procured from Eco-rehab environmental service Kuje, Federal Capital Territory Abuja. They were transported in a 50 liter plastic container to the Departmental laboratory. Upon arrival at the Laboratory, the fishes were emptied into an open 250 liter plastic tank where they were acclimatized for two weeks. During this acclimatization there were fed on commercial catfish feed (Aquamax) to satiation twice daily (9.00 and 17.00). Water was refreshed daily while weak and dead fish were also removed.

Experimental diet

The feedstuffs for the study included soya bean meal, maize meal, fishmeal, vegetable oil, and vitamin-mineral premix were obtained from the Kure market, Minna, Niger State while the raw and detoxified *Jatropha curcas* oil were sourced from the Department of Microbiology, Federal University of Technology, Minna. Four experimental diets containing forty-five percent (45 %) crude protein was formulated using the Pearson Square method of feed formulation. The diets comprised of 0% lipid inclusion (control)as diet 1, 12% raw *Jatropha curcas* oil as diet 2, 12% detoxified *Jatropha curcas* oil as diet 3 and 12% vegetable oil as diet 4(Table 1).

Experimental design

Twenty (20) *Clarias gariepinus* fingerlings mean weight 3.85g±0.09 were randomly distributed in twelve experimental tanks of 20 litres water capacity in a recirculatory system in triplicate in a complete randomized design. The fishes were fed with experimental diets at 3% body weight twice daily (9.00 and 17.00 hours) however, the feeding rate was adjusted fortnightly to meet the fish nutrient requirements for the period of 8 weeks. The water was changed daily by recirculation, the uneaten feed and faecal matters were siphoned, oven dried and subsequently stored in a deep freezer (-4^{o}C) for later analysis. Water quality parameters were monitored weekly for temperature using mercury bulb thermometer, conductivity was measured with a conductivity meter (Model-JENWAY 4010) while hydrogen concentration (pH) was monitored with a pH meter (Model: EIL 7045/46) according to the standard methods of APHA (1980).

Table 1: Composition of Experimental diets

Feedstuffs (%)	**Jatropha oil inclusion levels (%)**			
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
Fishmeal	421.10	363.70	363.70	363.70
Maize meal	458.80	396.20	396.20	396.20
Soy bean meal	100.00	100.00	100.00	100.00
Detoxified *Jatropha curcas* oil	0.00	120.00	0.00	0.00
Raw *Jatropha curcas* oil	0.00	0.00	120.00	0.00
Groundnut oil	0.00	0.00	0.00	120.00
Vitamin premix	20.00	20.00	20.00	20.00
Total	999.90	999.90	999.90	999.90
Proximate Compositions (%)				
Crude Protein	43.5	44.40	45.20	44.75
Crude Lipid	13.30	9.70	13.9	13.85
Crude Fiber	0.75	0.75	1.05	0.65
Ash	7.80	2.50	4.40	5.05
Dry matter	28.46	33.28	43.10	36.68

Chemical analysis

The carcass contents for initial and final treatments were analysed for their proximate compositions according to the method of AOAC (1990).

Biological evaluation

The biological parameters which included mean weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio were evaluated according to the method of Maynald (1979) and Halver 1989):

Weight gain: Weight gain = Final body weight (g)- initial body weight (g)

Specific Growth Rate (SGR): According to Brown 1957 was measure with the formula

SGR = Ln Mean Final Weight x Ln Mean Initial Weight x 100 /Duration of experiment (Days)

Feed Conversion Ratio (FCR): This is measure with the formula

$$FCR = \frac{\text{Weight of feed fed (gram)}}{\text{Weight gain of fish (gram)}}$$

Protein Efficiency Ratio (PER): This is express as:

$$PER = \frac{\text{Weight gain of fish}}{\text{Protein fed}}$$

$$\text{Apparent Net Protein Utilization (ANPU)} = \frac{\text{Carcass Protein gain (g)} \times 100}{\text{Protein fed (g)}}$$

$$\text{Mortality was evaluated as the expressed as \%Mortality} = \frac{\text{No of fish left in the tank}}{\text{No of fish stocked}} \times 100$$

Statistical analysis

Data obtained from the feeding trials were subjected to one-way Analysis of Variance (ANOVA). Means between the treatment were compared using Duncan multiple tests Duncan (1995) with the aid of Minitab 14.0 ($P<0.05$). The graphical analysis was plotted with Microsoft excel window 2007.

RESULTS

The final mean weight gain was higher for diet 2 (9.01g), 0 followed by diet 4 (6.00g) but lowest for diet 3 (1.39g) with significant difference ($P<0.05$) among the treatments. A similar trend was observed for values obtained for mean weight gain, however, there was no significant difference ($P>0.05$) in the mean initial weight for the fishes. Diet 2 had the best FCR value (1.29) followed by diets 4 (2.02) and 3 (3.31) while the poorest value was obtained from diet 1 (7.94).

The specific growth rate was highest for diet 2 (2.06%/day) followed by diets 4 (1.41%/day) and 3 (0.33%/day) with significant difference (P<0.05) among treatments while diet 1 was significantly lower (0.17%/day). The protein efficiency ratio for diet 2 was the best (9.54) followed by diet 4 (7.17) and diet 1 (2.73) while diet 3 exhibited a significant (P<0.05) low value (1.92). Diet 2 had the best apparent net protein utilization value (93.19%) followed by diet 4 (26.37%) with significant difference (P<0.05) between them while, diets 1 and 3 gave a negative value of -379.86% and -130.23% respectively. Furthermore, diets 2 and 4 had the highest survival percentages of 80% and 85% with no significant difference between them while diets 1 and 3 gave the lowest survival percentages of 8.33% and 11.67% respectively (Table 2).

The body composition values were significantly different (P<0.05) for the treatments. Fish fed with diet 2 exhibited highest body crude protein value (63.88%) followed by diets 4 (56.88%) and diet 1 (54.63%) which were not significantly different (P>0.05) from the initial carcass crude protein value (55.00%). The body lipid was found highest in fish fed with diet 4 (27.10%) while it was lowest with diet 1 (16.10%) and 3 (17.00%) respectively. The body ash was highest with diets 1 (28.95%) and 3 (28.68%) followed by dies 4 (18.905) and 2 (17.85%) with no significant difference (P>0.05) between the treatments while it was significantly lower (P<0.05) for the initial carcass value (11.11%). The body crude fiber was highest for diet 1 (5.40%) and lowest for the initial carcass value (1.29%). The moisture content was highest in fish fed with diet 1 (9.25%) followed by diet 3 (6.49%) and significantly low (P<0.05) for diet 4 (2.46%) Table 3.

Table 2: Growth performance of *Clarias gariepinus* fed detoxified *Jatropha curcus* oil

Growth Parameters	**Diet1**	**Diet 2**	**Diet 3**	**Diet 4**	**SE±**
Initial weight (g)	$3.85^{a} \pm 0.02$	$3.87^{a} \pm 0.03$	$3.75^{a} \pm 0.17$	$3.91^{a} \pm 0.02$	0.09
Initial weight (g)	$2.97^{b} \pm 2.58$	$9.01^{a} \pm 7.80$	$1.39^{b} \pm 0.41$	$6.00^{ab} \pm 5.21$	5.01
Mean weight gain (g)	$0.39^{b} \pm$ o.40	$6.44^{a} \pm 5.59$	$0.21^{b} \pm 0.39$	$3.38^{ab} \pm 2.95$	3.17
Feed fed (g)	$4.33^{b} \pm 3.84$	$15.33^{a} \pm 13.27$	$2.05^{b} \pm 1.56$	$11.21^{ab} \pm 9.74$	8.64
Feed of Conversion Rate	$7.94^{a} \pm 7.37$	$1.59^{b} \pm 1.38$	$3.31^{b} \pm 2.7$	$2.22^{b} \pm 1.92$	4.82
Specific Growth Rate (%/day)	$0.17^{c} \pm 0.17$	$2.06^{a} \pm 0.31$	$0.33^{c} \pm 0.36$	$1.41^{b} \pm 0.14$	0.26
Protein Efficiency Rate (PER)	$2.73^{c} \pm 0.40$	$9.54^{a} \pm 1.18$	$1.92^{d} \pm 0.84$	$7.17^{b} \pm 0.69$	0.82
Apparent Net Protein Utilization (%)	$-379.86^{c} \pm 0.02$	$93.19^{a} \pm 0.02$	$-30.23^{d} \pm 0.02$	$26.37^{b} \pm 0.02$	0.02
Mortality (%)	$91.67^{a} \pm$ 5.77	$20.00^{b} \pm 22.11$	$88^{a}.33 \pm 11.55$	$15.00^{b} \pm 5.00$	13.39

Mean data in the same row carrying similar superscripts are not significantly different (P>0.05)

Table 3: Body Composition of *Clarias gariepinus* fed detoxified and undetoxified *Jatropha curcus* oil

Nutrients	**Initial**	***Jatropha curcus* oil inclusion levels**				
		Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**SD (±)**
Crude protein	$55.00^{b} \pm 0.00$	$54.63^{b} \pm 0.00$	$63.88^{a} \pm 0.00$	$52.50^{c} \pm 0.00$	$56.88^{b} \pm 0.00$	0.00
Lipid	$20.09^{b} \pm 0.00$	$16.10^{c} \pm 0.00$	$19.50^{b} \pm 0.00$	$17.00^{c} \pm 0.00$	$27.30^{a} \pm 0.00$	0.00
Ash	$11.11^{c} \pm 0.00$	$28.95^{a} \pm 0.00$	$17.85^{b} \pm 0.00$	$28.68^{a} \pm 0.00$	$18.90^{b} \pm 0.00$	0.00
Crude fibre	$1.29^{d} \pm 0.00$	$5.40^{a} \pm 0.00$	$2.10^{c} \pm 0.00$	$2.70^{b} \pm 0.00$	$2.50^{b} \pm 0.00$	0.00
Moisture	$3.70^{c} \pm 0.00$	$9.25^{a} \pm 0.00$	$4.56^{b} \pm 0.00$	$6.49^{b} \pm 0.00$	$2.46^{c} \pm 0.00$	0.00

Mean data in the same row carrying similar superscripts are not significantly different (P>0.05)

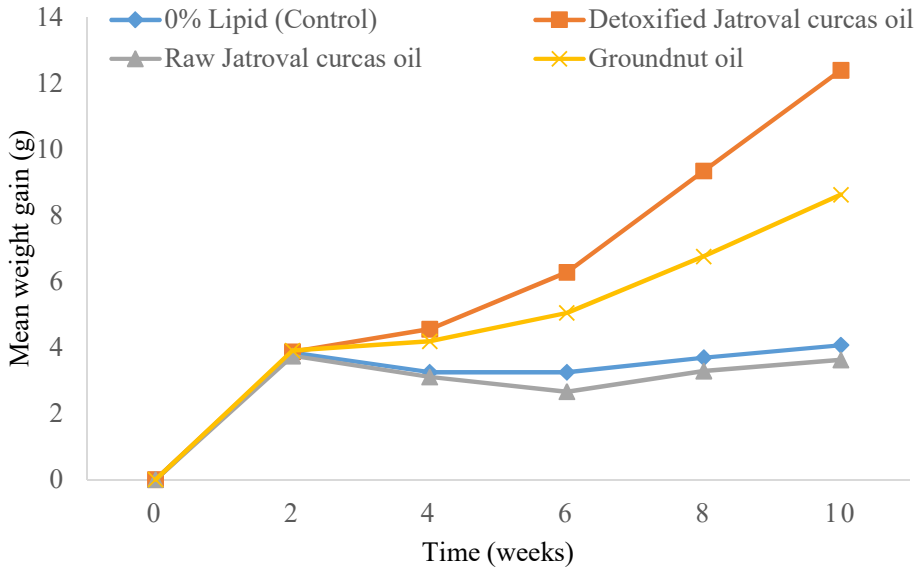

Figure 1. Growth pattern of Clarias *gariepinus* fed detoxified Jatropha *curcas* oil.

The finding of this study indicated that the fish utilized the dietary lipids introduced to them (Orire *et al.*, 2013). Their performances showed an affirmative involvement in the development of the fish on feeding diets as reported by Hoelihan *et al.* (2001). The poor performance of fishes fed raw *Jatropha curcas* oil indicated presence of toxic substances such as phorbos esters and anti-nutritional compounds like phytate, lectin and trypsin in the oil (Kassaye *et al*., 2013; Makkar and Becker, 2009; Devappa *et al*., 2010a; 2011). The presence of the toxins in the raw *Jatropha curcas* oil might be responsible for the inadequate ingestion of the diets to meet the dietary lipid requirement for fish growth, which resulted in the depressed growth performance (Azzaza *et al*., 2011 and Reddy and Pierson, 1994). The removal of toxins such as Saponin, Lectin, Phorbol Esters, Tannin, Trypsin inhibitors, Phytate, which are growth inhibitors in the detoxified *Jatropha curcas* oil in diet 2 could be responsible for better utilization of the lipid for growth (Makkar and Becker 1997; 1999; Ojediran, *et al.*, 2014). The high growth recorded for detoxified *Jatroval curcas* oil could also be attributed to its rich, fatty content in oleic and linoleic fatty acids (Foidl *et al.*, 1996; Gübitz and Trabi, 1999; King *et al.*, 2009). Higher survival rates of fish fed detoxified *Jatropha curcas* oil as against those fed raw *Jatroval curcas* oil can be attributed to low levels of toxins in the lipid (Hajos *et al*., 1995). The effect of lipid in fish growth can also be observed in the fish fed with control diets which had depressed growth and low survival rate (Figure 1) this is in agreement with the findings of Aderolu and Akinremi (2009), Sala and Balesteros (1997) and Nig (2004) who reported improved growth in catfish and tilapia fed dietary lipids of palm oil. Furthermore, the tolerance of detoxified *Jatroval curcas* oil at 12% inclusion level was evident in the carcass lipid which is lower than that of raw *Jatroval curcas* oil-based diet. This is in agreement with the findings of Babalola and Adebayo (1997) who reported higher feed intake and nonretarded growth in *Heterobranchus longifilis* fingerlings fed plant oil up to 12.5%. Phorbos esters have been reported as heat stable (Makkar *et al*., 2012) therefore, the detoxifying methods employed for the oil used in this experiment have proven to be effective in removing the toxin which made it available for utilization by the fish for growth.

Conclusion

The results obtained from this study support the use of *Jatroval curcas* oil if detoxified in the diet of Clarias *gariepinus* fingerlings. This can be an alternative and non-competitive dietary lipid for fish feed industry. Detoxified *Jatropha curcas* oil can replace vegetable oil in the diet of *Clarias gariepinus* fingerlings without adverse effect. Therefore, fish farmers and fish feed industry can adopt it as an alternative lipid sources in fish feed production provided an appropriate method of detoxification is adopted.

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