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Nutrient use and Response of African Catfish *Clarias Gariepinus* to Fishmeal Diets Containing Auto-Detoxified Mixtures of Jatropha Kernel Cake with Bovine Blood

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Nutrient use and Response of African Catfish *Clarias Gariepinus* to Fishmeal Diets Containing Auto-Detoxified Mixtures of Jatropha Kernel Cake with Bovine Blood

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Abstract- African catfish *Clarias gariepinus* fingerlings were used as a model to evaluate diets containing three auto-detoxified mixtures of Jatropha kernel cake with bovine blood (ADMJKC/bb) ingredients. The test ingredients and a control were used to produce seven isonitrogenous and isocalorific diets in which they either replaced 30% or 50% level of fishmeal. The Jatropha kernel cake mixed with bovine blood at a ratio of 2:1; heated, spread dried, remoistened and substituting 30% of fishmeal, and the Jatropha kernel cake mixed with bovine blood at a ratio of 3:1; unheated, spread dried, without remoistening, and substituting 30% of fishmeal), were similar to the control in feed conversion ratio and feed cost per gram of gain. The control significantly out-performed the $Y3_{50}$, $Z2_{50}$ $Z4_{30}$ and $Z4_{50}$ in feed efficiency, indicating thereby that at 30% fishmeal substitution, the 2:1 mixture of Jatropha kernel cake and bovine blood (heated) and the 3:1 mixture of Jatropha kernel cake and bovine blood (unheated) are biologically and economically satisfactory for *C. gariepinus* fingerlings, and could be recommended for *C. gariepinus* industrial rearing.

Keywords: auto-detoxified, jatropha -kernel -cake bovine -blood, clarias gariepinus.

I. Introduction

The African catfish (*Clarias gariepinus*) has served as a good model for evaluating animal feed resources when supplemented with the Jackbean (Osuigwe *et al*., 2005; 2006), poultry viscera (Toutou *et al*; 2018) and soybean meal (Davies and Gouveia, 2008; Abdel-Warith *et al*., 2020). This omnivorous scavenger, particularly amenable to farming practices of peasant small holders in the Tropics and sub-Tropics, was used in this study to test diets containing auto-detoxified mixtures of Jatropha kernel cake (a co-product following extraction of Jatropha oil from *Jatropha curcas* seeds) and bovine blood - ADMJKC/bb (Ewane *et al*; 2017). The Jatropha auto-detoxification process (Ewane *et al*., 2017) and its enrichment with bovine blood (Ewane *et al*., 2021) were meant to maximize the former's benefits to the resource-poor smallholder farmers. This study, which involved in-vitro and in vivo trials, was undertaken to assess the growth, nutrient utilization and economic response of the African catfish fed with diets containing auto-detoxified mixtures of Jatropha kernel cake enriched with bovine blood (ADMJKC/bb) at different levels of fishmeal substitution.

II. Materials and Methods

a) Experimental ingredients and diets

Auto-detoxified mixtures of Jatropha kernel cake and bovine blood (ADMJKC/bb) were produced as described elsewhere (Ewane *et al*., 2021), and three recommended ingredients (Table 1)were tested in iso-calorific and iso-nitrogenous diets, at 30% and 50% replacement levels for fishmeal.

Table 1: Auto-detoxified mixtures of Jatropha kernel cake (JKC) and bovine blood (bb) - ADMJKC/bb

Test ingredients	Code	Description and preparation protocol
Treatment 1	Y3	JKC : bb (2:1 v/v), heated, spread dried, and remoistened daily to 66% w/w dry matter
Treatment 2	Z2	JKC: bb (3:1 v/v) unheated, spread dried without remoistening
Treatment 3	Z4	JKC: bb (3:1 v/v). Unheated, spread dried, and remoistened daily to 66% w/w dry matter

For the purpose of this study, the control treatment was composed of fishmeal, blood meal, maize, palm oil, bone meal, and premix and titanium

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oxide, without any Jatropha or soybean meal. Whereas all the treatments had the same quantities of blood meal (3% w/w), palm oil (5% w/w), bone meal (1% w/w), titanium oxide (1% w/w) and premix (3% w/w), the quantity of ingredients in each formulation/treatment, given as parts per hundred of fishmeal (Table 2), varied with respect to the quantities of maize (16.2 to 36% w/w), soybean cake (0 to 19.8% w/w), ADMJKC/bb (0 to 25% w/w) and fish meal (25 to 50% w/w).The 30% ADMJKC/bb treatment level was based on the recommendation of Fakunle *et al* (2013) and Alatise *et al* (2014) for a test with *Clarias gariepinus* while the 50% ADMJKC/bb treatment level was based on the recommendation of Kumar *et al* (2011) for a test with carp.

With these ingredients, seven iso-nitrogenous and iso-calorific diets were prepared containing crude protein (38% w/w), metabolis able energy (3000 MCal/kg), and 1% titanium oxide as inert tracer. The materials were thoroughly ground; uniformly using a hammer millthen hand-mixed to produce a mash, and the latter compressed (with a pelletizer) into 2-mm diameter hard pellets. The pellets were sun-dried for 1 h and packaged in dark 50-μm thin polyethylene sheets, and stored at room temperature for eventual use.

Table 2: Composition of experimental diets

Treatments			Ingredients									
Fish meal	ADMJKC/ bb*	Code	Maize	Soybean cake	ADMJKC/ bb	Blood meal	Fish meal	Palm oil	Bone meal	TiO_2	Premix **	Total
100%	Control	-	36	0	0	3	50	5	2	1	3	100
70%	30% Y3	$Y3_{30}$	25	11	15	3	35	5	2	1	3	100
50%	50% Y3	$Y3_{50}$	16.2	19.8	25	3	25	5	2	1	3	100
70%	30% Z2	$Z2_{30}$	32	4	15	3	35	5	2	1	3	100
50%	50% Z2	$Z2_{50}$	29	7	25	3	25	5	2	1	3	100
70%	30% Z4	$Z4_{30}$	26.3	9.7	15	3	35	5	2	1	3	100
50%	50% Z4	$Z4_{50}$	18.5	17.5	25	3	25	5	2	1	3	100

* Auto-detoxified mix of Jatropha kernel cake and bovine blood

** Premix: composed (mg vitamin and mineral/kg premix): vitamin A 4,800,000 IU, vitamin D3 800,000 IU, vitamin E4800 mg, vitamin K 800 mg, thiamine 600 mg, riboflavin 2800 mg, vitamin B3 4800 mg, pyridoxine 600 mg, vitamin B12 4 mg, folic acid 200 mg, cobalt 160 mg, copper 1200 mg, iron 9000 mg, iodine 480 mg, magnesium 2730 mg, manganese 28000 mg, zinc 20000 mg

Table 3 shows that the overall proximate composition of the experimental test ingredients were considerably different in terms of nitrogen-free and ether extracts as well as the ash and crude protein contents and less so in terms of their dry matter and crude fibre contents.

In a similar manner, as opposed to their tannin contents, the overall proximate composition of anti-nutritional factors in the test ingredients were significantly different with respect to phytates content, trypsin inhibitor, saponins, crude phorbol ester and lectin activity (Table 3b).

Table 3: Proximate composition of test ingredients

Figures followed by the same letter in a row were not significantly different (P=5%)

a. *Nutritional factors*

Formulations	Dry matter (%)	Crude protein (%)	Ether extract (%)	Crude fibre (%)	Ash (%)	NFE† (%)
Control						
Treatment1(Y3)	92.09	34.78	39.64	11.56	7.31	5.38
Treatment 2 (Z2)	94.33	50.72	32.89	10.03	5.95	0.41
Treatment 3 (Z4)	93.71	41.81	37.36	11.43	7.16	2.24

b. *Anti-nutritional factors in test ingredients*

Formulations	Phytates (% dry matter)	Trypsin inhibitor (TIU/mg)	Saponins (g/100 g)	Tannin (%)	Crude Phorbol Esther (mg/g)	Lectin activity (mg/mL)
Control						
Treatment 1, Y3	1.71 [a]	3.99 [a]	0.10 [a]	0.044 [a]	0.36 [a]	8.50 [a]
Treatment 2, Z2	1.92 [b]	4.13 [a]	0.11 [a]	0.046 [a]	0.41 [b]	9.13 [b]
Treatment 3, Z4	2.29 [c]	4.31 [b]	0.13 [b]	0.047 [a]	0.47 [c]	9.68 [c]

† NFE = Nitrogen free extract

b) Experimental system and animals

For the feeding trial, modelled after the work of Kumar *et al* (2011), 700 African catfish fingerlings weighing between2.5 and5 g, were obtained from the Batoke-Limbe Station of the Institute of Agricultural Research for Development (IRAD) and transferred to the Ekona Centre of the same institute where feeding trials were conducted. . Hand aerators were used to supply oxygen to the fingerlings during transportation. Upon arrival at Ekona, they were rested for 6 h in two 50-litre tanks, then, randomly split in twenty one (21) groups of 20 fingerlings and each group placed in 35-litre aquaria to acclimatize, over a period of 14 days during which they were fed a commercial diet containing no vegetable protein.

The fish were all starved for 24h before the start of trials, Thereafter, the aquaria were thoroughly washed, rinsed and disinfected. Each fingerling was separately weighed and the five (5) with extreme weights in each group were retrieved and discarded, leaving 15 fingerlings in each tank (experimental unit). Each tank was thus a replicate and three tanks constituted a treatment. About two thirds of the water in each aquarium was siphoned and replaced daily (between 6 am and 8.30 am) with fresh water that had been stored for about 24 hat room temperature in the laboratory. Preliminary measurements showed no detectable levels of nitrite in the water before the start of the experiment. The water quality was monitored regularly and adjustments were made daily (between 3 and 10 pm) to keep the values within optimum range for fresh water fish culture (Temperature of 23-26°C, pH of 7- 8, dissolved oxygen of5 - 6.8 mg/L, conductance of 65.6 – 107 μhom/cm^3, total NH_3 of 0.1 -0.2 mg/L, nitrites of 0.02 – 0.08 mg/L and nitrates of 1–3 mg/L..

The weekly rations of the fish were composed of 5% of their body weight. The daily ration was supplied in five equal parts, and given at five specific periods during the day (9 am, 11.30 am, 2 pm, 4.30 pm and 7 pm), in order to encourage consumption of feed formulations and allow for elimination of less promising treatments. Weight and feed data was recorded for each fish.

Throughout, fish behaviour seemed normal for all the treatments as they were well dispersed and did not always clump into one side of the tank despite routine daily handling. However, fish fed $Z4_{50}$ test ingredient sometimes ate slowly and always left uneaten feed. This behaviour was not observed in the fishes fed the control and other ingredients.

c) Digestibility trial and measurements

The last 14 days of feeding trial were modelled into a digestibility trial during which the inert-marker (titanium dioxide) containing-diets were fed to the fish in accordance with the usual daily schedule. All leftover feed in each aquarium was siphoned (using a pipe) and discarded while the faeces were separately siphoned, poured into labelled beakers, and centrifuged at 4000g. The supernatants were discarded and the sediments (faeces) retrieved and deep-frozen at -20°C for subsequent analysis. Titanium dioxide in feed and faeces were determined using the method of Richter *et al.* (2003) while the nutrients' apparent digestibility coefficients (ADC) and the energy of the various diets were calculated according to (Cho *et al*., 1982). The apparent digestibility coefficients of test ingredients (ADCI) were then calculated based on the digestibility of the reference and test diets using Equations of (Bureau and Cho, 1999),

d) Body composition analysis

From each replicate, twenty (20) fish samples were used for the initial body composition analysis and three (3) for the final body composition analysis. In each case, the fish were sacrificed by immersion in ice slurry, then, autoclaved at 120°C for 10 min, homogenized using a domestic blender and portions retrieved for proximate compositional analysis.

e) Evaluation Parameters

Growth performance and nutrient utilization were assessed in terms of:

- Body mass gain (BMG): The percentage ratio of the change in body mass (Final body mass less Initial body mass) to the initial body mass
- Average daily gain (ADG): The ratio of the change in body mass (final body mass less initial body mass) to the number of trial days
- Specific growth rate (SGR): The percentage ratio of the change in body mass [Ln (final body mass) less Ln (initial body mass)] to the number of trial days

- Interval survival rate (ISR): The ratio of the fish mortality (Initial number of fish less the mortality at midway of Trial) to the initial number of fish
- Final survival rate (FSR): The ratio of the fish mortality (Initial number of fish less the mortality at end of Trial) to the initial number of fish
- Feed conversion ratio (FCR): The ratio of the dry feed intake to the body mass gain
- Protein efficiency ratio (PER): The ratio of the fresh body mass gain to the crude protein fed
- Protein productive value (PPV): The percentage ratio of the change in body protein (Final fish body protein less initial fish body protein) to the total crude protein consumed
- Lipid production value (LPV): The percentage ratio of the change in body lipid (Final fish body lipid less initial fish body lipid) to the total crude lipid consumed
- Metabolic growth rate (MGR):

$$= \frac{\text{Body mass gain in g}}{\left[\left[\frac{\text{Initial body mass in g}/1000^{0.8}+\text{final body mass in g}/1000^{0.8}}{2}\right]\right]}{\text{Number of trial days}}$$

Assuming the market value of a kilogram of *Clarias gariepinus* was 2,000 FCFA (~ 3.64 USD), economic performance was assessed in terms of:

- Cost of feed consumed, which was the product of quantity of feed intake and their unit cost
- Feed cost per gram of gain (USD/g), which was the ratio of the cost of feed consumed to the body mass gain
- Value of live weight gain (in USD), which was a thousandth of the product of the body mass gain and the unit cost of fish
- Economy of gain, which was the ratio of the cost of feed consumed and the value of live-weight gain.

f) Statistical analysis

The experiment was conducted using a Completely Randomized Design and all data were subjected to a one-way analysis of variance using the SPSS Software (IBM Corp., Released 2013). Significant differences between means were detected using the Duncan Multiple Range Test (DMRT).

III. Results

a) Growth performance, survival and feed utilization response to 3ADMJKC/bb ingredient based diets

The initial weights, final weights and body mass gain of *Clarias* fed test diets are shown in Table 4 while the growth performance, survival, feed utilization and feed assimilation are shown in Table: 5. the initial weight of the fishes averaged 2.8±0.19 g. The fishes fed the control diet significantly ($P<0.05$) had higher final weight and body mass gain than those fed the ADMJKC/bb ingredients based diets. The fishes fed the ADMJKC/bb at 30% replacement of fishmeal ($Y3_{30}$, $Z2_{30}$ and $Z4_{30}$) significantly ($P<0.05$)had higher final weights and body mass gains than their counterparts fed 50% level of fishmeal replacement ($Y3_{50}$, $Z2_{50}$ and $Z4_{50}$). However, fishes fed the $Z4_{30}$ ingredient significantly ($P<0.05$)had lower final weights and body mass gains than those fed similar levels of test ingredient Z230 and $Y3_{30}$.

Table 4: Body mass gain of *Clarias gariepinus* fingerlings fed with ADMJKC/bb ingredient based diets for 8 weeks

Treatment*			Initial weight(g)	Final weight (g)	Body mass gain (g)	
Fish meal	Proportion of ADMJKC/bb	Code				
100%	Control *	Control	2.8±0.08	12.20±1.09	9.40± 1.07	d
70%	30% Treatment 1	$Y3_{30}$	2.82±0.08	6.55±0.62	3.72± 0.61	c
50%	50% Treatment 1	$Y3_{50}$	2.92±0.08	4.03±0.46	1.11± 0.51	ab
70%	30% Treatment 2	$Z2_{30}$	2.89±0.12	6.52±0.65	3.63± 0.66	c
50%	50% Treatment 2	$Z2_{50}$	2.92±0.15	3.75±0.17	0.83± 0.27	a
70%	30% Treatment 3	$Z4_{30}$	2.99±0.04	5.09±0.29	2.10± 0.30	b
50%	50% Treatment 3	$Z4_{50}$	2.92±0.08	3.11±0.09	0.19± 0.01	a
Standard error of the mean			0.02	0.65	0.66	

Table 5: Growth performance, survival, feed utilization and feed assimilation of *Clarias gariepinus* fingerlings fed ADMJKC/bb ingredient based diets for 8 weeks

Values (means ± standard deviation) in a column followed by different superscript differ significantly (P < 0.05)

Treatment*			Growth Performance				Survival		Feed Utilization			Feed Assimilation	
Fish meal	Proportion of ADMJKC/bb	Code	Body mass gain (%)	Average daily gain (mg/fish/day)	Specific growth rate (%)	Metabolic growth rate (gkg$^{0.8}$ day^{-1})	Intervals survival rate (%)	Final survival rate (%)	Average daily feed (mg/fish/day)	Feed conversion ratio	Protein efficiency ratio	Protein productive value (%)	Lipid production value (%)
100 %	Control	T0	335±38 ^{d}	168 ± 19^{d}	2.62±0.15^{e}	8.69±0.51^{e}	100±0	82.2±3.9^{ab}	364±6^{e}	2.2±0.2^{a}	1.47±0.17 ^{f}	28.7±3.1 ^{e}	61.3 ± 6.7 ^{a}
70%	30% Treatment 1	$Y3_{30}$	132±22 ^{c}	66 ± 11^{c}	1.50±0.17^{d}	4.90±0.57^{d}	100±0	80.0±0^{a} ^{b}	234±12 ^{d}	3.6±0.4^{a}	0.87±0.11 ^{e}	16.5±1.6 ^{d}	41.0 ± 3.9 ^{a}
50%	50% Treatment 1	$Y3_{50}$	38±18 ^{ab}	20 ± 9^{ab}	0.57±0.24^{b}	1.82±0.78^{b}	98±4	93.3±6.7^{b}	181±12 ^{b}	11.0±0.4 ^{bc}	0.33±0.14 ^{c}	8.2±1.9 ^{b}	20.6 ± 5.2 ^{a}
70%	30% Treatment 2	$Z2_{30}$	126±24 ^{c}	65 ± 12^{c}	1.45±0.20^{d}	4.76±0.65^{d}	100±0	80.0±6.7^{ab}	230±1^{d}	3.6±0.6^{a}	0.85±0.13 ^{e}	16.1±1.9 ^{d}	40.7 ± 4.9 ^{a}
50%	50% Treatment 2	$Z2_{50}$	29±11 ^{c}	15 ± 5^{a}	0.45±0.15^{b}	1.42±0.47^{b}	100±0	84.4±7.7^{ab}	178±5^{b}	13.1±5.3^{c}	0.25±0.08 ^{ab}	7.0±1.1 ^{ab}	14.9 ± 3.0 ^{a}
70%	30% Treatment 3	$Z4_{30}$	70±11 ^{c}	38 ± 6^{b}	0.95±0.11^{c}	3.10±0.36^{c}	100±0	93.9±6.7^{b}	208±15 ^{c}	5.6±0.7^{ab}	0.55±0.07 ^{d}	11.4±1.1 ^{c}	28.8 ± 2.8 ^{a}
50%	50% Treatment 3	$Z4_{50}$	7±0 ^{a}	3 ± 1^{a}	0.11±0.00^{a}	0.35±0.01^{a}	98±4	71.1±19.2^{a}	145±3^{a}	43.0±1.2^{d}	0.07±0.00 ^{a}	4.5±0.1 ^{a}	9.2 ± 0.1 ^{a}
standard error of the mean			23.49	1	0.18	0.59	0.44	2.35	0.01	3.03	0.10	1.72	3.8

There was no significant difference (P>0.05) in final weight and body mass gain among all fishes fed the ADMJKC/bb at 50% level of fishmeal replacement ($Y3_{50}$, $Z2_{50}$ and $Z4_{50}$). However, fishes fed the Y3 ingredient at 50% replacement ($Y3_{50}$) were similar to those fed Z4 ingredient at 30% level of fishmeal replacement ($Z4_{30}$) in body mass gain. The percentage body mass gain and the average daily gains (ADG) followed the same trend as the body mass gains while the specific growth rate (SGR) and the metabolic growth rate (MGR) followed the same trend as the final weights. The $Z4_{30}$ ingredients significantly (P<0.05) induced better SGR and MGR than the $Y3_{50}$ ingredients.

The intermediary survival rates (ISR) were similar (P>0.05) for all the treatment groups including the control. However, $Y3_{30}$ and $Z2_{30}$ had similar final survival rates (FSR) with the control (P>0.05) and these were lower compared to $Y3_{50}$ and Z250 although the differences' were not significant (P>0.05). On the other hand, $Z4_{30}$ significantly (P<0.05) had better final survival rate than $Z4_{50}$.

Average daily feed (ADF) consumption for the control was significantly (P<0.05) higher than the ADMJKC/bb ingredients. The ADMJKC/bb ingredients based diets fed at 30% level of fishmeal replacement were significantly consumed more than their counterparts fed at 50% level of fishmeal replacement. The feed conversion ratio did not significantly (P>0.05) differ between the control and the ADMJKC/bb ingredients based diets fed at 30% level of fishmeal replacement. However, FCR for each ingredient fed at 30% level of fishmeal replacement was significantly (P<0.05) better than the same ingredient fed at 50% level of fishmeal replacement. The protein efficiency ratio (PER) was significantly (P<0.05) higher for the control compared to the ADMJKC/bb. Similarly, the ADMJKC/bb fed at 30% level of fishmeal replacement significantly (P<0.05) had higher PER than their counterparts fed ADMJKC/bb ingredients at 50% level of fishmeal replacement. Protein productive value (PPV) and lipid productive value (LPV) were all significantly (P<0.05) higher for the control than the ADMJKC/bb fed

fish. Also, the ingredients fed at 30% level of fishmeal replacement significantly ($P<0.05$) induced higher PPV and LPV than their counterparts fed at 50% level of fishmeal inclusion. However, $Z4_{30}$ had significantly ($P<0.05$) lower PPV and LPV values than $Z2_{30}$ and $Y3_{30}$.

b) Proximate composition of whole body carcass of Clarias gariepinus

The proximate composition of the whole body carcass of *Clarias gariepinus* fed experiment 6 diets is found in Table 6. The dry matter crude protein, crude fibre and ash contents were not significantly different ($P>0.05$) among the treatments. The ether extract content of the test fish varied from 5.94% for $Z4_{50}$ to 8.12% for the control, but there were no significant difference ($P>0.05$) between the control and the ADMJKC/bb fed fish except for $Z4_{50}$. Similarly, the control and the ADMJKC/bb fed fish were statistically similar in nitrogen free extract (NFE) except the $Z4_{50}$. The control was significantly ($P<0.05$) higher in gross energy compared to the fishes fed higher levels of the test ingredients ($Y3_{50}$, $Z2_{50}$ and $Z4_{50}$).

Table 6: Proximate composition of whole body carcass of *Clarias gariepinus* fingerlings fed ADMJKC/bb Ingredient based diets for 8 weeks

Values (means ± standard deviation) in a column followed by different superscript differ significantly ($P < 0.05$)

Treatment*			Dry matter (%)	Crude protein (%)	Ether extract (%)	Crude fibre (%)	Ash (%)	Nitrogen Free Extract(%)	Gross energy (Kcal/100g)
Fish meal	Proportion of ADMJKC/bb	Code							
Pre-Experimental Fish			72.05 ± 1.41	63.35 ± 1.12	6.02 ± 1.28^{c}	0.14 ± 0.01^{bc}	15.26 ± 1.53^{c}	15.23 ± 3.93^{c}	306.31 ± 1.11^{c}
100%	Control *	Control	76.18 ± 1.19	65.38 ± 0.95	8.12 ± 1.08^{a}	0.15 ± 0.05^{b}	15.44 ± 1.29^{bc}	10.91 ± 1.11^{ab}	323.21 ± 0.94^{a}
70%	30% Treatment 1	$Y3_{30}$	77.52 ± 1.34	65.21 ± 1.06	8.11 ± 1.22^{a}	0.16 ± 0.05^{b}	16.38 ± 1.45^{b}	10.15 ± 1.25^{a}	322.16 ± 1.05^{c}
50%	50% Treatment 1	$Y3_{50}$	77.13 ± 1.07	64.38 ± 0.85	7.52 ± 0.97^{b}	0.17 ± 0.04^{b}	16.52 ± 1.16^{a}	11.41 ± 1.0^{bc}	317.6 ± 0.84^{ab}
70%	30% Treatment 2	$Z2_{30}$	76.18 ± 0.80	65.11 ± 0.64	8.02 ± 0.73^{a}	0.15 ± 0.03^{b}	16.34 ± 0.87^{b}	10.39 ± 0.75^{ab}	322.12 ± 0.63^{c}
50%	50% Treatment 2	$Z2_{50}$	76.91 ± 1.05	64.38 ± 0.83	6.75 ± 0.95^{b}	0.21 ± 0.04^{a}	16.58 ± 1.13^{a}	12.08 ± 0.97^{bc}	314.69 ± 0.82^{bc}
70%	30% Treatment 3	$Z4_{30}$	77.19 ± 1.25	64.75 ± 1.00	7.72 ± 1.14^{b}	0.16 ± 0.05^{b}	16.39 ± 1.36^{b}	10.98 ± 1.17^{ab}	319.72 ± 0.99^{ab}
50%	50% Treatment 3	$Z4_{50}$	76.56 ± 1.21	63.78 ± 0.96	5.94 ± 1.09^{c}	0.22 ± 0.05^{a}	16.56 ± 1.31^{a}	13.51 ± 1.13^{c}	312.92 ± 0.95^{c}
Standard error of the mean			0.23	0.29	0.23	0.01	0.24	0.33	1.32

c) Digestibility of Diets and ADMJKC/bb ingredients

The digestible nutrients and energy of ADMJKC/bb test ingredients are shown in Table 7 The digestible dry matter of the ingredients fed at 30% level of fishmeal replacement was significantly ($P<0.05$) higher for all test ingredients than their counterparts fed at 50% level of fishmeal replacement. The digestible dry matter for Y3 and Z2 was similar ($P>0.05$) at both the 30% and 50% levels of inclusion and significantly ($P<0.05$) superior to Z4 at all the corresponding levels of inclusion.

The digestible crude protein was significantly ($P<0.05$) higher for ingredients fed at 30% level of fishmeal replacements compared to their counterparts fed at 50% level of fishmeal replacement. Specifically, however, the Z2 ingredients had significantly ($P<0.05$) higher digestible crude protein values at both the 30% and 50% levels of fishmeal replacement compared to the Y3 and Z4 ingredients. In the same light, the Y3 ingredients were significantly superior to the Z4 ingredients in digestible crude protein at both the 30% and 50% levels of fishmeal replacement. Irrespective of level of fishmeal replacement, ether extract digestibility was similar for the Z2 and Y3 ingredients which were all significantly ($P<0.05$) superior to the Z4 ingredient at both levels. However, the ether extract digestibility of $Z4_{30}$ was significantly ($P<0.05$) higher than $Z4_{50}$. The digestible energy content followed the same pattern as the dry matter digestibility.

Table 7: Percentage digestible nutrients and energy of ADMJKC/bb test ingredients fed to *Clarias gariepinus* fingerlings in experimental diets

Values (means ± standard deviation) in a column followed by different superscript differ significantly ($P < 0.05$)

Treatment*			Dry matter (%)	Crude Protein (%)	Ether extract (%)	Energy (MCal/kg)
Fish meal	Proportion of ADMJKC/bb	Code				
50%	30% Treatment 1	$Y3_{30}$	42.75 ± 0.84^{d}	84.42 ± 0.59^{d}	63.49 ± 1.34^{c}	64.84 ± 1.12^{d}
50%	50% Treatment 1	$Y3_{50}$	39.27 ± 1.08^{c}	73.98 ± 1.16^{c}	62.52 ± 1.92^{c}	58.39 ± 1.54^{c}
50%	30% Treatment 2	$Z2_{30}$	42.27 ± 0.84^{d}	85.91 ± 0.80^{d}	60.43 ± 1.47^{c}	64.70 ± 1.26^{d}
50%	50% Treatment 2	$Z2_{50}$	39.80 ± 1.32^{c}	83.36 ± 1.27^{d}	59.99 ± 2.59^{c}	59.15 ± 2.04^{c}
50%	30% Treatment 3	$Z4_{30}$	31.31 ± 1.40^{b}	57.60 ± 1.2^{b}	54.21 ± 2.42^{b}	32.37 ± 1.78^{b}
50%	50% Treatment 3	$Z4_{50}$	26.82 ± 1.56^{a}	30.63 ± 1.57^{a}	24.52 ± 3.92^{a}	13.26 ± 4.05^{a}
Standard error of the mean			1.45	4.81	3.33	4.71

d) Economic performance of Clarias gariepinus fingerlings on ADMJKC/bb based diets

The economic performance of *Clarias gariepinus* fingerlings fed ADMJKC/bb based diets is shown in Table 8. The financial value of the body mass gain for the control fish was significantly ($P<0.05$) higher than the fishes fed ADMJKC/bb based diets. The fishes fed 30% level of fishmeal replacement significantly ($P<0.05$) had higher financial value of body mass gain compared to their counterparts fed 50% level of fishmeal replacement, with the exception of the $Z4_{30}$ ingredient which did not differ significantly ($P>0.05$) from its $Z4_{50}$ counterparts. Similarly, the control significantly ($P<0.05$) had higher cost of feed consumed compared to the fishes fed ADMJKC/bb based diets. Also fishes fed ADMJKC/bb based diets at 30% significantly ($P<0.05$) had higher cost of feed consumed than their counterparts fed at 50% level of fishmeal replacement. The cost of feed consumed by $Z4_{30}$ was significantly ($P<0.05$) lower than that of $Z2_{30}$ and $Y3_{30}$. The cost of feed consumed by $Z4_{50}$ was significantly ($P<0.05$) lower than the cost of feed consumed by $Z2_{50}$ and $Y3_{50}$. In the same line the cost of feed consumed by $Z2_{50}$ was significantly ($P<0.05$) lower than the cost of feed consumed by $Y3_{50}$. The control and fishes fed ADMJKC/bb based diets at 30% of fishmeal replacement did not differ significantly ($P<0.05$) in feed cost per gram of gain. However, these fishes fed ADMJKC/bb based diets at 30% level of fishmeal replacement significantly ($P<0.05$) had lower feed cost per gram of gain compared to their counterparts fed ADMJKC/bb based diets at 50% level of fishmeal replacement. The most expensive gains were made by fishes fed $Z4_{50}$ ADMJKC/bb based diets while the least expensive gains were made by the control fishes (compare17.85 fcfa/g to 0.93 fcfa/g). The economy of gain followed the same trend as the feed cost per gram of gain.

Table 8: Economic performance of *Clarias gariepinus* fingerlings fed ADMJKC/bb based diets for 8 weeks.

Values (means ± standard deviation) in a column followed by different superscript differ significantly ($P < 0.05$)

Treatment*			Value of body mass gain (FCFA)	Cost of feed consumed (FCFA)	Feed cost per gram of gain (FCFA/g)	Economy of Gain
Fish meal	Proportion of ADMJKC/bb	Code				
100%	Control *	Control	18.79 ± 2.15^{d}	8.64 ± 0.15^{c}	0.93 ± 0.09^{a}	0.46 ± 0.05^{a}
50%	30% Treatment 1	$Y3_{30}$	7.44 ± 1.24^{c}	5.49 ± 0.27^{a}	1.49 ± 0.18^{a}	0.75 ± 0.09^{a}
50%	50% Treatment 1	$Y3_{50}$	2.22 ± 1.02^{a}	4.27 ± 0.28^{b}	4.62 ± 2.63^{b}	2.31 ± 1.31^{b}
50%	30% Treatment 2	$Z2_{30}$	7.27 ± 1.32^{c}	5.22 ± 0.25^{b}	1.46 ± 0.23^{a}	0.73 ± 0.12^{a}
50%	50% Treatment 2	$Z2_{50}$	1.67 ± 0.55^{a}	3.88 ± 0.12^{a}	5.10 ± 2.06^{b}	2.55 ± 1.03^{b}
50%	30% Treatment 3	$Z4_{30}$	4.20 ± 0.61^{b}	4.83 ± 0.22^{b}	2.32 ± 0.30^{a}	1.16 ± 0.15^{a}
50%	50% Treatment 3	$Z4_{50}$	0.38 ± 0.02^{a}	3.38 ± 0.07^{a}	17.85 ± 0.49^{c}	8.93 ± 0.24^{c}
Standard error of the mean			1.31	0.36	1.26	0.63

Control= Fish meal, blood meal, maize, palm oil, bone meal, premix and Titanium oxide, without any Jatropha meal and soybean meal.

IV. Discussion

The proximate composition of maize, fishmeal, soybean cake and blood meal used in this trial is within normal range and agree with values of Tacon *et al.* (2009). However, the crude protein values for the ADMJKC/bb appear lower than expected. Since bovine blood has more crude protein (81.50%) compared to JKC (29.83%), it was normal to expect that the Y3 ingredient should contain more crude protein than Z2 and Z4, but the converse was observed. Also, Z2 and Z4 were expected to be the same or closer. The difference in crude protein values among the ADMJKC/bb is an indicator that they are not just mixtures of JKC and bovine blood. They have surely undergone biochemical processes which are unique to each mixture. The Y3 and Z4 ingredients were remoistened daily at 66% dry matter while Z2 was not. The Z2 therefore was undergoing a process similar to solid state fermentation. However, the regular remoistening of Y3 and Z4 probably induced a different path of microbial succession that was responsible for the net loss of nitrogen and, consequently, lower crude protein. Such a loss was higher with the Y3 ingredient that had just two parts of JKC compared to the Z4 ingredient that had three parts of JKC. According to Philippot *et al.* (2013), denitrification is the main biological process responsible for the return of fixed nitrogen to the atmosphere, thus completing the N-cycle. Denitrifiers are taxonomically diverse microorganisms capable of reducing soluble nitrogen oxides into the gases N_2O and N_2. It is possible that Y3 and Z4 that were constantly remoistened attracted an active denitrification microbial population that was responsible for its lower than expected crude protein content.

Similarly the microbial succession path undertaken by the Z2 ingredient may be responsible for its comparatively lower level of ether extract. The Z2 ingredient could attract much lipase producing microbes which contribute in lowering the level of ether extract. It is also possible that *J. curcas* lipase activity which is endogenous in dormant and germinating seeds was enhanced by the Z2 treatment. Abigor *et al.* (2002) noted that endogenous *J. curcas* lipase, hydrolyses *J. curcas* oil, at twice the rate for palm kernel and coconut oils. Mendes and Castro (2005) also used commercially available lipase preparations from animal source to decrease fat and organic contents in dairy wastewater.

The anti-nutrients were lowest in Y3, moderate in Z2 and highest in Z4. However, the differences were not significant ($P>0.05$) except for lectin activity, where Z4 was significantly ($P<0.05$) higher than Y3. The lower level of anti-nutrients in Y3 could be as a result of its lower content of JKC in the mixture, or a combination of JKC content and the unique auto-detoxification path taken by this ingredient. However, the difference between Z2 and Z4 was solely dependent on the unique auto-detoxification path taken by each ingredient following the treatment applied.

All the diets which compared three ADMJKC/bb as ingredients at 30% and 50% levels of fishmeal replacement in this study were iso-nitrogenous and iso-calorific, and were similar in proximate composition with the diets for *Clarias gariepinus* reported by Fakunle *et al.* (2013), Alatise *et al.* (2014) and Musiba *et al.* (2014). Slow eating behaviour observed for $Z4_{50}$wassimilar to that reported by Kumar *et al.* (2011) for carp fed a poorly detoxified JKM diet at 75% level.

The control was significantly better than the ADMJKC/bb ingredients in all growth parameters. This was followed by the lower replacement levels of Y3 and Z2 ($Y3_{30}$ and $Z2_{30}$) which recorded similar performance but significantly ($P<0.05$) outperformed the $Z4_{30}$ and the higher replacement levels ($Y3_{50}$, $Z2_{50}$ and $Z4_{50}$). This result is not in agreement with Fakunle *et al.* (2013) and Alatise *et al*. (2014) who reported that a 30% level of including boiled Jatropha in the diets of *Clarias gariepinus* significantly ($P<0.05$)outperformed the control with 0% boiled Jatropha kernels. Several studies (Aregheore, *et al*., 2003; Martınez-Herrera *et al*., 2006; and Gogoi *et al.,* 2014) have concluded that moist heat has no significant effect on reducing phorbolesters which is the main toxic component in jatropha seeds.The poorer performance of the higher replacement levels ($Y3_{50}$, $Z2_{50}$ and $Z4_{50}$) as well as Z4 ingredient irrespective of levels is an indication that ADMJKC/bb ingredients still had some level of toxicity. Detoxified JKM has been reported to contain "residual toxicity". This was detected after a feeding trial with carp which revealed that the sensitivity of the HPLC method for determination of phorbol esters has to be enhanced (Kumar *et al.,* 2011). Working with Carp, Kumar *et al*. (2011) observed highest body mass gain, specific growth rate, and metabolic growth rate and energy production value for the group fed JKM detoxified for 60 minutes and replacing 50% fishmeal. These results were statistically similar to that for the control group and significantly ($P<0.05$) higher than for all test ingredient groups. Therefore, a 50% level of fishmeal replacement by detoxified JKC for *Clarias gariepinus* is achievable since *Clarias gariepinus* like carp is classified more as an omnivore. Moreover, carp is not able to utilize high level (more than 50% of FM protein replacement) of plant derived protein in the diet because of low palatability, high fibre and anti nutrients content (Kumar *et al*., 2011), whereas, *Clarias gariepinus* has recorded good performance on purely plant proteins (Musiba *et al*., 2014).

The intermediary and final survival rates were similar between the control and the ADMJKC/bb ingredients. Also, intake levels did not affect survival except for the Z4 where FSR was significantly ($P<0.05$)lower for $Z4_{50}$ than $Z4_{30}$. This may suggest that the Z4 ingredient retained a higher level of toxicity than

the Y3 and Z2 ingredients. The feed utilization data further buttress this suggestion because the FCR was statistically similar between the control $Y3_{30}$ and $Z2_{30}$ ingredient based diets. In addition, the feed assimilation data indicates that $Y3_{30}$ and $Z2_{30}$ were statistically similar in PPV and LPV, but significantly ($P<0.05$)superior to all other ADMJKC/bb ingredients.

The fish fed ADMJKC/bb based diets were similar ($P>0.05$) in whole proximate composition except in ether extract, NFE and energy where the control was significantly ($P<0.05$) different from the $Z4_{50}$ ingredient based diets. According to Petricorena (2014), chemical composition of fish varies greatly among species and from an individual fish to another, depending on age, sex, environment and season. In this study all other factors were held constant except type and level of ADMJKC/bb ingredients. The differences observed therefore resulted from the test ingredients. There was similarity of this result with the results obtained by Kumar *et al.* (2011) for carp. The higher ether extract and gross energy values for the control and the fishes fed lower levels of ADMJKC/bb ingredients based diets is an indication of better fat deposition in the tissues. At higher levels of inclusion, particularly the Z4 based ingredient, efforts at detoxifying anti-nutrients could contribute in reducing ether extract and gross energy of whole fish carcass. This suggestion is corroborated by the lower PPV and LPV for fish fed higher levels of ADMJKC/bb ingredients. The NFE values followed the same trend as ether extract and gross energy. The only difference is that NFE values were higher where EE and GE values were lower. The NFE values for *Clarias gariepinus* fingerlings recorded in this study are lower than those reported for another type of African catfish (*Heterobranchus bidorsalis*) by Akhirevbulu and Okonji (2013). This difference could be as a result of age and species differences. NFE is a measure of the readily available carbohydrates calculated by subtracting all proximate components from100.Therfore it is likely to suffer from errors compounded in measuring other proximate components.

In the present study, data on digestible nutrients and energy (dry matter, crude protein, ether extract and gross energy) of ADMJKC/bb test ingredients fed indicates that the Z4 ingredient, irrespective of levels, significantly ($P<0.05$) had the lowest digestibility. This therefore reduces its chances of being selected for further development.

The control was significantly ($P<0.05$) superior to the ADMJKC/bb ingredients based diets in terms of the financial value of body mass gain. Conversely, it also significantly ($P<0.05$) recorded a higher cost of feed consumed. However, when evaluated in terms of feed cost per gram of gain as well as the economy of the gain, the control was statistically similar ($P>0.05$) to the $Y3_{30}$ and $Z2_{30}$ ADMJKC/bb ingredients based diets. Feed cost per gram of gain is a parameter that combines biology with economics. It tells if the gains made biologically are financially expensive or not. The similarity of $Y3_{30}$ and $Z2_{30}$ ADMJKC/bb ingredients based diets with the control in feed cost per gram of gain is an indication that these levels of ADMJKC/bb ingredients can be recommended for *Clarias gariepinus* fingerlings. At the $Y3_{50}$ and $Z2_{50}$ levels, the elevated effects of anti- nutrients make it both biologically and economically not feasible for practical diets with *Clarias gariepinus* fingerlings. These results also confirm that the Z4 ADMJKC/bb ingredient is not biologically and economically practical for inclusion into diets of *Clarias gariepinus* fingerlings at either the 30 % or 50% level of fishmeal replacement.

V. Conclusion

Clarias gariepinus fingerlings fed diets containing either Y3 or Z2 ADMJKC/bb diets, where any replaces 30% of fishmeal, have both positive biological and economic performance comparable to the control. At higher levels of 50% fishmeal replacement, biological and economic performance reduces. After this study, the Y3 and Z2 ingredients have been recommended for further development

Abbreviations

ADMJKC/bb: auto-detoxified mixtures Jatropha kernel cake and bovine blood; ANOVA: analysis of variance; ADG: Average daily gain; BMG: Body mass gain; FCR: Feed conversion ratio; FSR: Final survival rate; ISR: Interval survival rate; JKC: Jatropha kernel cake; JKM: Jatropha kernel meal; LPV: Lipid production value; MGR: Metabolic growth rate; NFE: Nitrogen free extract; PER: Protein efficiency ratio; PPV: Protein productive value; SGR: Specific growth rate;Y3:Jatropha kernel cake and bovine blood, mixed at a ratio of 2:1. Heated, spread dried, remoisten to 66% dry matter; Z2: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Unheated, spread dried without remoistening; Z4: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Unheated, spread dried, Remoistened to 66% dry matter.

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Supporting Information

Table A: Proximate composition and anti-nutritional factors of ingredients used in the feeding trial

Parameter	Ingredient						
	Maize	Fish meal	Soybean Meal	Blood meal	Y3	Z2	Z4
Dry matter (%)	88.40	94.10	94.60	92.00	92.09	94.33	93.71
Crude protein (%)	8.00	66.80	45.20	81.50	34.78	50.72	41.81
Ether extract (%)	5.90	8.10	12.60	3.50	39.64	32.89	37.36
Crude fibre (%)	1.90	1.04	4.98	0.00	11.56	10.03	11.43
Ash (%)	4.20	13.80	6.50	12.36	7.31	5.95	7.16
NFE (%)	80.00	10.26	30.72	2.64	5.38	0.41	2.24
Gross energy (Kcal/100g)	394.3	442.3	419.7	486.0	485.61	460.77	467.74
Anti-nutrients							
Phytates (% dry matter)	ND	ND	ND	ND	1.71	1.92	2.29
Trypsin inhibitor (TIU/mg)	ND	ND	ND	ND	3.99	4.13	4.31
Saponins (g/100 g)	ND	ND	ND	ND	0.10	110	0.125
Tannin (%)	ND	ND	ND	ND	0.044	0.046	0.047
Lectin activity (inverse of mg meal per mL of the assay that produced haemagglutination	ND	ND	ND	ND	8.50	9.13	9.68
CrudePhorbol Esther (mg/g)	ND	ND	ND	ND	0.36	0.41	0.47

ND= Not Determined

Table B: Proximate composition of diets used in feeding trial which compared three ADMJKC/bb as ingredients at 30% and 50% levels of fishmeal replacement

Parameter	Treatment*						
	Control	$Y3_{30}$	$Y3_{50}$	$Z2_{30}$	$Z2_{50}$	$Z4_{30}$	$Z4_{50}$
Dry matter (%)	93.4	93.2	93.1	94.3	94.21	94.3	94.1
Crude protein (%)	38.12	38.02	37.99	38.68	38.08	38.15	38.04
Ether extract (%)	9.15	9.53	9.98	8.43	8.57	9.17	9.53
Crude fibre (%)	3.26	3.11	3.17	3.1	3.12	3.21	3.36
Ash (%)	7.14	8.41	8.53	8.45	8.74	8.39	8.55
NFE (%)	42.33	40.93	40.33	41.34	41.49	41.08	40.52
Metabolisable energy (Kcal/kg)	3032	3014.45	3001.85	3068.1	3091.5	3023.95	3002.5
Cost / Kg (fcfa)	424	415.75	420.65	402.15	390.25	413.75	414.75

Table C: Mean weekly water quality parameters of 35L rectangular tanks used in feeding trial

Week	Temp (°C)	pH	Dissolved Oxygen (mg l^{-1})	Conductance (μhom/cm^3)	NH_3 (mgl^{-1})	Nitrate (mg l^{-1})
1	24.0	7.3	5.8	80.9	0.1	0.8
2	24.5	7.3	5.7	84.8	0.1	0.9
3	24.5	7.4	5.6	85.5	0.1	0.8
4	23.5	7.4	5.7	85.7	0.1	0.8
5	24.5	7.5	5.8	85.7	0.1	0.8
6	24.5	7.6	5.8	85.8	0.1	0.8
7	25.5	7.8	5.8	80.6	0.1	0.8
8	25.0	7.8	5.8	80.6	0.1	0.8

Table D: Apparent Digestibility Coefficients (%) of dry matter, crude protein, ether extract and gross energy of ADMJKC/bb ingredient based diets fed to *Clariasgariepinus* fingerlings

Treatment*	Dry matter Digestibility	Crude Protein Digestibility	Ether Extract Digestibility	Gross Energy Digestibility
Control	$80.31^{c}\pm0.3$	$91.82^{e}\pm0.20$	$93.76^{d}\pm0.53$	$81.65^{d}\pm0.64$
$Y3_{30}$	$71.53^{b}\pm0.7$	$83.97^{d}\pm0.42$	$66.88^{c}\pm0.84$	$72.78^{c}\pm0.80$
$Y3_{50}$	$66.41^{a}\pm0.9$	$79.91^{b}\pm0.83$	$61.12^{b}\pm1.20$	$68.39^{b}\pm1.10$
$Z2_{30}$	$71.16^{b}\pm0.7$	$82.46^{c}\pm0.57$	$66.15^{c}\pm0.92$	$72.58^{c}\pm0.90$
$Z2_{50}$	$66.08^{a}\pm1.1$	$79.57^{b}\pm0.91$	$60.38^{b}\pm1.62$	$68.38^{b}\pm1.46$
$Z4_{30}$	$70.93^{b}\pm1.2$	$81.96^{c}\pm0.87$	$65.88^{c}\pm1.51$	$70.46b^{c}\pm1.27$
$Z4_{50}$	$65.42^{a}\pm1.3$	$73.52^{a}\pm1.12$	$49.62^{a}\pm2.45$	$61.24^{a}\pm2.89$
SEM	1.08	1.15	2.81	1.30

Values are means (n = 3) ± Standard deviation.
Mean values in the same column with different superscript differ significantly ($P < 0.05$)
SEM = standard error of the mean

*Seven treatments designated as follows:
Control= Fish meal, blood meal, maize, palm oil, bone meal, premix and Titanium oxide, without any Jatropha meal and soybean meal.
$Y3_{30:}$=50% of Fish meal replaced by 30% Y3 ADMJKC/bb
$Y3_{50}$=50% of Fish meal replaced by 50% Y3 ADMJKC/bb
$Z2_{30}$=50% of Fish meal replaced by 30% Z2 ADMJKC/bb
$Z2_{50}$=50% of Fish meal replaced by 50% Z2 ADMJKC/bb
$Z4_{30}$= 50% of Fish meal replaced by 30% Z4 ADMJKC/bb
$Z4_{50}$=50% of Fish meal replaced by 50% Z4 ADMJKC/bb