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Evaluation of Processed *Jatropha curcas* cake as food supplement on catfish (*Clarias gariepinus*)

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Abstract

Jatropha curcas seed cake was processed using different methods of detoxification treatments (Hexane extraction + 95% ethanol extraction+ Autoclaving; 2% sodium hydroxide; 92% ethanol extraction + Autoclaving; 2% sodium hydroxide + days fermentation).the proximate composition and antinutrient of the untreated and treated *Jatropha curcas* cake were determined. The crude protein (CP) content of the untreated *Jatropha curcas* cake was 10.12 ± 0.012g/100g while (Ethanol + Autoclaving) showed reduction of 8.16 ± 0.02g/100g (Hex+Autoclaving+Ethanol); (2% NaOH) and (NaOH+ fermentation) showed increased in protein contents of 10.16 ± 0.01g/100g, 12.62 ± 0.02g/100g, 12.81 ± 0.01g/100g respectively. Lipid content were 9.15 ± 0.02g/100g, 5.81 ± 0.01g/100g, 4.13 ± 0.01g/100g, 3.14 ± 0.01g/100g, 3.72 ± 0.02g/100g, 3.67 ± 0,02g/100g (Control; Ethanol+Auclaving; Hex+Autoclaving+Ethanol; NaOH; NaOH+fermentation) respectively. The antinutrient values were reduced more in the solvent treatments than the chemical treatment with Ethanol+Autoclaving causing the highest reduction in antinutrient levels compared to other treatments. Fish (*Clarias gariepinus)* divided into groups of 20 each were fed processed *Jatropha curcas* supplemented diets at a rate of 0, 5%, 10%, 20% respectively, with biweekly monitoring. All fish on 20% *Jatropha curcas* supplemented died within 24days irrespective of the processing method. After 56days, the average weight gain was 82.35 ± 0.74g (Control feed), 25.00 ± 1.33g (92%Ethanol+Autoclaving), 20.49 ± 1.07g (2%Sodium hydroxide+fermentation), 15.62 ± 0.63g (Hexane+ 95% Ethanol+Autoclaving) and (2% Sodium hydroxide). These findings suggest that processing with 92% ethanol prior to autoclaving is the best method for detoxifying *Jatropha curcas* cake utilized in fish feed..

Keywords: Supplement; Catfish (*Clarias garieinus*); *Jatropha curcas*

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Introduction

Aquaculture is an important weapon in the global fight against malnutrition and poverty, particularly in the developing countries [1]. Increase in human population in these countries, along with changing perceptions of healthy food in affluent regions, are set to increase the demand for food fish. Aquaculture production seems to be responding to the increased fish demand and have exclusively increase the world fish production by 20 million tones. In addition to being the fastest growing food production sector of the world aquaculture activities currently employ about 9 million people (FAO 2000).

Feeding is the most important task in the intensive pond production of catfish [2], their feeding behavior can be an important clue to general health and to other conditions in the pond. Nigeria been in the fish farming, the quest to make it interesting by using low cost feed with adequate qualities of nutritional balanced content and available in our environment will be of great improvement to farmers. Even when the natural feed forms the main source of nutrition, supplemental feeding with artificial feed is necessary to obtain increased production in pond.

Fish meal is a favorite source of the high protein in fish feed. There

are essential amino acids and fatty acids that are present in fish meal but not present in tissue from plant or other animals. The proportion of global fishmeal production used for fish feeds has increase from 10 to 35% in the last fifteen years (Hardy, 2000). The cost of this fish meal has been a problem even in industrially advanced countries, so considerable research is now underway to find suitable substitute in order to formulate cheaper and highly nutritious feeds (Hardy, 2000). Hence the need to look at new possible aqua-feed ingredients of the required high nutritional quality is therefore imperative. High cost, increasing demand and uncertain availability of fishmeal plus risk factors associated with disease from animal protein sources resulted in nutritionist studying alternative sources for inclusive into the of fresh water and marine species. Coupling with remedial measures for other problems where international attention is focused, multifunctional plant that requires low input and are capable of surviving under poor soil conditions offer viable solution to multiple problems [3].

Fish feed have been developed in some countries notably USA, Japan and Sweden for catfish, salmon fish, eel, carps, and shrimp. In most case the formulations are proprietary secretes and too expensive for use in developing countries. The high cost of some feed ingredients like fish meal has been a problem even in industrially advance countries and so considerable research is now underway in to find suitable substitute order to formulate cheaper and highly nutritious feeds.

Although there has been a lot of research work on the production of fish feed to meet the nutrient requirement for cultivable fish in Nigeria. Good quality fish feed pellets are sparingly used by fish farmers. Protein form the bulk of fish feed ingredients, as fish culture principally is to produce quality fish food for human consumption. Any management techniques that will reduce the cost of production will result to increase in profit. It can serve as employment opportunities for thousands of people directly or in directly.

Therefore it is necessary to investigate a combination of available ingredient within the locality to produce fish feed of low cost that can meet the nutrient requirements of fish. Plant species promoted internationally for multiple purpose include Jatropha curcas, Moringa oleifera, Mucuna pruriens, Leucaena leucocephala, Sesbania aculeate, Sesbinia bispinosa and Stylosanthies hamata to name a few. These plants are capable of resisting adverse soil and climatic conditions and still sustain a reasonably high primary and secondary production. Research reports available on some of them indicate the potential to develop products of high nutritional quality [3]. These products however, also contain high levels of antinutritional, toxic principle that keep herbivores at bay. Utilization of these plants as animals and fish feeds would therefore not only depend on their nutritional content, but also on the presence and levels of detoxification. A challenge for tropical aquaculture research is therefore to identify products from these plants having the required nutritionally quality and development via treatment method to make them suitable for addition to fish feeds [3].

Jatropha curcas is a valuable multi-purpose crop that alleviates soil degradation, desertification, and Jatropha is a highly adaptable species. Its strength as a crop comes from its ability to grow on very poor, dry soil. It is recommended, from an economic standpoint, to be cultivated in marginal lands. Jatropha oil is inedible and its cake is very rich source of protein and its price is not distorted by competing food uses. Apart from other applications and potential uses of Jatropha-medicinal, animal feed, and soap. No local market for Jatropha was identified. The seed cake has 58-60 % crude protein (53-55 % true protein content) and the level of essential amino acids except lysine is higher than the FAO reference protein. However, this seed cake has been found to be highly toxic to fish and rats. The toxic principles have been identified as phorbol esters and other anti-nutrients, Feeding studies on rats and fish established that the seed meal prepared from seeds collected from a wild variety of Jatropha curcas which originated from Mexico is non-toxic. The protein, energy, lipid and amino acid contents in the seeds of the non-toxic provenance are similar to those of toxic varieties. Phytate constitutes a major single anti-nutritive component of Jatropha meals which is not heat labile and can have adverse

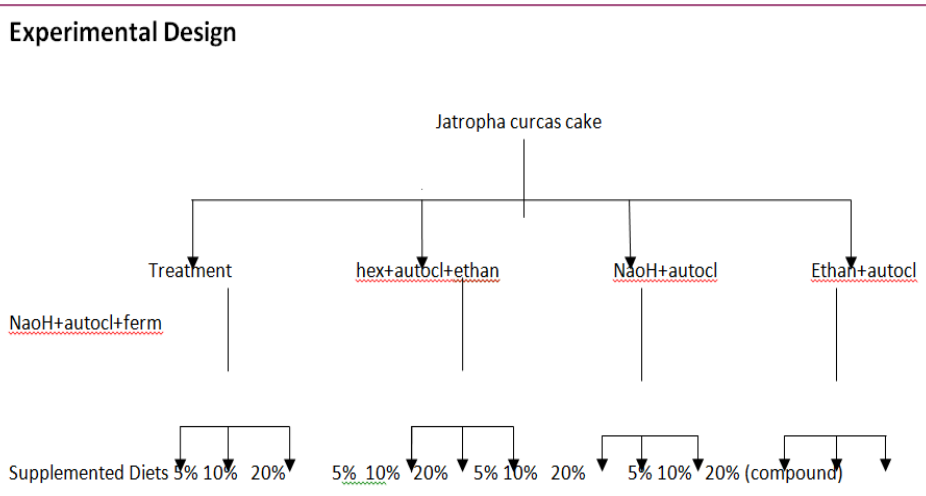

Figure 1 Experimental Design.

effects, whereas other anti-nutritional factors like trypsin inhibitors and lectin can be destroyed by heat treatment [4]. This work if successfully completed will provide suitable substitute for conventional feed which is likely to be cheaper and highly nutritious for the production of catfish and thus solving the problem of malnutrition in the country. It can also provide job opportunity.

Materials and Method

Experimental Design **(Figure 1).**

Group of Experimental Animal

1. Coppen (control feed)

2. 5% (Hexane extraction+ Autoclaving +Ethanol extraction treatment) supplemented diet

3. 5% (2% NaoH + Autoclaving treatment) supplemented diet

4. 5% (92% Ethanol + Autoclaving treatment) supplemented diet

5. 5% (2% NaoH + Autoclaving treatment +4days Fermentation treatment) supplemented diet

6. 10% (Hexane extraction+ Autoclaving +Ethanol extraction treatment) supplemented diet

7. 10% (2% NaoH + Autoclaving treatment) supplemented diet

8. 10% (92% Ethanol + Autoclaving treatment) supplemented diet

9. 10% (2% NaoH + Autoclaving treatment + 4days Fermentation treatment) supplemented diet

10. 20% (Hexane extraction+ Autoclaving +Ethanol extraction treatment) supplemented diet

11. 20% (2% NaoH + Autoclaving treatment) supplemented diet

12. 20% (92% Ethanol + Autoclaving treatment) supplemented diet

13. 20% (2% NaoH + Autoclaving treatment +4days Fermentation treatment) supplemented diet

Materials

- 250 fingerlings of *Clarias garepinus* (MSB fisheries Ltd)
- *Jatropha curcas* cake meal [NARICT Bassawa, Zaria]

Methodology

Detoxification of *jatropha curcas*

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Formulation of the Supplemented Diets

950g of coppens + 50g of detoxified *Jatropha curcas* cake =====5% feed supplement diet

900g of coppens + 100g of detoxified *Jatropha curcas* cake =====10% feed supplement diet

800g of coppens + 200g of detoxified *Jatropha curcas* cake =====20% feed supplement diet

These were mixed properly pelletized using meat grinder and packed in transparent cellophane bag

Reaging of Fish

Fish were obtained from MSB fisheries Ltd. Acclimatized for 14 days in a circular plastic bowl of 100litres capacity and all fed coppens (control feed) at 5% body weight of the whole fish weight. After acclimatization period (14days), fish were divided into thirteen (13) groups each in 50litres jerry-can that was cut open at the top and properly labeled. Filled with 45 litres of water which was allowed to stand for 24 hours in order to allow for dechlorination. Each jerry can contain 20 post fingerlings. The pH and temperature were monitored throughout the feeding trials.

The fish were fed 5%`10% and 20% supplemented diets, each of the four (4) processed *Jatropha curcas* cake for fifty-six(56) days. Their weights in each group were taken in triplicate biweekly. Feeding was done manually by hand twice each day at 5% weight of the fish. Fish were sacrifices and oil quality determined

Monitoring of Growth Indices in Fish

Fish growth is measured in terms of weight gain .Hence a balanced diet that meets fish nutritionally requirement is essential at all time. This was recorded every two [2] weeks**.**

Statistical Analysis

Data obtained were analyzed by analysis of variance [ANOVA] and student t-test at 95% confidence level [P=0.05]

Proximate analysis of detoxified *jatropha curcas* cake and supplemented diets

Determination of lipid content (a.o.a.c.1980)

Principle: This is the continuous extraction of fat content from the simple using suitable solvent e.g. petroleum either in a soxhlet extractor. In this principle, non-polar components of the sample are easily extracted into the organic solvent, ether.

Determination of crude fiber (a.o.a.c.1980)

Principle: This involves sequential digestion of the sample with dilute acid and alkali solutions. The residue obtained was ignited to obtain crude fiber.

Determination of crude protein kjeldahl method (a.o.a.c.1980)

Principle: The principle of this method is to digest the organic matter with sulphuric acid in the presence of a catalyst, render the reaction alkaline, then distil and titrate the liberated ammonia.

The Determination of Moisture Content (a.o.a.c.1980)

Principle: The method employed the determination of moisture in the sample is the measurement of the loss in weight due to drying at a temperature of about 105 ^{0}Clarias A watch glass was

washed and dried in an oven at 105 ^{0}c ;it was cooled and weighed empty.

Ash Content Determinatio (a.o.a.c.1980)

Principle: the term ash refers to the residue left after combustion of the oven dried sample and is a measured of the total mineral content

Determination of Carbohydrate

The total of protein, moisture content, ash content and lipid content subtracted from 100 gives the carbohydrate, and this is referred to as estimation by difference (PEARSON, 1976)

Determination of Antinutrient in Detoxified *Jatropha Curcas Cake*

Determination of trypsin inhibitor (ti) (on wuke, 2005)

Determination of total phenolics (tannins) (shahide and naczk 1989)

Determination of lectin using purification by affinity chromatography (felsted *et al* 1975)

Results

Figures 2-6.

Discussions

The protein content of the solvent treatment shows no significant difference from the untreated *Jaropha curcas* seed cake whereas the sodium hydroxide treatment and the pro- fermentation sodium hydroxide treatment shows increment in its value these could be due to the release of some bounded protein by the NaOH treatment employed.

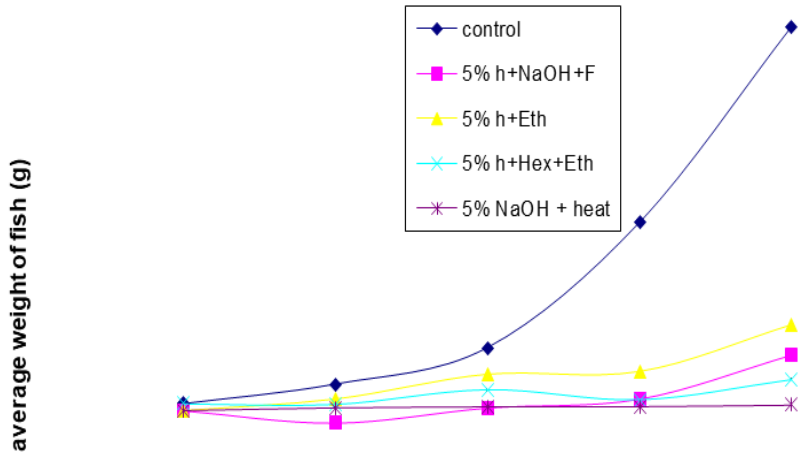

Figure 2 Show the graph of growth rate of *Clarias garepinus* on 5% diet for eight weeks. 0-2weeks control had the highest growth followed by (Eth+Autocl) then (Hex+Autocl+Etha), (NaOH+Autocl) and (NaOH+Autocl+Ferm) having the lowest. Week 2-4 (Etha+Autocl) still had highest growth followed by (Hex+Autocl+Etha), (NaOH+Autocl+Ferm) and (NaOH+Autocl) both having lowest. Week 4-6 all lower compare to control but (Etha+Autocl) highest in growth rate followed by (NaOH+Autocl+Ferm) then (Hex+Autocl+Etha) and (NaOH+Autocl) having the lowest.

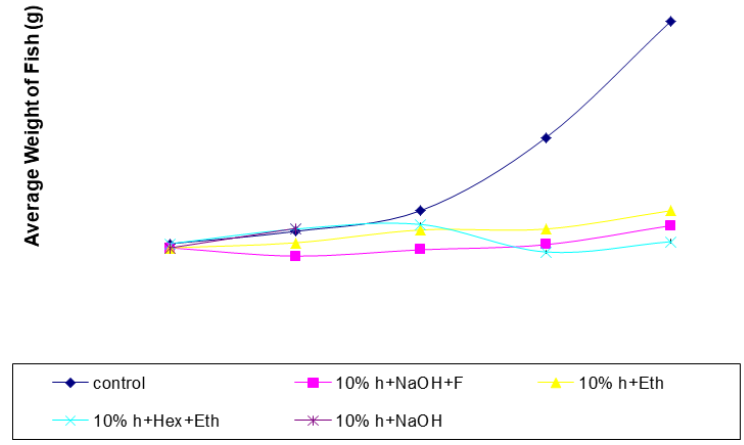

Figure 3 Comparism of growth rate for 10% supplemented diets on *clarias garepinus* (shows the graph of growth rate of Clarias garepinus on 10% diet. At weeks 0-2 all showed increased in weight except (NaOH+Autocl) that had decrease in weight. Weeks2-4 Control had the highest followed by (Hex+Autocl+Etha) then (Etha+Autocl) and (NaOH+Autocl+Ferm),while (NaOH+Autocl) on 10% all died at the end of weeks 2-4.in weeks 4-6 control still the highest followed by (Etha+Autocl) then (NaOH+Autocl+Ferm) and (Hex+Autocl+Etha) having the lowest. A wider margin in weight was observed in the control compare to all the treatments,followed by (Etha+Autocl) then (NaOH+Autocl+Ferm) and (Hex+Autocl+Etha) having the lowest).

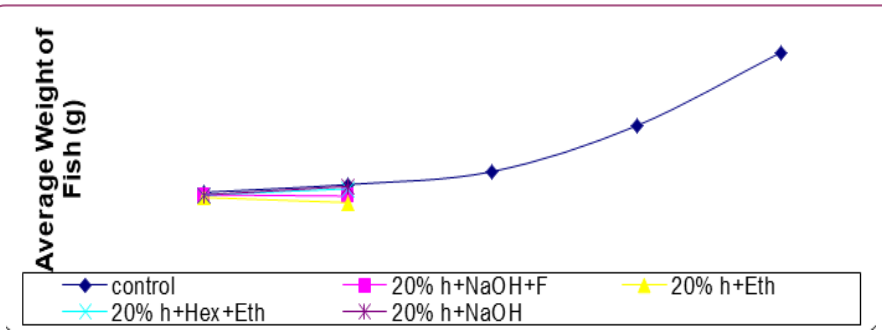

Figure 4 Comparism shows the graph of growth rate of Clarias garepinus on 20% diet control had the highest for weeks 0-2 followed by (NaOH+Autocl) then (Hex+Autocl+Etha) (NaOH+Autocl +Ferm) and (Etha+Autocl) with the lowestIrrespective of the various treatments all the fish died at end of weeks 0-2.

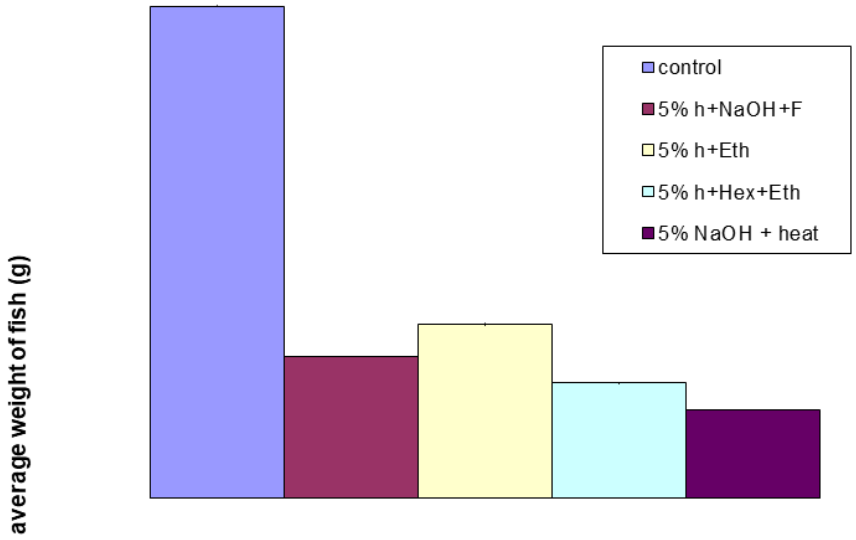

Figure 5 Shows the chart of comparism total growth rate of Clarias garepinus on 5% diet at weeks 8. Control had the highest followed by (Etha+Autocl) then (NaOH+Autocl+Ferm) , (Hex+Autocl+Etha) and (NaOH+Autocl) with the lowest growth rate.

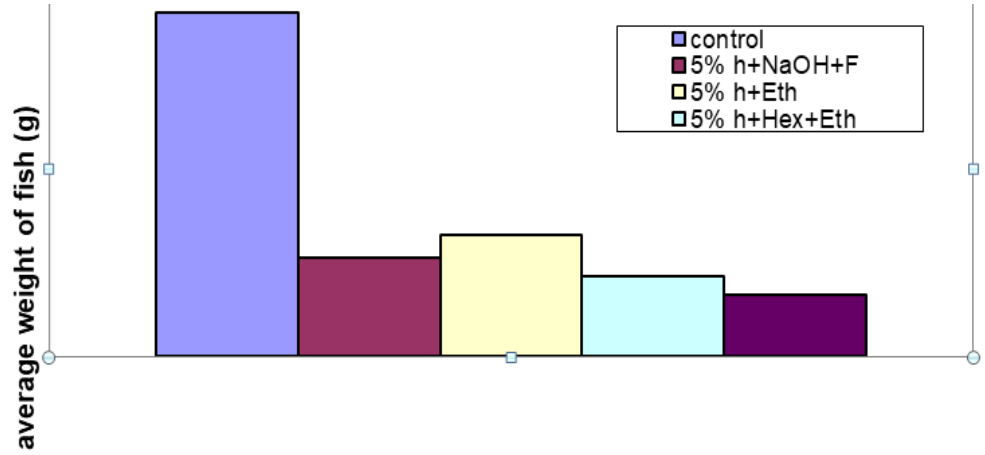

Figure 6 Show the chart of comparisonof the total growth rate of *Clarias garepinus* on 10% dietat 8 weeks Control had the highest followed by (Etha+Autocl) then (NaOH+Autocl+Ferm) and (Hex+Autocl+Etha) having thelowest value.

Substantial amount of total phenolics, lectins and trypsin inhibitors have been reported in *Jatropha curcas* by Makkar and Becker 2001 this agrees with their detection in this experiment, all treatment used in detoxification shows significant difference from the untreated *Jatropha curcas* seed cake. Monogastric and other warm blooded animals are known to be adversely affected

by the high proportion of phenolics substance present in feeds by reduction of feed intake, growth and nutrient availability and increase in endogenous loses of nitrogen through feaces. Also observed significant growth reduction in fish fed diets containing low level of total phenolics. The level in various supplemented diets may have negatively affected the growth paraments of fish fed such diets in this experiment. The slight reduction of the concentration of phenolics in treated *Jatropha curcas* seed cake may have contributed to the poor growth.

Lectin of the various treatments shows reduction from the untreated *Jatropha curcas* seed cake but then there was significant difference between the solvent extraction and the sodium hydroxide extraction treatments. Photoheamagglutinin in nut and legumes are considered as potential antinutrients and are known to decrease growth performance of animals. Many fish species have been reported to be sensitive to trypsin inhibitors. Similarly, and found that fish are sensitive to protease inhibitors. In this experiment although trypsin inhibitors were reduced respectively by all the treatments, the significantly reduced growth performance in *Clarias garepinus* fingerlings fed diets implies that residual trypsin inhibitors and lectins in the various treatments may have led to the poor performance. Related works on *Jatropha curcas* also reported the presence of other antinutrients such as phytates, saponins, these antinutritional factors which are mostly thermalstable may have contributed to the poor growth performances experienced in fish fed with the various methods used in detoxifying *Jatropha curcas* seed cake. The better performance of *Clarias garepinus* fed solvents extracted and pro-fermentation sodium hydroxide treatments of 5% and 10% supplemented diets of *Jatropha curcas* seed cake might be due to the high biological value of the protein derived from the higher proportions of fish meal contained therein the control feed (coppens) and relatively low levels of antinutritional factors contained in the detoxified *Jatropha curcas* seed cake in the experimental diets.

The result of this study shows that (Etha+Autocl)is the best method of detoxifying *Jatropha curcas* cake utilized in this experimental trial even though 5%, 10% and 20% of the various treatment of *Jatropha curcas* seed cake did not significantly affect the growth performance of *Clarias garepinus* fingerlings.

Recommendations

- The processed *Jatropha carcus* cake should be tested on other animals (chicken and rats)
- Further processing techniques should be employed
- Study that could get rid of the phorbol ester and curcin without affecting the protein content should be investigated

Conclusion and Suggestion

Jatropha curcas a multipurpose plant being promoted internationally as it has a potential of been use as biodiesel, research are on extensively on how to refine and optimize this important treasure, so also its cake after extraction was found to be very rich in protein with a value of 53-58% and was reported to contain toxic substances and antinutrients which makes it not fit for direct consumption without detoxification.

The question is which method will best detoxify this *Jatropha curcas* in other t make it fit for use as no enough record was available on the best method that qualify for this purpose. So many methods on detoxification are suggested but no one of them was said to completely detoxify it. This can only be answer if research is set to investigate the best method, which led to the scope of this experimental design to probe into the various methods available and used *Clarias garepinus* as a model

From the result obtained and discussed on the previous sections, one can say (Ethanol+Autoclaving) treatment was the best method of detoxification among the four treatments employed. Even though there was a wide from all the treatment as compared to the Control feed in this experiment.

Studies that will enhance and validate the safe use of *Jatropha curcas* cake in animal feed, especially economic animals should be looked into. Since *Jatropha curcas* is promoted internationally to look into the area of alternative fuel, it can as well solve the problem of malnutrition and sustain the economic well-being of the ever growing population of our Country, Nigeria and other Africa Countries.

Therefore it is further suggested that:

- Further research on the genetic composition to enhance it production in larger quantities
- Also the possibility of growing the nontoxic variety of *Jatropha curcas* will go a long way to really brighten the vision of this experimental designin our Country and other Africa Countries.

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