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Research Article

Fermented *Parkia biglobosa* seed Modulates Growth Performance, Feed Utilization and Proximate composition of Acetaminophen-Exposed *Clarias gariepinus*

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ABSTRACT

This study evaluates the effect of *Parkia biglobosa* seed on growth performance, feed utilization and proximate composition of *Clarias gariepinus* fingerlings exposed to acetaminophen toxicity**.** Formulated diets (FD1, 2, 3, 4, 5, 6, and 7) containing 0, 15, 20, 25, 30, and 35%, respectively of *Parkia biglobosa* seed were fed to twenty *C. Gariepinus* fingerlings per tank (10 L). Tank 2, 3, 4, 5, 6, and 7 had 300 mg of acetaminophen added daily. The feeding was twice daily at 2% body weight of the fish for eight weeks after the initial weight and length of the fish were taken. The results revealed a significant ($p > 0.05$) increase in protein, fiber and fats of formulated feeds (12.23±0.02, 50.25±0.21 and 7.50±1.42 respectively)% as compared to Acetaminophen-exposed fish fed with normal feed (10.23±0.01, 31.26±1.02 and 5.78±1.01)% of protein, fiber and fats respectively. Also, a significant ($p > 0.05$) increase in weight gain was observed in fish-fed formulated diets with varying graded inclusion of *P. biglobosa* seed (1.78±1.11gram) compared to Acetaminophen-exposed fish fed with normal feed having weight (-12.80±1.11gram). All formulated meals provided vital nutrients for growth and feed efficiency utilization in *C. gariepinus* exposed to toxic agents. However, formulated diets with 25, 30, and 35% inclusion of fermented *P. biglobosa* seed look more promising compared to others. Consumption of diets supplemented with fermented *P. biglobosa* seeds by the fish would improve its nutritional constituents despite its constant exposure to toxic agents.

Keywords: *Clarias gariepinus;* Fishmeal; Growth performance; *Parkia biglobosa* seed*;* Proximate composition

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INTRODUCTION

The termed inflammation is created as a response to conventional liver damage (Coussens & Werb, 2000). A very important mediator for the formation of inflammation is prostaglandin (Ricciotti & Fotz-Gerald, 2011). The common drugs used in treating acute and chronic inflammation are aspirin, ibuprofen, paracetamol, etc. Paracetamol, scientifically known as acetaminophen is generally used to combat pains (Graham & Scott, 2005).The acetaminophen mechanisms of action are still unclear. Its accepted mechanism of action is the prevention of the production of prostaglandin (Klinger-Gratz & Ralvenius, 2018). The metabolism of acetaminophen is determined by the drug dose and the patient's age (Mitchell *et al.,* 2011). Paracetamol metabolizes into cysteine metabolites and glucuronide sulfate in a healthy individual. It is relatively safe when the therapeutic doses are taken but in high doses, it becomes hepatotoxic (Eriksson *et al.,* 1992). The non-toxic metabolites generated during paracetamol metabolism were majorly through the conjugation process in the liver (Graham & Scott, 2005). There used to be conjugation saturation that produces excess acetyl para amino

phenol (APAP) during acute mega doses of the drug. The APAP produced is converted into a toxic metabolite to damage the liver through the activity of enzymes of cytochrome (Caparrotta *et al.,* 2018).

All over the world, the rapidly growing sector in the production of human foods is the aquaculture industry, and is considered the major food production sector (FAO, 2010; Ombugadu *et al.,* 2021). Orire & Ricketts (2013) have demonstrated that sustainable aquaculture practice depends on the effective formulation of nutritionally balanced fishmeal with the required nutrition of the fish at a lower cost. Fish farmers need to have better knowledge of the nutrition required by species of fish to produce nutritious feeds to achieve maximum growth and boost immune levels (Ombugadu *et al.,* 2021; Chenemerem *et al.,* 2022). Fish's higher growth rate lowers the cost of fish production. Previously, the supplementations of plant products as the source of protein and nutrients in aquafeed production have been demonstrated. In this regard, *Sesame indicum* seed meal (Yakubu*et al.,* 2020), sunflower meals (Ahmad *et al*., 2021), *Citrullus lanatus* Seed meal (Ombugadu *et al.,* 2021), Fermented Cottonseed Meal (Gu*et al.,* 2021) have been used in the preparation of fishmeal. Before now, researchers used plant products as the sources of protein, antioxidants, and nutrients in the production of fish feed. A complete or partial replacement of soybean in fishmeal with alternative proteins and antioxidant-rich stuff does not adversely affect fish wellness and production (Effiong *et al.,* 2019). Thus, replacing soybeans in fish feeds with locally available antioxidant and protein-rich stuff could promote the fish's health condition, production performance, and eliminate stress and disease conditions that might affect their production performance. In this study, the fermented African condiment from locust bean seed (*Parkia biglobosa*) meal was selected to replace soybeans as an alternative source of protein, antioxidants and nutrients.

Parkia biglobosa is a leguminous plant found in the Savannah area of Africa (Ojewumi *et al*., 2017). The seeds of the locust bean are the most valuable part of the plant with a high content of lipids (29%), protein (35%), and carbohydrates (16%), and are a good source of fat, antioxidants, and calcium for rural dwellers (Ntui *et al.,* 2012). Ojewumi *et al.* (2018) have demonstrated that the bark, leaves, flowers, roots, fruit, and seeds of *Parkia biglobosa* could be effectively used for the treatment of many diseases.

In Africa, there are many fermented foods, including fermented food condiments which are classified based on the food items from which they are derived (Olasupo *et al.,* 2010). Condiments are spices or food preparations added to food to improve its taste/flavor. The flavoring condiments are usually the product of fermentation processes caused by microorganisms on the proteins in leguminous seeds like African locust beans (Olasupo *et al.,* 2010). Fermented condiments from African locust bean seeds are locally called Iru by Yarubas, Dawadawa by Hausas, and Ogiri by Ibos (Olasupo *et al.,* 2010).The protein-rich and tasty food condiments derived from *P. biglobosa* are been used in making soup and stew in some parts of African countries (Danial, 2018).The fruit pulp, the leaves, and the seeds are been used in animal feed (Heuzé *et al.,* 2018). Obe *et al*. (2019) reported the use of bark extract from *P. biglobosa* tree for the treatment of wounds, bronchitis, pneumonia, and malaria fever. This study was therefore carried out to evaluate the Growth Performance, Feed Utilization, and Proximate composition of *Clarias gariepinus* fingerling suffering from acetaminophen-induced hepatotoxicity and then fed with Fishmeal Substituted with fermented seed of *Parkia biglobosa*.

MATERIALS AND METHODS

Study Area

This work was performed in the Animal House of the Department of Biochemistry and Biotechnology in the Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, Lapai, Nigeria. Lapai is located on latitude 8° 35' N and longitude 8° 32' E, altitude 181.53 m above sea level with an annual mean temperature of 34 °C, relative humidity of 40-86%, and average daylight of 9-12 hours as reported by NIMET (2011).

Sample Collection

Parkia biglobosa seeds were purchased from the Suleja market, Suleja and Fishmeal and Containers were acquired from Kure market Minna, Niger state in January 2022. The botanical identity of samples was confirmed at the Department of Biology, Ibrahim Badamasi Babangida University Lapai, Niger State.

Sample Preparation

Feedstuffs (*Parkia biglobosa* seeds), were cleaned to remove dirt and foreign materials using the hand-picking method, fermented for 24hrs, air-dried, and de-shelled manually. The de-shelled samples were finely ground to small sizes using mortar and pestle.

Feed Formulation and Pellet Preparation

The composition of soybean meal and formulated experimental feeds used for the treatment following the procedures of Olurin *et al*. (2006), and Ombugadu *et al.* (2021) are shown in Tables 1 and 2. The feed ingredients were pulverized by a mill (Poly Brizer Co., China) in the Aquatic Feed Plant (BehdanehShomal Co., Babolsar, Mazandaran, Iran) with a diameter of

less than 200 µm. The pulverized mixtures were pelletized by a feed pelletizer machine (Garma Electric, Amol, Mazandaran, Iran) with a diameter of 4 mm. The pelletized feeds were then air dried and stored in air-tight polythene bags until required.

The Formulated Feed

About six (6) dietary treatments were prepared from the processed feeds containing varying inclusions of fermented *Parkia biglobosa* seed (Tables 1 & 2). Formulated diet1 (FD1), control has 0% of *P. biglobosa* seed meal inclusion, formulated diet 2 (FD2) has 15% inclusion of *P. biglobosa* seed meal, formulated diet 3 (FD3) has 20%inclusion of *P. biglobosa* seed meal, formulated diet 4 (FD4) has 25% inclusion of *P. biglobosa* seed meal, formulated diet 5 (FD5) has 30% inclusion of *P. biglobosa* seed meal, formulated diet 6 (FD6) has 35% inclusion of *P. biglobosa* seed meal. Thus, the formulated feed samples were subjected to proximate analysis using the method of Olurin *et al.* (2006), which was modified by Ombugadu *et al*. (2021); Chenemerem *et al.* (2022).

Table 1: Experimental diet compositions

Ingredients	FD1 (0%)	FD2 (15%)	FD3 (20%)	FD4 (25%)	FD5 (30%)	FD6 (35%)
Soya beans	28	28	28	28	28	28
Fishmeal	16	16	16	16	16	16
Groundnut cake	40	25	20	15	10	0
P.biglobosa seed	0	15	20	25	30	35
Maize	4	4	4	4	4	4
Vitamin premix	2	2	2	2	2	2
Sodium chloride	2	2	2	2	2	2
Cassava flour	8	8	8	8	8	8
Total	100	100	100	100	100	100

FD: Formulated diet

Table 2: Quantitative gross composition of diet (2kg)

Ingredients	FD1 (0%)	FD2 (15%)	FD3 (20%)	FD4 (25%)	FD5 (30%)	FD6 (35%)
Soya beans	0.64	0.64	0.64	0.64	0.64	0.64
Fishmeal	0.28	0.28	0.28	0.28	0.28	0.28
Groundnut cake	0.72	0.42	0.32	0.22	0.12	0.00
P. biglobosa seed	0.00	0.33	0.40	0.50	0.60	0.72
Maize	0.17	0.17	0.17	0.17	0.17	0.17
Vitamin premix	0.02	0.02	0.02	0.02	0.02	0.02
Sodium chloride	0.01	0.01	0.01	0.01	0.01	0.01
Cassava flour	0.16	0.16	0.16	0.16	0.16	0.16
Total	2.00	2.00	2.00	2.00	2.00	2.00

FD: Formulated diet

Procurement and Acclimatization of Experimental Fish

This study was conducted around July and August 2023. A total of one hundred and forty (140) of *C. gariepinus* fingerlings of mixed-sex but of the same age were bought from Lapai Gwari fish Village, along Lapai – Paiko-Minna road, Niger State. The average weight and length of the fish were 148.2±2.14g and 28.23±1.21, respectively. The fishes were transported in an aerated aquarium into the Department of Biology, Ibrahim Badamasi Babangida University, Lapai, Niger State. Fishes were acclimatized to experimental conditions for one week before the feeding trial and fed with a commercial fish meal (2mmVital feed, company, Nigeria) three times daily as described by Fakunle *et al*.(2013).

Experimental conditions

Upon fish adaptation to the conditions of rearing, they were divided into seven (7) experimental treatments. That is seven (7) glass tanks (10 L) with 20 *C. gariepinus* fingerlings per rear tank and each rear tank was replicated three (3) times. All rearing tanks were equipped with an aerator and thermometer, and water-regulating valves. The siphoned of leftover feed in the aquarium water was done every day to avoid infections and mortality during the period of the experiment (Kumar *et al.,* 2011). The paracetamol-induced oxidative stress model was achieved using the method modified by Ndatsu *et al.* (2020).

Experiment Design and Treatments

C. gariepinus fingerlings were randomly picked and grouped into seven (7) different aquarium tanks (10 L), namely,Tank1, 2, 3, 4, 5, 6, and 7 with twenty (20)

C. gariepinus placed in each tank (Kumar *et al.* 2011; Nisarat *et al*., 2021; Antony *et al.,* 2022). The feeding trial is shown in Table 3. Each aquarium tank was fed with each formulated diet (Table 2), individually, as shown in Table 3, which was replicated three (3) times. The feeding trial commences as below after fish are starved for 24 hours.

Table 3: Experimental Treatment

AT (10 L)	NF	FD	Paracetamol
		FD (20 g/kg/day)	300mg/kg/day
1	20	FD1 (Positive control)	-
2	20	FD2 (Negative control)	+
3	20	FD3: Formulated diet with 15% *P. biglobosa* seed meal	+
4	20	FD4: Formulated diet with 20% *P. biglobosa* seed meal	+
5	20	FD5: Formulated diet with 25% *P. biglobosa* seed meal	+
6	20	FD6: Formulated diet with 30% *P. biglobosa* seed meal	+
7	20	TD7: Formulated diet with 35% *P. biglobosa* seed meal	+

AT: Aquarium tank; NF: Number of fish; FD: Formulated diet (formulated diet)

At the beginning of the experiment, the weight and length of the fish (Initial weight and length) were observed and recorded. The fish were fed on experimental diets twice (2) a day at 2% of their body weight for eight (8) weeks. The mean final weight and length of the fish were taken after the 56th day of the experiment and the mortality observed was also recorded. This was done following the internationally accepted principle for laboratory animal use and care (NIEHS. 1985). At the end of 8 weeks, the proximate analysis of the fish was also done and recorded.

Determination of Proximate Composition of Feed

This was done before the feeding trial using the method described by AOAC (1990); Effiong *et al.,* (2019), and Ombugadu *et al*. (2021).

Moisture Content

This was determined by oven-drying the sample after the Petri dish was cleaned, oven-dried, and weighed using a weighing balance (W_1). Thereafter, 4g of individual samples were weighed in a pre-weighed Petri dish as W_2, separately. The sample in each pre-weighed dish was taken into the oven for drying at 70^{o}C for 5 hours. This was followed by taking the samples into desiccators to cool for 25 minutes and weighing them until constant weight (W_3) was attained.

$$\text{Moisture (\%)} = \frac{\text{Weight lost by drying}}{\text{Sample weight taken}} \text{X } 100$$

Crude protein content

This was done using the Micro-Kjedahl Apparatus. The method involves three stages, namely; digestion, distillation, and titration. Digestion was done in the fume cupboard by adding 0.5g of each sample with 10ml of H_2SO_4into digesting tubes while selenium was added to the tube as a catalyst. The digestion was continued in the fume cupboard until the digestion changed colourless. The digest was allowed to cool and made up to 50ml using distilled water before the percentage of nitrogen was determined. Into the distillation apparatus, 5ml of the sample was pipetted, and 10ml of 40% NaOH was added. Also, into a 100ml conical flask, 5ml of 2% boric acid was placed and three drops of phenolphthalein as an indicator was added. Thereafter the titration was performed using 0.01M of HCL.

The calculation of protein content was done using the formula:

$$\text{Nitrogen (\%)} = \frac{\text{Titre value x 0.1 HCl x0.014 x 100 x 10}}{\text{Weight of sample used}} \text{X } 100$$

The content of fish crude protein was calculated by multiplying 6.25 with nitrogen content.

Crude protein (%) = Nitrogen x 6.25

Crude Fat Content Determination

The Soxhlet apparatus was used to determine crude fat. Briefly, 120 ml of petroleum ether was introduced into the flask and boiled at 55^{o}C. The filter paper removed from the desiccators was weighed as W_1 and 0.4g of the sample was weighed onto filter paper as W_2. Thereafter, the extraction processes took place for 4 hours and the oil residue obtained was the oil content. Then the filter paper in the desiccators was removed and weighed as W_3.

$$\text{Lipid (\%)} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \text{X } 100$$

Note: W_1 = Weight of the filter paper; W_2 = Weight of filter paper sample; W_3 = Weight of sample after extraction.

Ash content determination

This is done by ashing the sample. The empty crucible was weighed as W_1 and 2g of the sample was placed in the crucible and weighed as W_2. The crucible containing the sample was then placed in the muffle furnace set at 500^{o}C for 1hour. The crucible was removed from the furnace, cooled, and weighed as W_3.

$$\text{Ash}\ (\%) = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \text{X}\ 100$$

Note: W_1 = Crucible weight; W_2 = Crucible and sample weight; W_3 = Weight of the crucible and ash sample.

Crude Fibre Determination

Briefly, exactly 1g (W_1) of the sample was weighed inside the 1L of the conical flask with 100ml of 2% of H_2SO_4 and boiled at 100°C for 20 minutes. Thereafter, the boiled mixture was filtered and washed using hot distilled water. The filtrate inside the flask was topped with 200ml of 2% NaOH, boiled for 20 minutes, and filtered. Then the residue collected was scraped on the crucible, dried at 102°C, allowed to cool, and weighed as W_2. The dried residue was ashed again at 500°C for 80 minutes inside the furnace, allowed to cool, and weighed as W_3.

$$\text{Fibre}(\%) = \frac{\text{W2} - \text{W3}}{\text{W1}} \text{X}\ 100$$

Carbohydrate Content Determination

This was determined by subtracting the total sum of %crude protein, %fat, %ash, and %crude fiber from 100.

Growth Performance Determination

This involves the growth and nutrient utilization parameters of the experimental animal's calculations following the method of Kumar *et al.* (2011), which was modified by Yakubu *et al*. (2020) using the appropriate formulae as follows;

Weight Gain (WG)

During the experimental treatment, the weight gains by each group were recorded. That is individually, the weight of fish was taken using a sensitive weighing balance and the respective average weight per group was calculated.

$$\text{MWG} = \text{MWf} - \text{MWi}$$

Note: MWf = Mean final weight; MWi = Mean initial weight

Average Weight Gain Percentage (%MWG)

The calculation was done using the formulae:

$$\%\text{MWG} = \frac{\text{Wf} - \text{Wi}}{\text{Wi}} \text{X}\ 100$$

Note: Wf = final average weight; Wi = Initial average weight

Percentage (%) Survival Rate (SR)

$$\%\text{Survival rate (SR)} = \frac{\text{Total Fish Survived}}{\text{Total fish used}} \text{X}\ 100$$

Percentage (%) Specific Growth Rate (SGR)

$$\%\text{Specific weight gained (SGR)} = \frac{\text{LogWf} - \text{LogWi}}{\text{T (day)}} \text{X}\ 100$$

W_I = Initial Weight

W_F= Final Weight

Log = Logarithm

T = Time taken in days

Percentage (%) Feed Conversion Ratio (FCR)

$$\%\text{Feed conversion ratio(FCR)} = \frac{\text{Total food given (g)}}{\text{Total weight gained (g)}}$$

Percentage (%) Gross Feed Conversion Efficiency (GFCE)

$$\%\text{Gross feed conversion efficiency(GFCE)} = \frac{1}{\text{FCR}}$$

Feed Intake (FI)

It was done by subtracting leftover feed from the feed supplied

$$\text{FI} = \text{W0} - \text{WI}$$

Note: W0 = Weight of feed supplied, W_I = The left over feed

Percentage (%) Mortality Rate (MR)

$$\%\ \text{Mortality rate (MR)} = \frac{\text{Number of dead fish}}{\text{Number of fish stocked}} \text{X}\ 100$$

Data Analysis

The mean±standard deviation was used to present the data recorded and one-way analysis of variance (ANOVA) was used to analyse the data. Differences were considered statistically significant at $P<0.05$ (Zar, 1996).

RESULTS

In Table 4, the proximate compositions of formulated diets are presented. Significant ($P>0.05$) variations in the proximate composition were exhibited by the diets with varying inclusion of fermented *P. biglobosa* seed meal. High moisture (MC) ranged from 8.01 – 8.18% with no significant ($P<0.05$) difference recorded among FD3 (diet with 15% *P. biglobosa* seed meal), FD4 (diet made up of 20% *P. biglobosa* seed meal), FD5 (diet with 25% *P. biglobosa* seed meal), FD6 ((diet with 30% *P. biglobosa* seed meal), and FD7 (diet composed of 35% *P. biglobosa* seed meal) when compared to the control group (FD1); a diet without *P. biglobosa* seed meal. Higher crude fiber (CF), ash (AS), and crude protein (CP) contents ranged from 12.02, – 12.23%, 4.32 – 4.53%, and 50.05 – 50.25%, respectively, with no significant ($P<0.05$) difference observed in FD5, 6, and 7, while FD3 and 4 showed no significant ($P<0.05$) difference in CF, AS, and CP among the treatments. FD1shows lower CF, AS, and CP contents with 10.23, 1.51, and 31.23%, respectively as compared to others. Furthermore, elevated %lipid (LP) contents with no significant ($P<0.05$) differences were noted in FD6 and 7 (7.48 and 7.50%, respectively). These were followed by no significant differences in lipid composition in FD3, 4, and 5 at 6.01, 6.11, and 6.40%, respectively. Carbohydrate (CA) compositions were noted to be higher with no significant ($P<0.05$) difference in FD1 at 44.81%, while FD3 and 4 had CA contents of 30.22

and 30.00%, respectively, as compared to others. The CA contents of 18.98% were recorded in FD5, while FD6 and 7 showed no significant (P<0.05) difference in values of CA contents (17.71 and 17.53%, respectively) as shown in Table 4.

Table 4: Proximate composition of fish meal with graded inclusion of fermented *P. biglobosa* seed meal

FD	Proximate composition (%)					
	MC	CF	AS	CP	LP	CA
FD1	7.12±0.21^{b}	10.23±0.01^{c}	1.51±0.23^{c}	31.23±0.52^{c}	5.10±0.51^{c}	44.81±0.23^{a}
FD2	7.10±0.32^{b}	10.23±0.01^{c}	1.93±0.30^{c}	31.26±1.02^{c}	5.78±1.01^{c}	43.70±0.23^{a}
FD3	8.01±0.35^{a}	11.12±0.01^{b}	2.34±0.21^{b}	42.30±0.23^{b}	6.01±1.56^{b}	30.22±0.23^{b}
FD4	8.13±1.03^{a}	11.24±0.02^{b}	2.21±0.14^{b}	42.31±2.01^{b}	6.11±1.23^{b}	30.00±0.23^{b}
FD5	8.13±1.12^{a}	12.02±0.02^{a}	4.42±0.23^{a}	50.05±2.02^{a}	6.40±2.01^{b}	18.98±0.25^{c}
FD6	8.05±2.02^{a}	12.13±0.02^{a}	4.53±0.21^{a}	50.10±0.34^{a}	7.48±1.24^{a}	17.71±0.25^{d}
FD7	8.17±2.03^{a}	12.23±0.02^{a}	4.32±0.11^{a}	50.25±0.21^{a}	7.50±1.42^{a}	17.53±0.23^{d}

Values are mean±standard deviation. Means with different superscripts within a column are significantly (p>0.05) different. FD: Formulated diet, MC: Moisture content, CF: Crude fiber content, AS: Ash content, CP: Crude protein content, LP: Lipid content, CA: Carbohydrate content.FD1: Formulated diet without *P. biglobosa*, FD2: Formulated diet without *P. biglobosa* FD3: Formulated diet with 15% *P. biglobosa*, FD4**:** Formulated diet with 20% *P. biglobosa*, FD5: Formulated diet with 25% *P. biglobosa*, FD6: Formulated diet with 30% *P. biglobosa*, FD7: Formulated diet with 35% *P. biglobosa*

In Table 5, the results of growth performance were presented. The results revealed that there were significant (P>0.05) differences in the growth performances of *C. gariepinus* served the formulated diets. The higher mean weight gained (MWG) and mean length gained (MLG) of 2.00mg and 4.20cm were respectively observed in *C. gariepinus* given FD1 which had no graded inclusion of fermented *P. biglobosa* seed meal and acetaminophen intoxication. High values of MWG ranging from 1.13 – 1.78mg, with no significant (P<0.05) differences were recorded in the groups of fish fingerlings served FD 4 (20% *P. biglobosa* seed meal and 100mg acetaminophen), FD5 (25% *P. biglobosa* seed meal and 100mg acetaminophen), FD6*(*30% *P. biglobosa* seed meal and 100mg acetaminophen), and FD7 (35% *P. biglobosa* seed meal and 100mg acetaminophen) as compared to fingerlings served FD3 (15% *P. biglobosa* seed meal and 100mg acetaminophen). These were followed by a group of fingerlings served FD3 while fingerlings given FD2 (0%*P. biglobosa* seed meal with 100mg acetaminophen) had the least significant (P<0.05) value of MWG (-12.80mg). Likewise, fingerlings fed FD5, 6, and 7 recorded lower mean length gained (MLG)ranging from3.33 – 3.63m compared to fingerlings fed FD3, while fingerlings fed FD2 gave no significant (P<0.05) difference in MLG (Table 5).

Table 5: Growth performance of *C. gariepinus* fingerlings fed graded inclusion of fermented *P. biglobosa* seed meal upon acetaminophen intoxication

FD	MIW (mg/kg/bw)	MFW (mg/kg/bw)	MWG (mg/kg/bw)	MIL (m)	MFL (m)	MLG (m)
FD1	101.00±2.11^{a}	103.00±1.23^{a}	2.00±1.23^{a}	23.12±0.02^{a}	27.32±0.02^{a}	4.20±0.01^{a}
FD2	101.00±2.01^{a}	88.20±1.12^{d}	-12.80±1.12^{d}	23.12±1.02^{a}	21.23±0.02^{e}	-2.00±0.01^{e}
FD3	101.00±2.50^{a}	101.20±1.01^{c}	0.20±1.49^{c}	23.12±0.01^{a}	23.53±0.02^{d}	0.41±0.01^{d}
FD4	101.00±1.05^{a}	102.13±0.23^{b}	1.13±0.29^{b}	23.12±0.01^{a}	26.45±0.02^{c}	3.33±1.01^{c}
FD5	101.00±1.20^{a}	102.34±1.50^{b}	1.34±0.30^{b}	23.12±0.02^{a}	26.45±0.02^{b}	3.33±1.01^{b}
FD6	101.00±2.01^{a}	102.60±2.30^{b}	1.60±1.30^{b}	23.12±0.02^{a}	26.67±0.03^{b}	3.55±1.01^{b}
FD7	**101.00±1.01^{a}**	**102.78±1.21^{b}**	**1.78±1.11^{b}**	**23.12±1.03^{a}**	**26.75±0.10^{b}**	**3.63±1.01^{b}**

Values are mean±standard deviation. Means with different superscripts within a column are significantly (p>0.05) different. FD: Formulated diet, MIW: Mean initial weight, MFW: Mean final weight, MWG: Mean weight gained, MIL: Mean initial length, MFL: Mean final length, MLG: Mean length gained,FD1: Formulated diet without *P. biglobosa*, FD2: Formulated diet without *P. biglobosa* + acetaminophen, FD3: Formulated diet with 15% *P. biglobosa* + acetaminophen, FD4**:** Formulated diet with 20% *P. biglobosa* + acetaminophen, FD5: Formulated diet with 25% *P.*

biglobosa + acetaminophen, FD6: Formulated diet with 30% *P. biglobosa* + acetaminophen, FD7: Formulated diet with 35% *P. biglobosa* + acetaminophen.

The results of nutrient utilization are shown in Table 6. The results revealed that the nutrient utilization differed significantly (P>0.05) among the diets fed *C. gariepinus* fingerlings as compared to FD2 without *P. biglobosa* + acetaminophen (100mg). High values of specific ratios (SR) (100%) with no significant (P<0.05) differences were observed in FD1 without *P. biglobosa*+ acetaminophen, FD5 (25% *P. biglobosa* + 100mg acetaminophen), FD6 (30% *P. biglobosa*+100mg acetaminophen, and FD7 (35% *P. biglobosa* + 100mg acetaminophen) as compared to other diets. While the SR values (5.00%) in FD3 (15% *P. biglobosa*+ 100mg acetaminophen) and FD4 (20% *P. biglobosa* + 100mg acetaminophen) were not significantly (P>0.05) differ as compared to other diets. FD2 (0%*P. biglobosa* + acetaminophen) recorded no significant (P<0.05) difference in SR values as compared to others (Table 6). The fish fed FD2 recorded a significantly (P>0.05) elevated value (100%) of mortality (MR) followed by those fed FD3 and FD4 both of which showed no significant (P<0.05) difference in their MR when compared with other diets. FD1, 5, 6, and 7 showed no significant (P<0.05) in their MR values throughout the experiment. The % feed intake (FI) of the animals fed FD4, 5, 6, and 7 showed no significant (P<0.05) difference and recorded the highest values of FI with 4.21 – 4.50% as compared to others. These were followed by the groups of animals fed FD3 and 4 while animals fed FD2 had a significantly (P<0.05) low FI value of 2.54% (Table 6). The group of animals fed FD1, 3, 4, 5, 6, and 7 recorded the highest values of feed conversion ratios (FCR) of 1.00 – 1.80%, which are not significantly (P<0.05) different as compared to animals fed FD2, which had FCR values of -16.00% (Table 6). High significant values of specific growth rate (SGR) were obtained in groups of fish fed FD1 and 2 and are not deferred significantly when compared to others. These were followed by that of fish fed FD4, 5, and 6 with the SGR values ranging from 2.02 – 2.90% and all were not different significantly (P>0.05) from each other. The gross fed conversion ratios (GFCR) were high but not differ significantly in fish-fed FD3, 4, 5, 6, and 7, which ranged from 0.10 – 0.90% compared to others. Likewise, the group of fish given FD1recorded 0.00% of GFCR while the group fed FD2 had a -0.06 value of GFCR (Table 6).

Table6: Nutrient utilization of *C. gariepinus* fingerlings fed fish meal with graded inclusion of fermented *P. biglobosa* seed meal upon acetaminophen intoxication

FD	SR (%)	MR (%)	FI (%)	FCR (%)	SGR (%)	GFCR (%)
FD1	100±0.00^{a}	0.00±0.00^{c}	4.50±1.01^{a}	1.00±1.01^{a}	3.60±0.01^{a}	0.00±0.01^{f}
FD2	0.00±0.00^{c}	100.00±0.00^{a}	2.54±I.02^{c}	-16±1.02^{b}	-2.90±0.01^{d}	-0.06±0.01^{g}
FD3	5.00±2.50^{b}	10.00±1.01^{b}	3.10±1.49^{b}	1.00±1.02^{a}	0.36±1.02^{c}	0.10±1.01^{e}
FD4	5.00±0.50^{b}	10.00±0.21^{b}	3.40±0.29^{b}	1.80±1.01^{a}	2.02±1.02^{b}	0.60±1.03^{d}
FD5	100±0.00^{a}	0.00±0.00^{c}	4.21±0.30^{a}	1.50±1.02^{a}	2.40±0.03^{b}	0.70±1.03^{c}
FD6	100±0.00^{a}	0.00±0.00^{c}	4..46±1.30^{a}	1.30±0.02^{a}	2.90±0.03^{b}	0.80±1.10^{b}
FD7	100±0.00^{a}	0.00±0.00^{c}	4.47±1.01^{a}	1.12±0.01^{a}	3.20±0.01^{a}	0.90±1.02^{a}

Values are in mean±standard deviation. Means with different superscripts within a column are significantly (p>0.05) different. SGR: specific growth rate, GFCE: Gross feed Conversion efficiency, FI: feed intake, FCR: feed conversion rate, MR: Mortality Rate, SR: survival rate. %WG: Percentage weight gain. FD: Formulated diet**,** FD: Formulated diets, FD1: Formulated diet without *P. biglobosa*, FD2: Formulated diet without *P. biglobosa* + acetaminophen, FD3: Formulated diet with 15% *P. biglobosa* + acetaminophen, FD4**:** Formulated diet with 20% *P. biglobosa* + acetaminophen, FD5: Formulated diet with 25% *P. biglobosa* + acetaminophen, FD6: Formulated diet with 30% *P. biglobosa* + acetaminophen, FD7: Formulated diet with 35% *P. biglobosa* + acetaminophen.

Table 7 shows the proximate composition of treated fingerlings (carcasses). It reveals that there were significant (P>0.05) differences in carcass' proximate composition with respect to varying levels of fermented *P. biglobosa* inclusion. *C. gariepinus* fingerlings fed FD5 (25% *P. biglobosa* + 100mg acetaminophen), FD6 (30% *P. biglobosa* and 100mg acetaminophen), and FD7 (35% *P. biglobosa* + 100mg acetaminophen) recorded no significant (P<0.05) difference. The moisture content (MC) and crude fiber (CF) content ranged from 19.05 – 19.15% compared to other treatment diets. This is followed by the fingerlings fed FD3 and 4 with MC and CF contents ranged from 18.12 to 18.23% and 8.10 to 8.14%, respectively, as compared to those of control groups. Fingerlings fed FD1 recorded high MC and CF of 17.06 and 7.52%, respectively, as compared to those fed FD2 with 16.12% (MC) and 6.71% (CF). No

significant (P<0.05) increase in levels of ash (AS) and crude protein (CP) contents was observed in fingerlings fed FD1, 4, 5, 6, and 7 with 1.05% - 1.28% (AS) and 42.12 – 42.50 (CP) when compared to other. Fingerlings fed FD2 and 3 recorded significantly (P>0.05) lower values of AS (0.67 – 0.91%) and CP (38.67 – 39.34%). Lipid (LP) contents in *C. gariepinus* served FD1, 5, 6, and 7 were elevated and not different significantly from each other compared to other treatments. Likewise, no significant difference in LP values ranged (1.76 – 1.86%) was observed in *C. gariepinus* fed FD2, 3, and 4. The *C. gariepinus* served FD2 gave a significantly(P>0.05) higher value of carbohydrate (CA) of 35.07% as compared to others, followed by those fed FD3 and 4 with 30.60% and 29.18% CA, respectively. No significant (P<0.05) difference was observed in CA contents of treatments fed FD1 and 5 having 26.80 and 26.58%, respectively, when compared to those fed FD6 and 7 with CA contents of 25.98 and 25.49%, respectively (Table 7).

Table 7: Proximate composition of *C. gariepinus* (carcasses) fed fish meal with graded inclusion of fermented *P. biglobosa* seed meal upon acetaminophen exposure

FD	Proximate composition (%)					
	MC	CF	AS	CP	LP	CA
FD1	17.06±0.01^{c}	7.52±0.02^{b}	1.30±0.02^{a}	42.50±0.01^{a}	2.82±0.12^{a}	26.80±0.02^{b}
FD2	16.12±0.01^{d}	6.71±0.01^{c}	0.67±0.02^{b}	38.67±0.01^{b}	1.76±0.12^{b}	35.07±0.02^{a}
FD3	18.23±0.02^{b}	8.10±0.03^{b}	0.91±0.02^{b}	39.34±0.01^{b}	1.82±0.12^{b}	30.60±0.02^{a}
FD4	18.45±0.02^{b}	8.14±0.03^{b}	1.05±0.01^{a}	41.32±1.01^{a}	1.86±0.02^{b}	29.18±0.01^{b}
FD5	19.05±0.02^{a}	9.02±0.02^{a}	1.11±0.01^{a}	42.12±1.01^{a}	2.12±0.02^{a}	26.58±0.01^{b}
FD6	19.12±1.02^{a}	9.14±0.01^{a}	1.24±0.01^{a}	42.25±1.01^{a}	2.27±0.02^{a}	25.98±0.01^{b}
FD7	19.15±1.02^{a}	9.20±0.01^{a}	1.28±0.01^{a}	42.34±1.01^{a}	2.54±0.02^{a}	25.49±0.01^{b}

Values are in mean±standard deviation. Means with different superscripts within a column are significantly different (p>0.05). FD: Formulated diet**,** MC: Moisture content, CF: Crude fiber content, AS: Ash content, CP: Crude protein content, FA: Fat content, CA: Carbohydrate content, FD1: Formulated diet without *P. biglobosa*, FD2: Formulated diet without *P. biglobosa* + acetaminophen, FD3: Formulated diet with 15% *P. biglobosa* + acetaminophen, FD4**:** Formulated diet with 20% *P. biglobosa* + acetaminophen, FD5: Formulated diet with 25% *P. biglobosa* + acetaminophen, FD6: Formulated diet with 30% *P. biglobosa* + acetaminophen, FD7: Formulated diet with 35% *P. biglobosa* + acetaminophen.

DISCUSSIONS

The significant (P>0.05)difference recorded on the proximate composition of all formulated diets (FD) as compared to the control group (Table 4) could be attributed to environmental factors. This is in tandem with the findings of Olujobi (2012) who reported that the nutrient constituents of *P. biglobosa* fruits may be probably affected by location. Significantly (P>0.05) higher values of all nutrients obtained in the formulated diets (FD2, FD3, FD4, FD5, and FD6) is a reflection of varying graded inclusion of fermented *P. biglobosa* seed meal in the formulated diets. Higher nutrient compositions in *P. biglobosa* fruits have been reported by Obe *et al.* (2019). The preparations of *P. biglobosa* fruits using varying processed methods improve nutrient depositions (Obe *et al*., 2019). It has been reported that boiled *P. biglobosa* seeds had more nutrient composition than raw ones (Femi-Ola *et al.,* 2008). The fermentation process liberates nutrient availability by retarding the level of anti-nutritional factors (Abdullahi and Rasheedat, 2014). It has been demonstrated previously, that lower tannin levels were established in fermented *P. biglobosa* seeds than raw ones (Abdullahi and Rasheedat, 2014). Higher moisture contents noted in all diets with graded levels of fermented *P. biglobosa seed* meal than that of the control group (FD1) could be reflected by proportion and blend interaction. Food qualities are greatly maintained by the moisture and water activity of the product (Peters *et al.,* 2016). Tor *et al*. (2018) reported that elevated moisture content is a reflection of the shelf life of the product and may be an indication of a dry-packed soup condiment. The significant increase of crude fiber and ash compositions recorded in formulated diets with varying graded inclusion of *P. biglobosa* seed meal than the control diet (FD1) may be responsible for its high mineral contents and protection ability against gastrointestinal and metabolic disorders in animals (Yakubu *et al*., 2020). Increased levels of crude protein level experienced in all formulated diets with graded levels of fermented *P. biglobosa* seed meal than the control diet could probably be due to the fermentation process the product was subjected to. This finding justifies all formulated diets as good sources of amino acids. Crude protein levels acquired in this study (42.30 – 50.25%) were not in agreement with the levels of protein (41.14%, 25.57 – 30.12%, 31.00 – 40.00% and 30.04 – 31.07% obtained in

processed and raw *P. biglobosa* as reported by Obe *et al.* (2019), Femi-Ola *et al*. (2008), Anyanwu *et al.* (2012) and Olujobi (2012) respectively. Olujobi (2012) has suggested that varying values of CP may probably be due to environmental conditions. Suggesting that location does greatly affect the nutrient contents of *P. biglobosa* fruits. The higher levels of CP demonstrated in this study are greater than the levels of protein (40.00% and 37.69%) reported by NSRL (2013) and Ogbemudia *et al.* (2017) respectively, in soybean meal. This has justified the ability of fermented *P. biglobosa* seed meal to substitute soybean in fish meal preparation (Obe *et al.,* 2019). Elevated levels of lipid/fat in all formulated diets with varying inclusion of *P. biglobosa* seed meal reported in this study suggest their appropriateness as diet source for fat in generating energy. Fats content of food affect the shelf life of the meal products. Rancidity in foods is characterized by fat that forms some compounds with unpleasant smells (Peters *et al*., 2016). Low levels of carbohydrate contents established in all formulated diets as compared to control diets suggested their lower source of immediate energy provision for the functioning of normal cells than the control diets.

A significant difference ($P<0.05$) generally, was exhibited in the growth performance of *C. gariepinus* between the formulated diets and the control diets (Table 5). Increased growth performance (both mean weight and length gained) noted in *C. gariepinus* fed FD1 when compared to FD2 could be attributed to no inclusion of 100mg of acetaminophen in diet 1. Indicating that absence of acetaminophen could be a cause of relief for *C. gariepinus* from oxidative-related stress. Absence of reactive oxygen species (ROS) generation in the *C. gariepinus* fed FD1 suggests a free-stress environment, and ability to feed well with enhancement of growth performance. This finding is in agreement with the report of Abdullahi and Rasheedat (2014). In contrast, low/no significant growth performance observed in *C. gariepinus* fed FD2 may be due to the mega doses of acetaminophen administered during the period of the experiment. Suggesting that acetaminophen administered might have caused ROS generation in *C. gariepinus* fed FD2, which results in the creation of environmentally unfriendly condition to the survival of fish due to the presence of oxidative-related stress. This ROS generated due to acetaminophen induce stress might have caused all *C. gariepinus* not to be able to feed well causing retardation of growth performance. This is in support of the findings by Ndatsu *et al.* (2013) who reported that concurrent intake of higher doses of paracetamol triggers ROS generation that causes oxidative-related stress in animals (Ndatsu *et al*., 2013). Furthermore, the significant decrease and elevation of mean weight and length gained recorded in all *C. gariepinus* fed diets with varying inclusion of fermented *P. biglobosa* seed meal + 100mg of paracetamol than those fed FD1 and FD2, respectively could be reflections of the addition of graded inclusion of fermented *P. biglobosa* seed meal. The inclusion of fermented *P. biglobosa* seed meal at different levels could prevent the effect of ROS that might be generated by mega doses of acetaminophen. This study shows that fermented *P. biglobosa* seed meal has higher contents of plant-based protein, vital bioactive compounds with reasonable ability to neutralize the effects of oxidative stress produced by acetaminophen in *C. gariepinus* (Abdullahi and Rasheedat, 2014; Tor *et al*., 2018; Oboh *et al*., 2008; Gu *et al*., 2021; Chenemerem *et al.,* 2022). Higher content of fiber, ash, and protein which are good sources of organic minerals and proteins were reported by Yakubu *et al*. (2020). Tor *et al*. (2018) have reported that the processed *P. biglobosa* seed meal can be regarded as a nutritious dry-packed soup condiment. Higher total phenolic content, reducing power, free radical scavenging ability, and improved liver health and protection were reported in fermented *P. biglobosa* seed by Oboh *et al*. (2008). Improved liver health and protection have been demonstrated in rats fed fermented *P. biglobosa* seed (Andrew *et al*., 2018, Fatima *et al.,* 2021). Oboh *et al*. (2008) and Salit *et al*. (2014) have demonstrated increased nutritive values and antioxidant properties in fermented *P. biglobosa* seed and plant parts, respectively. Increased % specific rate (SR), feed intake (FI), feed conversion ratios (FCR), and specific growth rate (SGR) and no mortality rate (MR) and gross feed conversion ratios (GFCR) observed in *C. gariepinus* fed FD1 as compared to other diets could signify free oxidative stress environment due to absence of high doses of acetaminophen to generate ROS, which subsequently, improved their feed efficient performance. Contrarily, 0% and 100% of SR and MR, respectively, and decreased significance % value of FI, FCR, SGR, and GFCR recorded in fish-fed FD2 could suggest the effects of the inclusion of mega doses of acetaminophen administered during the period of the experiment (Table 6). Interestingly, a significant increase of all feeding performances demonstrated in *C. gariepinus* served with FD incorporated with different grades of fermented *P. biglobosa* seed meal compared to *C. gariepinus* given FD2 may be attributed to protective potential provided by different grades of fermented *P. biglobosa* seed meal against unfavorable oxidative

stress-related environmental to conditions created by paracetamol administration to the fish during the experiment. *P. biglobosa* plant parts/products have been reported to contain higher value of antioxidant properties with the potential to scavenge/destroy the side effects of ROS produced by mega doses of paracetamol (Meraiyebu *et al*., 2013, Fatima *et al*., 2021). Aging, liver, and cancer diseases are good examples of degenerative diseases associated with oxidative stress (Ndatsu *et al*., 2013, Meraiyebu *et al*., 2013 and Fatima *et al.,* 2021). Increased contents of total phenols, flavonoids, and vital bioactive compounds in *P. biglobosa* plant parts could reflect its protective ability against hepatocyte damage caused by N-acetyl-benzoquinone imine (NAPQI), metabolites produced as a result of paracetamol metabolism from mega dosed (Oboh *et al*. 2008, Salit *et al*. 2014, Andrew *et al*., 2018 and Fatima *et al*., 2021). All plants with higher levels of phenolic and flavonoid contents possess high antioxidant properties (Ndatsu *et al*., 2013, Andrew *et al.,* 2018, Fatima *et al.,* 2021).

Furthermore, significantly elevated values of nutrient compositions in *C. gariepinus* fed FD1 compared to others imply efficient utilization of nutrients for their growth due to the absence of ROS production by mega doses of acetaminophen which was not included during feeding trials. Excellent utilization of diet by FD1 treatment reflected the high level of crude protein content in the body of *C. gariepinus.* In contrast, significantly lower levels of nutrient composition recorded in the body of *C. gariepinus* fed FD2 compared to others could be attributed to poor feed utilization due to ROS production by mega doses of paracetamol which was included during the experiment. Interestingly, a significant increase of nutrient compositions exhibited in the body of *C. gariepinus* fed all formulated diets with varying graded inclusion of fermented *P. biglobosa* seed meal compared to those fed FD2 reflects proper utilization of diets for fish growth in treatment FD3 to 7 (Table 7). This efficient utilization of the diets could be responsible for higher protein contents recorded in the body of fish as reflected in the inclusion of fermented *P. biglobosa* seed meal. This showed that fermented *P. biglobosa* possesses some bioactive compounds with protective properties. This finding is in line with the work of Abdullahi and Rasheedat (2014), Andrew *et al*. **(**2018) and Fatima *et al*. (2021) all of who reported that carcass composition is a reflection of served diet to the fish.

CONCLUSION

From the findings of this research, all formulated diets with varying graded inclusion of *P. biglobosa* seed meal can provide vital nutrients needed for growth and feed efficiency utilization in *C. gariepinus* upon exposure to toxic agents. However, formulated diets with 25, 30, and 35% inclusion of fermented *P. biglobosa* seed meal look more promising compared to others and would be better replacements for the high-cost fishmeal in feed manufacturing industries. Hence, consumption of these diets prepared from fermented *P. biglobosa* seeds by animals would improve derivable nutrients in them even when exposed to toxic agents.

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Declaration of Competing Interest

The authors declare that there are no conflicting interests as far as this work is concerned.

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