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Growth Performance of Heteroclarias Juveniles Fed Graded Levels of Autoclaved Castor Seed (Ricinus communis L.) Cake Based Diets

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Abstract

An 84 day feeding trial was conducted on the juveniles (42.51±0.09g) of hybrid catfish, *Heteroclarias,* in order to evaluate the nutritional potential of 30 minutes autoclaved castor seed cake isonitrogenous (40% crude protein) and isocaloric (3212Kcal/Kg) diets replacing soybean meal at 0, 12.5, 25, 37.5 and 50%, the diets being designated D1, D2, D3, D4 and D5 respectively. Prior to this, the castor seeds (*Ricinus communis* L.), ZiboCastor No. 3 variety, collected from Ado-Ekiti metropolis, Nigeria were subjected to 0, 20, 30 and 40 minutes autoclaved at 121°C, dehauled, grounded, oil extracted to form cake, then the proximate and antinutrients analysis were carried out. The preliminary tests on the seeds revealed 30 minutes level of autoclaved seeds was the best. The growth performance result showed that there were significant differences ($p<0.05$) in the mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (NPU) among the various diet levels with the progressive decline in the values of each parameter (except FCR that showed progressive increment) along the trend of increment of castor seed cake inclusion (D1, 0%; D2, 12.5%; D3, 25%; D4, 37.5% and D5, 50%). The survival rate followed the same trend of declination with the highest percentage recorded for D1 (93.33±6.67%), followed by D2 (83.33±3.33%), D3 (76.57±3.33%), D4 (66.67±3.33%) and D5 (60.00±5.77%) respectively. Hence the best growth performance and nutrient utilization was shown in D1 (control) followed by D2. It is therefore recommended that autoclaved castor seed cake at 121°C be included at not more than 12.5% inclusion level to reduce cost of feed without necessarily compromising fish growth rate.

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Introduction

Over the years, continuous efforts are being made across the globe in fish nutrition to ensure the use of non-conventional plant materials in feed formulation. This became necessary owing to the scarcity of high quality conventional feed materials coupled with high competition between man and animals for protein of plant origin [21, 46, 11, and 2]. Most investigations made so far focused at maximizing the nutritional potentials of unconventional plant materials which include cotton seed meal [28]; olive mill waste meal [39]; mango leaves meal [30]; *Bauhinia* and locust meal [20], *Moringa* leaf meal [19] and castor seeds, *Ricinus communis* L. [7; 15 and 41] among others. Through the expansion of these research efforts, balance diets of high quality protein from plant materials are now available to fish at cheaper rate.

However, the antinutritional factors in these plant materials have been the problem in their inclusion in fish diets [53]. [21] reported boiling of castor seeds for 50 minutes at 100^{o}C not only recorded the least reduced toxic substances in it but its subsequent inclusion in the diets of *Oreochromis niloticus* fingerlings and improved its growth performance. Similarly, [6] investigated growth performance of African catfish, *Clarias gariepinus* (Burchell) fingerlings which also showed 50 minutes boiled castor seeds meal inclusion in the diets with the best growth performance. In spite of the foregoing, it is expedient that other methods of processing methods (such as autoclaving, fermentation, soaking, toasting, among others) and inclusion levels (timing and/or percentage of inclusion) should be looked into in the feeding trials of same or other fish species. Hence, the objective of this study is to determine the effect of autoclaved castor seed cake meal on the growth performance and nutrient utilization of hybrid catfish, Heteroclarias. This species has feeding habit that is adaptable to artificial culture medium and grows appreciably on formulated feeds [2 and 31].

Aim of the Study

The study is aimed at processing *Ricinus communis* L. seeds through different levels of autoclaving and its inclusion in the diets of Heteroclarias juveniles.

Materials and Methods

Study Location

The study was conducted at the experimental station of Department of Fisheries and Aquaculture, Ekiti State University, Ado-Ekiti, Nigeria.

Experimental Set Up

An 84 day feeding trial was conducted on the hybrid catfish, *Hetero-clarias.* The experimental design adopted was a complete randomized design. A total of 180 fish were purchased. However, 150 fish were randomly selected and distributed into tanks at a stocking rate of 10 fish per tank. The 150 randomly selected fish were assigned to 5 treatments, control inclusive at one treatment per experimental diet with 3 replicates per treatment.

Sample Collection, Identification and Processing

Castor seeds (*Ricinus communis)*, ZiboCastor No. 3 variety from dehiscenced mature capsules of the plant were fetched within Ado-Ekiti metropolis, Nigeria, and used for this research. The plant capsule and seed samples were identified at the Herbarium of the Department of Plant Sciences and Biotechnology, Ekiti State University, Ado-Ekiti. The collected seed samples were subjected to autoclaving at 121^{o}C for 20, 30 and 40 minutes, in order to examine the best level, using the method of [10]. 2kg raw seeds sample were parboiled for 2 minutes in water at 60ºC in order to ease dehulling. 600g was removed at each time interval. Samples were sun-dried separately to a constant weight, dehaulled and oil extracted. The samples were then packed in air tight polythene bags against the subsequent proximate analysis.

Sources of Ingredients and Diets Preparation

The feed preparation was carried out using the method described by [23 and 5]. The best treatment level (30 minutes autoclaved at 121 ^{o}C castor seed cake) was used as protein source to progressively replace soybean meal in isonitrogenous (40% CP) and isocaloric (3212Kcal/Kg) basis at graded inclusion levels of 0, 12.5, 25, 37.5 and 50% for D1, D2, D3, D4 and D5 respectively. Diet (D1) comprised of soybean meal with no addition of castor seed cake, DL-methione and Lysine to serve as the control. Each of the diets (D2-D5) was enriched with DL-methionine and Lysine at 0.75%

each [2]. The cake was added to other ingredients, purchased from Metrovet Feed Mill, a commercial feed store in Ado-Ekiti. The ingredients include fish meal, yellow maize, cod liver oil, starch binder and vitamin premix.

In preparing the diets, dry ingredients were ground to a powdery form in a Wiley mill to enhance optimum utilization and digestibility. Diets were thoroughly mixed with cod liver oil and pelleted using Hobart A 200 pelleting machine with a 2.0mm die. Diets were sun dried and packed in labelled air tight containers and kept in a cool place prior to use. (Table 1)

Data Collection and Analysis

Proximate analysis of the raw seeds, autoclaved seeds and formulated diets were determined by using the methods of [17]. Anti-nutritional factors such as tannin, phytate, oxalic acid and lectin of the raw and processed seeds were determined using the procedures described in [16, 1, 51 and 54] respectively. The growth parameters examined include the mean body weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) using the procedure of 19]. The equation for each is represented below:

$MWG = W_2 - W_1$

Where W_2 = Final body weight

W_1 = Initial body weight

$$SGR = \frac{100\ (L_nW_2 - L_nW_1)}{t} \qquad [35]$$

Where W_2 = Final Weight

Table 1. Compositions of the feeds for Heteroclarias (%)

	D1 (control)	D2	D3	D4	D5
Fish meal	30	30	30	30	30
Soya bean meal	50	37.5	25	12.5	0
Castor seed cake	0	12.5	25	37.5	50
Yellow maize	13.5	13.5	13.5	13.5	13.5
Cod liver oil	2	2	2	2	2
*Vit. Premix	2	2	2	2	2
Starch binder	1	1	1	1	1
L-methionine	0.75	0.75	0.75	0.75	0.75
L-lysine	0.75	0.75	0.75	0.75	0.75
Total % cal.	100	100	100	100	100
Cp cal. %	40	40	40	40	40
Kcal/kg	2874.84	2827.34	2779.84	2732.34	2684.84

* Vitamins /mineral premixes contain:- vitamin B_{12}, riboflavin, Vit C, D_3, K and E, panthothenic acid, nicotinic acid, chlorine chloride, folic acid, selenium, phosphorus, calcium, iodine, copper, zinc, manganese, iron , terramycin antioxidant and anticaking agent.

W_1 = Initial weight

t = Period of experiment in days

L_n = base of natural logarithm

FCR = Amount of Feed Fed / Weight gain (g) [18]

PER = Weight gain / Protein fed

where protein Fed =

% protein in the diet x total diet consumed / 100 [45]

NPU= Fish protein gain x 100 / Protein Fed [27]

Where Protein gain = Final body Protein – Initial body protein

Protein consumed = Total Dietary protein fed

Mortality/Survival Rate (SR) = initial number of fish stocked – mortality x 100 / Initial number of fish stocked [38]

Fish Feeding and Culture

180 juveniles of *Heteroclarias* catfish (42.51±0.09g) were obtained from ABUAD fish farm in Ado-Ekiti, Ekiti State. They were brought to the laboratory immediately and put in large water baths for two weeks for acclimatization. Commercial diet (Coppen 40%CP) was used to feed the fish during the period of acclimatization. Subsequently, the fish were stocked randomly into 15 glass aquaria tanks (70cm x 45cm x 30cm) using 10 fish/tank in three replicates for a five treatment feeding trials. Each glass tank contained 70litres of borehole water with aerators. The water was changed every other day through siphoning. The setups were covered with wire mesh to prevent incursion. The fish were fed 5% of their body weight twice daily at 08.00-09.00am and 17.00-18.00pm throughout the 84 days. Weight of the experimental fish was measured bi-weekly to calculate their response to feed [34].

Water Quality Determination

Water parameters such as temperature, pH and dissolved oxygen will be measured weekly using HANNA instruments Model: HI-98129, HI-987130 and HI-3810 respectively.

Statistical Analysis

Data obtained will be subjected to a two-way analysis of variance (ANOVA) to determine the significance of the variations between parameters to be examined at (P<0.05). Means obtained will be segregated using Duncan's multiple range tests (DMRT) with the aid of SPSS version 20.

Results

Water Quality

Table 4 shows the mean values for water temperature, pH, and dissolved oxygen (D.O) during the feeding period for the treatments. There was no significance difference (P>0.05) in the mean values for temperature throughout the feeding trials with which ranged between 24.98±0.23^{0}C and 25.38±0.2^{0}C. There were slight variations in pH values in the aquaria during the experiment with no significant difference (P>0.05) except in treatment 5 (6.72±0.09) when compared with treatment 1 to 4 which ranged between 6.46±0.0 - 6.51±0.05. The mean dissolved oxygen values recorded showed there was no significance difference (P>0.05) among the treatments with values ranging between 5.67±0.09 - 5.87±0.07.

Proximate Analysis of the Raw and Autoclaved Castor Seed Cake

The proximate composition of the autoclaved castor seed at 0, 20, 30 and 40 minutes time intervals is presented in Table 2. The mean moisture contents are significantly different (p>0.05) in all the treatments including the control. However, the sample autoclaved at 20 minutes was highest (5.53±0.03) while the control recorded the least value (4.85±0.03). In the same vein, the crude protein, fats, ash, crude fibre and nitrogen free extract (NFE) contents were significantly different (p<0.05) among the various levels of autoclaving, 0, 20, 30 and 40 minutes time intervals. Crude protein and fats were highest in raw (control) (38.29±0.11, 28.65±0.15) and lowest at 20 minutes (5.13±0.07, 16.53±0.13) respectively. Ash was highest at 20 minutes treatment (5.47±0.02) and lowest in 30 minutes treatment (4.60±0.03). Crude fibre was highest in CSC autoclaved for 20 minutes (3.53±0.08) and least at 0 minutes (2.08±0.05). NFE was recorded highest at 20 minutes autoclaved (48.29±0.23) and lowest in control (16.50±0.14).

Antinutrients Determination

The antinutritional composition of the autoclaved castor seed at 0, 20, 30 and 40 minutes time intervals is presented in Table 3. The mean values recorded for phytate, oxalate, tannin and lectin contents were significantly different ($p<0.05$) among the treatments. Phytate, oxalate, tannin and lectin were highest in the control, 0.42±0.02, 8.58±0.02, 10.54±0.06 and 7.85±0.06 respectively. The lowest values (0.18±0.00, 2.41±0.01 and 0.004±0.001) for phytate, oxalate and lectin were recorded for 30 minutes autoclaved.

Growth Performance and Survival

The mean biweekly growth curve trend of Heteroclarias juveniles fed different levels of autoclaved castor seed cake based diets is shown in Figure. 1 while the general growth performance and nutrients utilization of the fish is shown in table 5. There were significant differences ($p<0.05$) in growth performance of the fish. Mean weight gain showed similar pattern to that of SGR with the best values in fish fed D1 (66.22±0.36, 1.11±0.01) respectively. Feed utilization, expressed as the feed conversion ratio (FCR), was significantly ($p>0.05$) different in all the treatments with the least mean value (4.28±0.04) in fish fed D1 (the control). The dietary protein utilization, expressed as the protein efficiency ratio (PER) and net protein utilization (NPU) were significantly highest ($p<0.05$) in fish fed D1 (0.58±0.00, 4.46±0.04) respectively. The results further indicated a progressively diminished growth rate from D2 to D5. There was a progressive decline in survival rate of the fish subjected to the increased diet levels of castor seed cake from 0% (D1) to 50% (D5) with the highest in D1 (93.33±6.67%) and lowest D5 (60.00±5.77%). (Table 2, Table 3, Table 4, Table 5, Table 6)

Discussion

Water Quality

The temperature value range recorded in this study is lower than 27.3^{0}C reported by [12] in a growth study of *Clarias gariepinus*, fed varying inclusion levels of *Leucaena leucocephala* leaf meal but nearly tally with the range of 24.83 – 25.17^{0}C observed by [6]. The difference might be probably as a result variation of experimental sites. [37] stressed that temperature fluctuations can affect the growth of fish in terms of their metabolism, oxygen consumption, ammonia and carbon dioxide production, feeding rate as well as food conversion. The pH recorded in this study revolved around and within the report of [25) and 4] who recommended that optimum pH level for maximum productivity of cultured tropical fish should be between 6.5 and 9. The safe range of 5-9 was recommended by [50]. The pH range was higher than the 6.11-6.30 reported by [19] in their study on growth performance of *Clarias gariepinus* fed soaked *moringa oleifera* leaf meal.Also, the DO recorded is within the range of 3.30 to 12 mgl-1 reported by [40 and 6] on the growth of rainbow trout (*Oncorhynchus mykiss*) in raceway system and growth of *Clarias gariepinus* respectively. [24] recommended DO of >5ppm as essential for good fish production.

Proximate Composition of Raw and Autoclaved Castor Seed Cake

The protein content reported in this work (38.29±0.11) is higher than that reported by [13, 44 and 36], 33.09%, 30.82% and 23.00% respectively, in raw undecorticated castor seeds. The observed differences may be attributed to differences in geographical distribution and variety [14].

Among the processed seeds in this study, the 30 minutes autoclaved seeds gave the highest mean value (36.98±0.16). All the processing levels reduced the protein content of castor seed. This may be attributed to the effect of temperature during the autoclaving periods [33, 9 and 29]. Crude fiber was least in control sample followed by sample autoclaved at 30 minutes. The low values recorded in this study was an improvement over 4.71% and 6.42% reported by [43 and 36] respectively. This disparity could be as a result of variety, geographical location and probably processing techniques. The values of fats recorded in this study are in tandem with the report of [48 and 13] which indicated plant-based food that provides more than 12 % of its caloric value from protein is considered as a good source of proteins.

The low ash recorded supports [44 and 26]

Table 2. Percentage proximate composition of autoclaved Castor seed at different time intervals

	Treatments			
Parameter	$RCSC_{00}$ (Control) (00 minute)	$ACSC_{20}$ (20 minutes)	$ACSC_{30}$ (30 minutes)	$ACSC_{40}$ (40 minutes)
Moisture	4.85 ± 0.03^{d}	5.53 ± 0.03^{a}	5.29 ± 0.01^{c}	5.44 ± 0.02^{b}
Protein	38.29 ± 0.11^{a}	15.13 ± 0.07^{d}	36.25 ± 0.13^{b}	21.43 ± 0.18^{c}
Fats	28.65 ± 0.15^{a}	16.53 ± 0.13^{d}	22.76 ± 0.16^{c}	25.63 ± 0.23^{b}
Ash	4.79 ± 0.02^{c}	5.47 ± 0.02^{a}	4.60 ± 0.03^{d}	5.20 ± 0.02^{b}
Crude Fibre	2.08 ± 0.05^{d}	3.53 ± 0.08^{a}	2.65 ± 0.09^{c}	3.23 ± 0.03^{b}
NFE	16.50 ± 0.14^{d}	48.29 ± 0.23^{a}	23.18 ± 0.19^{c}	32.65 ± 0.47^{b}

Values shown are means ± standard error. Means with different letters along the same column are significantly different at $p<0.05$

Table 3. Anti-nutritional composition (mg/100g) of autoclaved Castor seed at different time intervals

		Treatments		
Parameters	$CSCR_{00}$(Control) (00 minute)	$CSCA_{20}$ (20 minutes)	$CSCA_{30}$ (30 minutes)	$CSCA_{40}$ (40 minutes)
Phytate (mg/g)	0.42 ± 0.02^{a}	0.23 ± 0.01^{c}	0.18 ± 0.00^{d}	0.26 ± 0.02^{b}
Oxalate (mg/g)	8.58 ± 0.02^{a}	3.13 ± 0.03^{c}	2.41 ± 0.01^{d}	3.76 ± 0.04^{b}
Tannin (mg/g)	10.54 ± 0.06^{a}	6.46 ± 0.04^{b}	6.23 ± 0.03^{c}	6.14 ± 0.04^{d}
Lectin (HU)/mg)	7.85 ± 0.06^{a}	0.22 ± 0.01^{b}	0.004 ± 0.001^{c}	0.021 ± 0.003^{c}

Values shown are means ± standard error. Means with different letters along the same column are significantly different at $p>0.05$

Table 4. Mean Values of water quality parameters in all treatments for Heteroclarias juveniles.

	Treatments				
Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Temperature	24.98 ± 0.23^{a}	25.01 ± 0.12^{a}	25.31 ± 0.17^{a}	25.37 ± 0.22^{a}	25.38 ± 0.23^{a}
pH	6.46 ± 0.05^{b}	6.44 ± 0.04^{b}	6.39 ± 0.06^{b}	6.51 ± 0.05^{b}	6.72 ± 0.09^{a}
Dissolved oxygen	5.78 ± 0.07^{ab}	5.57 ± 0.16^{ab}	5.87 ± 0.07^{a}	5.67 ± 0.09^{ab}	5.51 ± 0.09^{b}

Values shown are means ± standard error. Means with different letters along the same row are significantly different at $P<0.05$

Table 5. Growth performance and nutrients utilization of Hetero-clarias juveniles fed with experimental diets.

Parameters	Treatments				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
IMW (g)	42.49±0.16[a]	42.73±0.31[a]	42.48±0.12[a]	42.33±0.20[a]	42.53±0.22[a]
FMW (g)	108.48±0.43[a]	82.15±0.41[b]	74.71±0.72[c]	58.54±0.25[d]	56.53±0.56[e]
MWG (g)	66.22±0.36[a]	39.76±0.42[b]	32.23±0.60[c]	16.21±0.20[d]	14.00±0.78[e]
SGR	1.11±0.01[a]	0.79±0.00[b]	0.68±0.01[c]	0.38±0.01[d]	0.34±0.02[e]
FCR	4.28±0.04[d]	5.98±0.08[c]	6.77±0.10[c]	12.45±0.20[b]	14.15±0.80[a]
PER	0.58±0.00[a]	0.41±0.00[b]	0.36±0.01[c]	0.20±0.00[d]	0.18±0.01[e]
NPU	4.46±0.04[a]	4.26±0.08[b]	3.04±0.05[c]	1.15±0.03[d]	0.94±0.01[e]
MTY (%)	93.33±6.67[a]	83.33±3.33[ab]	76.67±3.33[bc]	66.67±3.33[cd]	60.00±5.77[d]

IMW (Initial mean weight); FMW (Final mean weight); MWG (Mean weight gain); SGR (Specific growth rate); FCR (Feed conversion ratio); PER (Protein efficiency ratio); NPU (Net protein utilization) and MTY (Mortality rate)
Values shown are means ± standard error. Means with different letters along the same row are significantly different at $p<0.05$

Table 6. Proximate composition of *Heteroclarias* carcass before and after feeding trial

Parameters (%)	Treatments					
	Initial	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	4.24±0.01	6.26±0.06[a]	5.83±0.03[b]	5.11±0.01[c]	4.86±0.02[d]	4.75±0.01[e]
Protein	40.12±0.04	45.23±0.03[a]	44.22±0.06[b]	42.80±0.04[c]	41.06±0.02[d]	40.87±0.01[e]
Fats	11.85±0.03	13.17±0.02[a]	11.44±0.02[b]	11.07±0.03[c]	10.53±0.02[d]	10.10±0.01[e]
Ash	13.99±0.04	14.59±0.07[e]	18.77±0.07[d]	20.24±0.04[c]	22.38±0.02[b]	22.77±0.02[a]
Crude fiber	1.15±0.02	1.42±0.02[a]	1.37±0.01[b]	1.31±0.01[c]	1.25±0.01[d]	1.19±0.01[e]
NFE	28.65±0.04	19.31±0.07[d]	18.38±0.09[c]	19.46±0.09[c]	19.93±0.03[b]	20.31±0.04[a]

Values shown are means ± standard error. Means with different letters along the same row are significantly different at $p<0.05$

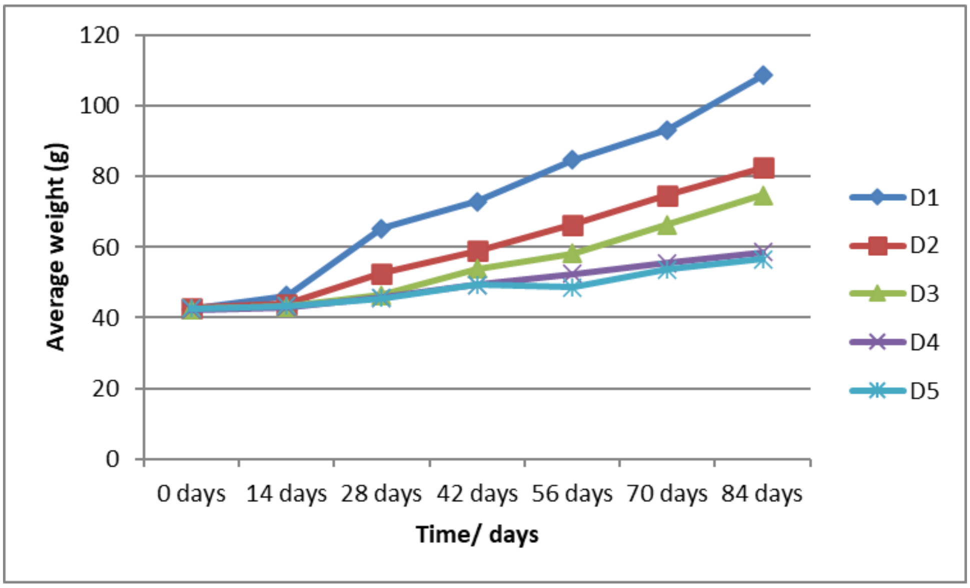

Figure 1. Mean biweekly growth curve trend of Heteroclarias juveniles fed different levels of castor seed cake based diets.

report of a similar ash value of low as 5.0%. The least ash value in 30 minutes autoclaved seeds makes it stands a better chance as energy source among other treatments since ash does not involve in total digestible nutrients (TDN) [8]. The moisture contents of the processed castor seed meals were generally low. This agreed with [52] who reported low moisture below 15% content is required as safe storage limit for plant food materials. The value of NFE recorded (23.18±0.19) in 30 minutes autoclaved seeds was close to 27.45% reported of [22] against roasted Bauhinia seeds. It is considered better because of its corresponding highest protein (36.98±0.16) value. This is an indication that it will enhance a high value of TDN [8].

Antinutrients Determination of Raw and Autoclaved Castor Seed Cake

The progressive drastic reduction ($p < 0.05$) observed in all the antinutrients determined, phytate, tannins, oxalate and lectin, is an indication that the various levels of autoclave processing, 20, 30 and 40 minutes at 121^{o}C is suitable for detoxification of castor seeds and any other antinutrients-rich plant materials. Phytic acid, the hexaphosphate ester of myo-inositol, is a major phosphorus storage constituent of most cereals, legumes and oilseeds [47 and 42]. The high percentage reduction of phytate (0.18, 57.14%), oxalate (2.41, 71.1%) and lectin (0.004, 99.95%) than tannin recorded in 30 minutes autoclaved seeds corroborates with the findings of [19] which showed soaking reduced the level of oxalate than tannin and hence en30 minutes makes it best among the processed levels.

Growth Performance and Survival

In this study it was observed that the growth of Heteroclarias juveniles was marred with high inclusion levels of autoclaved castor seed cake as replacement of soya meal in their diet. The diets and fish carcass fat which increased with the increase in the plant materials in the diet could have inhibited their growth. This is in tandem with the findings of [3 and 2] who inferred poor feed intake retarded growth of fish. Despite the success of drastic reduction of the antinutrients compared with the raw seeds, the levels of treatment might not have addressed the minimum reduction level required for the growth inhibitory substances to become harmless, particularly the low percentage of reduction of tannin 40.89% (6.23) of the raw constituent value (100% (10.64)) in this study could have contributed to this poor response. Tannins are astringent, bitter plant polyphenols which interfere with iron absorption through a complex formation with iron when it is in the gastro-intestinal lumen which decreases the bioavailability of iron [49]. Also, the reduction of phytate recorded in this study, although run in tandem with the findings of [19], culminated in far above 0.05% which [32] inferred if beyond the limit could be harmful to fish growth. The increase trend of the FCR across board might be due to low fibre content

Conclusion

From the results of this study it could be concluded that the growth performance of Heteroclarias (hybrid catfish) decreases with increase in inclusion level of autoclaved castor seed cake in the diet. It is therefore recommended that autoclaved castor seed cake be included at no more than 12.5% level without compromising fish growth. Further studies on the utilization of castor seed cake in the diets of grow- out fish as non-conventional plant material will maximize its inclusion in fish feed as an alternative source of plant protein

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