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Effects of Supplementing Fish Meal with *Sesame indicum* on Functional Properties, Phytotoxins and Hematological Compositions of *Clarias gariepinus*

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ABSTRACT: This study analyzed the effects of supplementing fish meal with *Sesame indicum* on functional properties, phytotoxins and hematological compositions of *Clarias gariepinus*. A total of 150 of *C. gariepinus* fingerlings were grouped into 6 tanks with 20 *C. gariepinus* per tank. Each tank of fish was served with prepared fish meals supplemented with various levels of beniseed (*S. indicum*), namely, DT1 (commercial diet), stands as the control group, DT2 (0% beniseed with 100% soya bean meal, DT3 (25% beniseed with 75% soya bean meal, DT4 (50% beniseed with 50% soya bean, DT5 (75% beniseed with 25% soya bean and DT6 (100% beniseed with 0% soya bean, individually. The functional properties of each diet and hematological indices of the treated fish were determined. All prepared diets have improved the functional properties and their phytotoxins level remains within the permissible limit as compared to control diets (DT1). The formulated diets have potentially influenced the hematological indices analyzed compared to the control diet (DT1). At any level of *S. indicum* inclusion in the fish meal of *C. gariepinus* there was a potential improvement of the functional properties, hematological parameters and maintaining the levels of phytotoxins not to rise above the permissible limit. Thus, experimental diets (DT5 and DT6) with 75 and 100% *S. indicum* would be promising candidates which may be used for the development of the product in various food industries.

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In developing countries, a lot of edible plants in nature are used as sources of food because of the adequate nutrients provided to the animals. Beniseed *(Sesamum indicum* L) falls into the family of Pedaliaceae, which is commonly cultivated in some of the Asian and African countries. It contains high edible oil, protein and mineral elements and considered as a quality food for older ages (Peters *et al*., 2016). The seeds are fried, used in soup making and the fermented seeds are used in making soup condiments for consumption in Nigeria. Beniseed is considered a healthy food additive that prevents diseases and enhancing wellbeing. Its uses as nutraceutical are prevention of cancer and heart disorders (Uaboi *et al*., 2008). Improved plasma gamma-tocopherol production and activity of vitamin E that aids in cancer and heart disorder prevention have been reported (Conney *et al*., 2001). Furthermore, it was reported to contain Sesamin and sesamolin, the special beneficial fibers and have the potential to lower cholesterol in animals (Peters *et al*., 2016). The African fish (Clarias spp.) is an Africa native fish, which is one of the best freshwater species commonly cultured in the tropical climate due to its favorable characteristic behaviors exhibited. Among these are, it feeds almost on everything (Ali *et al*., 2005). The Clarias gariepinus has gained consumer's acceptance than the other cultured freshwater species (Balogun and Fasakin, 1996). Improve growth and production of this fish is depending on the quality of its feed. During economic production, improper quality feed has adverse effects on fish growth rates, disease occurrence and fish production (latise et al., 2006). Thus, the production of this economic fish in Africa countries is the dependence of proper, less cost and nutrient balanced diet provision (Olapade and George, 2019). In fish farming, feed remains the largest ingredient in aquaculture production and it remains the major component of fish feed and this has made the cost of fish farming high. As a result of this, there is a need to examine the inclusion of less cost locally available stuffs in the *C. gariepinus* diets. The supplementation of fish meal with the plant-based protein sources have been reported to reduce the cost of fish feed (Olapade and George, 2019; Mohapatra and Patra, 2004). Substitution of fish meal with both animal and plant-based protein sources have been reported Ovie, 2007). However, the over replacement of fishmeal with animal and plant-based protein foodstuffs were described to have a negative effect on fish growth (Ovie, 2007). This effect on the growth performance of fish may be due to the presence of antinutritional factors (Olapade and George, 2019). Besides, improved growth performance and feed utilization is a sign of the good health of the fish (Keke Anene, 2011; Effiong *et al*., 2014). Also, the toxicological effects, monitoring of environmental conditions and fish health condition analysis are easily detected through the analysis of hematological indices (Adewoye,2010). The stress and disease conditions that affect the cultivation of fish and its production performance are easily examined using hematological techniques (Tavares-Dias and Barcellos, 2005). Therefore, this study is aimed to determine the functional properties, phytotoxins compositions and hematological levels of fish meal supplemented with *Sesame indicum* fed *Clarias gariepinus*.

MATERIALS AND METHODS

Sample Collection: The sesame seed, maize, fish meal, groundnut cake, vitamin premix, Sodium Chloride common salt, Soya bean, and the experimental tank were all collected at Lapai main market, Niger state in June 2019. Botanical identity was confirmed by the Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria.

Sample Preparation: The foodstuffs were finely grounded and mixed in the plastic bowl into dough form using hot water, with cassava starch as binding material. The mixture was then pelleted by passing it through a mincer of 2 mm die to produce a 2 mm diameter size of pellets. These were sun-dried to about 10% moisture content, packed in polyethylene bags and kept safe dry for use. The soya bean meal consisted of fish meal, soya bean, maize and other ingredients as presented in table 1.

Table 1. Formulation of soya bean meal

Ingredients	Combination (%)
Soya beans	48.4
Fish meal	21.5
Maize	11.4
Groundnut cake	15.2
Vitamin premix	1.3
Sodium chloride	2.2
Total	100

Feed formulation: Briefly, about six (6) experimental diets at varying sesame seed and soya beans meal compositions, namely, diet 1, 2, 3, 4, 5 and 6 were formulated (Table 2). The diet 1 is composed of 25% sesame seed meal with 75% soya bean meal, Diet 2 is composed of 50% sesame seed meal with 50% soya bean meal, Diet 3 is composed of 75% sesame seed meal with 25% soya bean meal, Diet 4 is 100% sesame seed meal with 0% soya bean meal, Diet 5 is 0% sesame seed meal with 100% soya bean meal and commercial feed was used as control.

Table 2. Experimental diet formulation

DT	Dietary Treatment (%)					
	DT1	DT2	DT3	DT4	DT5	DT6
Sesame seed	CD	0	25	50	75	100
Soya bean meal		100	75	50	25	0

DT: Diet treatment, CD: Commercial diet,

Animals: A total of one hundred and fifty of Catfish fingerlings (*C. gariepinus*) of mixed-sex and the same age (mean weight of 4.5±0.20g) were purchased from Lapai Gwari fish Village, along Lapai – Paiko-Minna road, Niger state. The fishes were transported in an aerated aquarium into the department of Biology, Ibrahim Badamasi Babangida University, Lapai, Niger State. Fishes were allowed to acclimatize to experimental conditions for one week before the feeding trial and were fed commercial fish feed meal (2mmVital feed, company, and country) three times daily. After the acclimatization, the fishes were starved for 24 hours and the average weight and length of all fish in each rearing tank was taken as an initial average weight and length. The water temperature, pH level, hardness of water and available oxygen of the aquarium were monitored throughout the experimental period. The leftover feed in the aquarium water was siphoned every day to avoid infections and mortality during the period of the experiment.

Experimental treatments and design: The total of a hundred and fifty of *C. gariepinus* fingerlings obtained was selected randomly into six (6) rearing tanks with 20 fingerlings of *C. gariepinus* per rearing tank (Kumar *et al*., 2011). Each rearing tank (T1, T2, T3, T4, T5, and T6) was assigned to an experimental diet as shown below, and all were replicated three (3) times and covered with a net to prevent the predators. The feeding trial commences after fish were starved for 24 hours before the introduction of experimental diet to each rearing tank at the varying percentage of sesame seed and soya beans meals inclusion; DT1 was served commercial diet only, DT2 was served with 0 and 100% of sesame seed meal and soya beans meal, respectively, DT3 was served with 25 and 75% of sesame seed meal and soya beans meal, DT4 was served with 50 and 50% of sesame seed meal and soya bean meal, DT5 was served with 75 and 25% of sesame seed meal and soya bean meal, DT6 was served with 100 and 0% of sesame seed meal and soya bean meal, respectively. The fishes were fed with the experimental diet three times a day at 4% of the body weight for twelve (12) weeks. At the end of 12 weeks, the experimental fishes starved for 12 hrs before harvest and subjected to various analyses. The Functional properties: Bulk density (BD), Oil absorption capacity (OAC) and Water absorption capacity (WAC) of the formulated diets were analyzed using the method of Peters *et al*. (2016) and Shaba *et al*. (2015). Anti-nutritional parameters (Oxalate and phytate) of the formulated diets were analyzed following the method of Peter *et al*. (2016) and Shaba *et al*. (2015). The method of Effiong *et al*. (2014) with little modification was used for blood sampling and analysis.

Statistical analysis: All analyses were carried out in triplicate and data were presented in mean±standard deviation. Data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

The functional properties result of the prepared diets is presented in Table 3. It reveals that the WAC, BD, and OAC for all fish meals supplemented with graded levels of *S. indicum* significantly elevated numerically than that of the DT1 (control). Diets (DT5 and DT6) recorded higher WAC (201.1 and 242.3 g/ml) and BD (4.64 and 4.86g/ml), respectively, which were not significantly deferred from each other but significant difference ($p<0.05$) from other experimental diets (Table 3).

Table 3. Functional properties of fish meal supplemented with different % of *S. indicum* meal.

FP (g/ml)	Dietary treatments					
	DT1	DT2	DT3	DT4	DT5	DT6
WAC	120.6 ± 0.05^{d}	149 ± 0.04^{c}	154.5 ± 0.04^{c}	170.8 ± 0.03^{b}	201.1 ± 0.05^{a}	242.3 ± 0.06^{a}
BD	0.40 ± 0.03^{d}	3.01 ± 0.03^{c}	3.63 ± 0.05^{b}	3.90 ± 0.04^{b}	4.64 ± 0.04^{a}	4.86 ± 0.05^{a}
OAC	143.3 ± 0.03^{c}	148.5 ± 0.04^{c}	158.1 ± 0.04^{b}	166.5 ± 0.04^{a}	164.8 ± 0.04^{a}	167.5 ± 0.04^{a}

Data are presented as mean±standard deviation. Means with the different superscript within a row are significantly different (p<0.05). FP: functional properties, BD: bulk density, OAC: Oil absorption capacity, WAC: water absorption capacity, DT1: commercial diet (control), DT2: 0% sesame seed meal with 100% soya bean meal, DT3: 25% sesame seed meal with 75% soya bean meal, DT4: 50% sesame seed meal with 50% soya bean meal, DT5: 75% sesame seed meal with 25% soya bean meal, DT6: 100% sesame seed meal with 0% soya.

Upon processing and storage, the physicochemical characteristics that affect the activity of the food system was termed functional properties (Peters *et al*., 2016). The progressive increase of WAC in increasing replacement levels of *S. indicum* in the formulated diets compared to the control diet (DT1) could signify the non-starch component in the formulated diets. It was suggested by Aboubakar et al. (Aboubakar *et al*., 2008) that water absorption capacity is a reflection of the no-starch component of processed food. The absorption of water capacity (WAC) signifies the variations in the weight of the formulated diet before and after its absorption of water or simply its water retention or absorption capacity. This remains an index of the water quantity that the formulated food can accommodate, and it plays a significant role during food preparation (Peters *et al.,* 2016, Shaba *et al*., 2015). Increased water absorption capacity was reported in flour formed from an African yam bean and jackfruit (Peters *et al*., 2016). The protein content of food can also contribute to its capacity to absorb water (Oladele and Aina, 2007). Therefore, WAC of food is an indication of either to incorporate more or not of protein to the formulated food. Likewise, a numerical increase in bulk density with an increasing percentage of *S. indicum* in the experimental diets than the control diet (DT1) may signify the higher amount of fiber content in them than that of the control diet. Peters *et al*. (2016) have reported that the increased bulk density was observed in formulated flour from breadfruit and beniseed flours. Bulk density (BD) is the ratio of the weight of the formulated food to the volume in g/ml or g/cm^3. It is determined by the heaviest of food (Shittu *et al*., 2005). The packaging of food, handling of raw materials and application methods in the food industry is determined by the bulk density of the food Shittu *et al.*, 2005). Hence, formulated diets (DT5 and DT6) with high bulk density could nutritionally improve digestion processes in fish with diseases of the immature digestive system and thereby enhance their growth performance and production. Furthermore, the OAC values for all diets are arranged in the following order; DT6>DT5>DT4>DT3>DT2>DT1 (Table 3). The OAC in DT4 (166.5 g/ml), DT5 (164.8g/ml) and DT6 (167.5g/ml) were higher and not deferred significantly ($p>0.05$) than each other but numerically higher significantly than the other diets involved in this experiment (Table 3). Furthermore, increased oil absorption capacity recorded in all diets substituted with varying percentage of *S. indicum* inclusion compared to control diet may imply the quantity of oil accommodated after the formulation. This result complies with the one reported by Peters *et al*. (2016) that increased OAC was a reflection of increasing *S. indicum* inclusion in composite flour. High crude oil contents were observed in composite flours with an increasing percentage of *S. indicum* inclusion Peters *et* *al*.,2016). Oil absorption capacity (OAC) signifies the variations in the weight of diet before and after the absorption of oil and has an essential role in the food industry as fats can provide flavor to improve food palatability and storage stability (Ubbor and Akobundu,2009). Subsequently, table 4 revealed the results of the antinutritional analysis with the values of oxalate was increased significantly ($p<0.05$) with an increasing percentage of *S. indicum* meal in the experimental diets. Thus, DT5 (5.34 mg/100g), and DT6 (5.42 mg/100g) were the oxalate values recorded, which are significantly differed ($p<0.05$) as compared to other diets used in this study (Table 4).

Table 4. Antinutritional properties of fish meal supplemented with different % of *S. indicum* meal.

AN (mg/100g)	Dietary treatments					
	DT1	DT2	DT3	DT4	DT5	DT6
Oxalate	2.20±0.03[d]	3.65±0.04[c]	3.85±0.04[c]	4.25±0.03[b]	5.34±0.05[a]	5.42±0.06[a]
Phytate	0.66±0.03[a]	0.59±0.03[b]	0.54±0.05[b]	0.46±0.04[c]	0.41±0.04[c]	0.36±0.05[d]

Data are presented as mean±standard deviation. Means with the different superscript within a row are significantly different (p<0.05). AN: antinutritional properties, DT1: commercial diet (control), DT2: 0% sesame seed meal with 100% soya bean meal, DT3: 25% sesame seed meal with 75% soya bean meal, DT4: 50% sesame seed meal with 50% soya bean meal, DT5: 75% sesame seed meal with 25% soya bean meal, DT6: 100% sesame seed meal with 0% soya.

The progressive increase in the values of oxalate contents recorded in all diets supplemented with different percentages of *S. indicum* inclusion as compared to the control group (DT1) could reflect the added oxalate content of the fish meal to that of the beniseed. No significant increase of oxalate contents obtained in DT5 and DT6 as compared to other experimental diets may be possibly due to their higher percentage of *S. indicum* than the others. This result agreed with that obtained by Peters *et al*. (2016). High consumption of oxalate in humans causes a reduction in plasma calcium, corrosive gastroenteritis, renal damage, and shock (Uche *et al*., 2014; Peters *et al*., 2016). However, the values for phytate recorded in this study revealed a significant decrease with an increasing percentage of *S. indicum* in the experimental diets. The value for phytate recorded are arranged in the following order; DT1> DT2>DT3>DT4>DT5>DT6 (Table 4). Phytate contents of all diets were significantly reduced by the increasing substitution of *S. indicum*. This could be due to the nutrient-nutrient interaction with increasing *S. indicum* levels. High phytate content in the food reduces mineral bioavailability by forming insoluble complexes with minerals (Ekwenye and Okorie, 2011; Shaba *et al*., 2015). This result is in line with what reported by Peters *et al*., (2016) that the lower the phytate content the higher the *S. indicum* substituted. Thus, the antinutrient results obtained in this study were generally below the toxic levels. Table 5 shows the results of hematological indices in *C. gariepinus* after dietary treatment with fish diets containing different percentages of *S. indicum* meal. It reveals that all indices in the treated fish observed were numerical increased significantly ($p<0.05$) with an increasing percentage of *S. indicum* in the experimental diets (Table 5), but not differed significantly ($P>0.05$) from each other as compared to the group of fish fed the control diet (DT1).

Table 5: Hematological profile of *Clarias gariepinus* treated with diets containing varying % of *S. indicum* meal.

HP	Dietary treatments					
	DT1	DT2	DT3	DT4	DT5	DT6
WBC ($\times10^2/\mu L$)	145.6±0.01[b]	202.7±0.01[a]	203.5±1.10[a]	203.7±1.20[a]	203.8±0.01[a]	203.6±0.10[a]
RBC (($\times10^6/\mu L$)	2.54±1.00[b]	3.76±0.01[a]	4.45±0.01[a]	4.47±1.00[a]	4.38±1.00[a]	3.98±0.01[a]
Hb (g/dL)	11.3±1.00[b]	18.4±0.01[a]	18.3±1.00[a]	18.4±0.01[a]	17.5±0.01[a]	17.8±1.00[a]
HCT (%)	32.4±0.02[b]	43.4±0.01[a]	42.5±1.00[a]	42.7±0.01[a]	43.3±0.01[a]	42.8±0.01[a]
MCV (fL)	125.3±0.01[b]	145.6±1.00[a]	146.4±0.02[a]	146.2±0.02[a]	145.6±0.01[a]	145.9±1.00[a]
MCH (pg)	37.6±0.01[b]	42.6±0.01[b]	43.4±0.01[a]	42.5±0.01[a]	42.7±0.01[a]	42.9±0.01[a]
MCHC (g/dL)	35.5±0.01[b]	41.6±0.01[a]	42.3±0.01[a]	41.6±0.01[a]	41.7±0.01[a]	41.8±0.01[a]
PLT (($\times10^3/\mu L$)	112.4±0.01[b]	135.6±0.01[a]	135.7±0.01[a]	133.7±0.01[a]	134.5±0.01[b]	133.7±0.01[a]

Data are presented as mean±standard deviation. Means with the different superscript within a row are significantly different (p<0.05). HP: hematological parameter, DT1: commercial diet (control), DT2: 0% sesame seed meal with 100% soya bean meal, DT3: 25% sesame seed meal with 75% soya bean meal, DT4: 50% sesame seed meal with 50% soya bean meal, DT5: 75% sesame seed meal with 25% soya bean meal, DT6: 100% sesame seed meal with 0% soya.

Generally, high significant ($p<0.05$) values of white blood count observed in this study in all treated fish is a reflection of all formulated diet's ability to improve their immunity levels. These values obtained are significantly greater compared to $52.00\times10^2/\mu L$ reported by George *et al*. (2012). Also, high values of RBC observed in all treated fish, which do not differ significantly ($p>0.05$) from each other, but significantly differ ($p<0.05$) from that of fish served with the control diet signify that *S. indicum* included at varying percentage is a blood builder and has the tendency to combat anaemia due to its protein content. Higher RBC was reported in *C. gariepinus* fed diets replaced with animal-based protein sources (Effiong *et al*., 2014). Adequate consumption of protein increases RBC production and prevents the occurrence of anaemia (Muhammad *et al*., 2015). The absorption and transportation of oxygen in the living system are a function of RBC. Its reduction in the count can lead to weakness and death in the living system (Effiong *et al*., 2014; Muhammad *et al*., 2015). Furthermore, the corresponding increased values of Hb recorded in all treated fish, which did not significantly differ ($p>0.05$) from each other, but differed significantly ($p<0.05$) compared to that of the control group could possibly reflect the high demand for oxygen in the blood of fish. It may also show that the inclusion of *S. indicum* in the fish meal is a way forward to combat anaemia. An increase in Hb concentration means high oxygen demand in the blood and its decrease in concentration signifies a anaemia development (Effiong *et al*., 2014). The level of Hb in the blood provides sensitive techniques for detecting conditions of disease in fish (Muhammad *et al*., 2015). The high numerical values for other hematological parameters (MCV, MCH and MCHC and PLT) obtained in this study imply a good condition of the fish blood and lack of adverse effects generated by the *S. indicum* inclusion in the fish meal. It's also a sign that the high protein content of the experimental diets favored the indices. The finding complies with the statement that protein molecules are excellent nutrients block and blood builders. This result complies with that reported by Effiong *et al.* (2014) that high protein diet favored the blood indices. All these indices are secondary responses of irritation by an organism. MCV is used to detect the level of RBC, while MCH is for HB level estimation and MCHC level signifies normal red blood swelling (Effiong *et al*., 2014). Finally, table 6 presents the results of white blood differential cells in *C. gariepinus* after dietary treatment with fish diets containing different percentages of *S. indicum* meal. It reveals that all the white blood components studied in this experiment in the treated fish except for monocytes were numerically increased significantly ($p<0.05$) with an ascending percentage of *S. indicum* in the experimental diets (Table 6).

Table 6. White blood cell differential levels (x10^2/μL) of *Clarias gariepinus* treated with diets containing varying % of *S. indicum* meal.

WBC (×10^2/μL)	Dietary treatments					
	DT1	DT2	DT3	DT4	DT5	DT6
Leukocytes	140.3±0.01^b	204.1±0.001^a	203.5±1.00^a	203.6±1.20^a	204.4±0.01^a	204.3±0.10^a
Lymphocytes	75.5±1.00^b	86.6±0.01^a	88.4±0.01^a	88.6±1.00^a	88.8±1.00^a	88.8±0.01^a
Neutrophils	0.73±1.00^b	0.82±0.01^a	0.80±1.00^a	0.83±0.01^a	0.81±0.01^a	0.84±1.00^a
Monocytes	2.02±0.02^a	1.71±0.01^b	1.68±1.00^b	1.65±0.01^b	1.70±0.01^b	1.67±0.01^b
Eosinophils	0.46±0.01^b	0.76±1.00^a	0.78±0.02^a	0.77±0.02^a	0.80±0.01^a	0.84±1.00^a
Basophils	0.04±0.01^b	0.06±0.01^a	0.07±0.01^a	0.08±0.01^a	0.08±0.01^a	0.08±0.01^a

Data are presented as mean±standard deviation. Means with the different superscript within a row are significantly different (p<0.05). DT1: commercial diet (control), DT2: 0% sesame seed meal with 100% soya bean meal, DT3: 25% sesame seed meal with 75% soya bean meal, DT4: 50% sesame seed meal with 50% soya bean meal, DT5: 75% sesame seed meal with 25% soya bean meal, DT6: 100% sesame seed meal with 0% soya

Higher significant values for leukocytes, lymphocytes, neutrophils, eosinophils, and basophils were observed in all fish fed fish diets containing different percentage of *S. indicum* meal than that of the group of fish fed the control diet (DT1). The figures recorded were not a significant difference ($p>0.05$) from each other (Table 6). Conversely, the monocyte values recorded in fish fed diets containing different percentages of *S. indicum* were numerically reduced compared to the fish group served the control diet (DT1). All the values observed in all fish served diets with different levels of *S. indicum* meal were not significantly differed ($p>0.05$) from each other (Table 6). Generally, the improved immune systems and the capacity of any animal to resist against vulnerable diseases are determined by the levels of components of white blood cells (leukocytes, lymphocytes, monocytes, and neutrophils (Effiong *et al*., 2014). The high levels of these components observed compared to those of the control group comply that all the experimental fish involved did not show any sign of adverse health defect. This could further explain the ability of formulated diets substituted with varying levels of *S. Indicum* to provide better health conditions for the *C. gariepinus*. High significant ($p<0.05$) values of white blood count observed in this study in all treated fish is a reflection of all formulated diet's ability to improve their immunity levels. These values obtained are significantly greater compared to 52.00×10^2/μL reported by George *et al*. (2012).

Conclusion: All findings from this study have shown that at any level of *S. indicum* inclusion in the fish meal of *C. gariepinus* there was a potential improvement of the functional properties, hematological parameters and maintaining the levels of phytotoxins not to rise above the permissible limit. Thus, experimental diets (DT5 and DT6) with 75 and 100% *S. indicum* would be promising candidates which may be used for the development of the product in various food industries.

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