



ASERUM BIOCHEMICAL PROFILES AND IMMUNO-STIMULATORY POTENTIALS OF *Clarias gariepinus* JUVENILES FED FISH MEAL CONTAINING DIFFERENT INCLUSION LEVELS OF *Sesame indicum* SEED MEAL

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ABSTRACT

Sesame indicum L. is a class of the *Pedaliaceae* family of plants that contains good eatable oil, protein, mineral (calcium), and antioxidants and has been recommended as good food for ages. Beni seeds possess a nutritional content that is favour of digestibility, growth, and feed utilization among domesticated animals. The serum biochemical profiles and immuno-stimulatory potentials of *Clarias gariepinus* juveniles fed *Sesame indicum* seed meal-based diets were carried out. Serum marker enzymes (ALT, AST, ALT, GGT, and LDH), serum chemistry; {glucose (GLU), total protein (TPN), bilirubin (BIL), cholesterol (CHL), albumin (ALB), creatinine (CTN), and immuno-stimulatory potentials; haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC), lymphocytes (LYM), neutrophils (NEU), monocytes (MON), eosinophil (EOS), and basophils (BAS) in *C. gariepinus* fed *Sesame indicum* seed meal-based diets were serum biochemical indices analyzed. High levels of serum marker enzymes {ALT (18.12-22.43U/L), AST (20.13-20.57U/L), ALT (18.62-22.65U/L), GGT (1.72-2.40U/L), LDH (104.35-110.01U/L)}, serum chemistry {Glucose (72.42- 75.34mg/dL), protein (7.01-7.04g/dL), bilirubin (0.44-1.52mg/dL), cholesterol (77.22-82.02mg/dL), albumin (2.46-3.65g/dL), creatinine (0.16-0.23mg/dL), and immuno-stimulatory potential (not greater than the permissible level) were observed in *C. gariepinus* fed *S. indicum* seed meal-based diets compared to those fed the control diet (DT1) only. Based on these results, the inclusion of *S. indicum* in the diets of *C. gariepinus* could therefore be saved and look promising in terms of health benefits.

Keywords: Biochemical profiles; *C. gariepinus*; fish meal; *S. indicum*

INTRODUCTION

To improve the food security status of the people, fish culture farming has a key role to play. In the developing countries and those with problems of food-deficiency, about 2.9 billion people depend on fisheries as animal based-protein sources (Ayoola 2010). The rapid growth of species of catfish has rendered them a great potential to satisfy fish demand and reduces fish import, improves job

opportunities, and poverty reduction (Williams et al. 2007). Fish are mostly found in the aqueous surroundings therefore the analysis of physiological changes can easily be ascertained using their blood samples (Ezeri et al. 2004; Ndatsu et al., 2020_a).

In fish farming development, the assessment of their physiological parameters is the vital methods used to distinguish between healthy and diseased/stressed species of fish (Adesina

2017; Yakubu *et al.*, 2020). The physiology of the fish, blood, and quality of diet served needed to be understood. Assessment of blood components is used as a parameter to ascertain the status of fish health in detecting physiological changes upon exposure to any stressful situation. The variations in the levels of blood/serum components as compared to those of the normal levels might be used for interpreting the state of metabolism and the health condition of fish (Adesina 2017, Taheri Mirghaed *et al.*, 2022)

The low level of blood parameters may signify abnormality in fish. In fish, the value of serum compositions is determined to know their normal ranges and the level of disease conditions (Edori *et al.* 2013; Antony *et al.*, 2022). The assessment of fish haematology is helpful in diagnostic tests and determines the Biochemical parameters of *C. gariepinus* for the new dietary feeds to know the side effects of stress conditions. Serum/blood assessment is a way of determining the state of the physiology of fish in other to analyze the effects of fish meal and stress-related conditions in the health of fish (Belko-olusoyi *et al.* 2006; Ndatsu *et al.*, 2020_b). Alterations in serum components of fish after exposure to toxic-related agents are the stage of stressful signs in fish that provides useful foresight against the abnormal condition that might affect the health of fish (Belko-olusoyi *et al.* 2006). It has been established that serum/blood assessment is one of the key role factors to behold in the evaluation of fish diets (Adepanisi and Ajayi 2004; Nisarar *et al.*, 2021).

The most commonly used plant-base protein source in the formulation of fish meal is soybean (Idowu and Afolayan 2013, Adesina 2017; Yakubu *et al.*, 2020). There are surplus plants in nature that can be used as a source of plant-based protein to replace soybean, which are castles, available and help lower the cost

of fish meal (Adesina 2017). This research considered the inclusion of *Sesame indicum* (Beni seed) meal for replacing soybean as a source of plant-based proteins. *S. indicum* L. is a class of the *Pedaliaceae* family of plants and commonly cultivated in Asian and African countries (Garba et al. 2000). It contains good eatable oil, protein, mineral (calcium), and antioxidants and has been recommended as good food for ages (Alobo 2001; Yakubu *et al.*, 2020). The fried and fermented seeds are used in making soup for human consumption (Uaboi et al. 2008). Its consumption in Nigeria is highly demanding due to global decrease in animal proteins, high demand for cholesterol-free and saturated fats lower foods. The acceptability of sesame foods in Nigeria could be reflective of its lower cost, availability and recognition of health benefits (Peters *et al.* 2016; Yakubu *et al.*, 2020). Beni seed has been loudly used as a protein supplement in the diet of fishes. This is because of the nutritional content that is favorable for the digestibility, growth, and feed utilization among domesticated animals (Adeniyani et al. 2013). Past studies have demonstrated that complete or partial substitution of fish meal with alternative proteins does not adversely affect the health of fish (Ayoola 2010). Hence, by promoting the inclusion of locally available protein-rich stuff in fish meals may increase the fish health.

Therefore, this study was carried out to evaluate the effects of different inclusion levels of *sesame indicum* seed on serum biochemical profiles and immuno-stimulatory potentials of *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 collected at Lapai

main market, Niger state in June 2019. Botanical identification of plant was confirmed by the Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State.

Sample Preparation

This was prepared using the methods adopted by Fowler *et al* (2019), Ndatsu et al. (2020_a) and Yakubu et al. (2020). Briefly, the feedstuffs 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 2 mm diameter size using pelleting machine (Hobart A-200TGmbH, Rhen-Bosch, Offenburg, Germany). These were sun-dried to about 10% moisture content, packed in polythene 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

(Fowler et al., 2019; Ndatsu et al., 2020_a; Yakubu et al., 2020)

Feed Formulation

This was done following the methods of Ndatsu *et al.* (2020_b) and Taheri Mirghaed *et al.* (2022). Briefly, about five (5) dietary treatments with varying graded levels (0, 25, 50, 75, and 100%) of *S. indicum* seed meal (SSM) compositions were replaced for soybean meal (SBM) in the dietary treatments (DT), namely, 1, 2, 3, 4, 5 and 6 were formulated (Table 2). The DT1 is purely, commercial diet, DT2 is composed of 0%

SSM with 100% SBM (control), DT3 is having 25% SSM with 75% SBM, DT4 is composed of 50% SSM with 50% SBM, DT5 is composed of 75% SSM with 25% SBM, and DT6 is 100% SSM with 0% SBM (Table 2).

Experimental Animals

A total of one hundred and twenty (120) *C. gariepinus* of mixed-sex and the same age (mean weight of 332.16.5±5.20 g and a length of 34.25±0.43 cm) were purchased from Lapai Gwari fish Village, along Lapai – Paiko-Minna road, Niger state. The fishes were transported in an aerated container 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 (20mg/100kg) three times daily. 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 Treatment and Design

The fishes were selected randomly into six (6) rearing tanks (10 L) with 20 *C. gariepinus* per rearing tank (Kumar et al. 2011; Nisarath et al., 2021; Antony et al., 2022). Each rearing tank (RT1, RT2, RT3, RT4, RT5, and RT6) was assigned to a dietary treatment (Table 2), 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 as follows:

Table 2: Feeding trial and treatment

RT	DT (20 g/day)	NF
1	Commercial diet	20
2	0% SSM + 100% SBM	20
3	25% SSM + 75% SBM	20
4	50% SSM + 50% SBM	20
5	75% SSM + 25% SBM	20
6	100% SSM + 0% SBM	20

RT: rearing tank, DT: dietary treatment, NF: number of fish, SSM: sesame meal, SBM: soya bean meal

The fishes were fed dietary treatment (20g/kg) once (1) in a day for four (4) weeks. Animal experiments were conducted following the laboratory animal use and care (NIEHS 1985).

Blood Sampling

At the end of 4 weeks, the dietary treated fish starved for 12 hrs before harvest for blood sample collection. The method of Effiong *et al.* (2019); Magouz *et al.* (2021) with little modification was used for blood sampling and analysis. The blood samples were taken to Clinical Pathology Laboratory, General Hospital, Lapai, Niger State and centrifuged to have serum for the analyses of serum enzyme markers, chemistry, and immuno-stimulatory potential of the dietary treatments was examined using blood samples collected from dietary treated fish.

Biochemical Analyses

This was conducted following the methods of Nisarar *et al.* (2021). The marker enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and bilirubin) concentrations were measured using Roche Modular Auto-analyzer (Cobas® 8000 modular analyzer series). Total protein, albumin, glucose, total cholesterol, and creatinine were analyzed by using standard random laboratory kits (Sigma-Aldrich, USA).

Immuno-Stimulatory Effect of Dietary Treatments on Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC)

The method of Chidume *et al.* (2002); Magouz *et al.* (2021) with slight modification was used to perform the TLC and DLC determination. Briefly, the blood sample was collected directly from the dietary treated fish for TLC by cell diluting pipette (capillary tube). The TLC was done by making 1:20 dilution of the blood samples with white cell diluting fluid and counting with the aid of a counting chamber under the microscope (x10 magnification). The DLC was determined by making a thin film of the blood samples on microscopic slides and staining with Leishman's stain. The films were air-dried at ambient temperature and examined microscopically under oil immersion (x100 magnification).

Statistical Analysis

The data were presented as the mean±standard deviation. Data were analyzed using a one-way analysis of variance (ANOVA) and the least significant difference (LSD) test was used to evaluate the significant difference. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The results of liver enzyme levels in *C. gariepinus* fed dietary treatments embedded with graded percentages of *S. indicum* meal are shown in Table 4. ALT levels in *C. gariepinus* fed DT2 (22.43U/L), DT3 (21.60U/L), and DT6 (21.65U/L) did not differ significantly ($P < 0.05$), but differed from those fed DT4 (18.16U/L) and DT5 (18.12U/L) while *C. gariepinus* fed DT1 had lower levels of ALT compared to others. *C. gariepinus* fed DT2 DT3, DT4, DT5, and DT6 had higher levels of AST (20.13, 20.54, 20.26, 20.23, and 20.57U/L), respectively,

which are not differ significantly, from each other, but differed from that fed DT1 (12.43U/L). The levels of ALP recorded in fish fed DT2, DT3, and DT6, respectively, were not a significant difference ($P<0.05$) from each other, but differed from those fed DT4 (18.66U/L), and DT5 (18.62U/L) while *C. gariepinus* fed DT1 recorded a lower value of ALP (12.31U/L) (Table 4). Similarly, the GGT (2.40, and 2.03U/L) and LDH (110.01, and 112.23U/L) levels recorded in *C.*

gariepinus fed DT2, and DT3, respectively, had no significant difference ($P<0.05$) from each other, but differed significantly, as compared to that fed DT1 (1.54, 102.25U/L), DT4 (1.76, 104.37U/L), DT5 (1.72, 104.35U/L), and DT6 (1.05, 106.21U/L), respectively. The levels of all serum biochemical markers in *C. gariepinus* fed formulated diets were higher significantly than those fed commercial diets (DT1) (Table 3).

Table 3: Serum Enzyme Marker Levels of *C. gariepinus* fed Varying levels of *S. indicum* inclusion.

DT (%)	Serum enzymes				
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
DT1	11.24±0.01 ^c	12.43±2.11 ^c	12.31±1.00 ^c	1.54±1.20 ^b	102.25±0.01 ^b
DT2	22.43±1.00 ^a	20.13±0.31 ^a	22.15±1.00 ^a	2.40±0.31 ^a	110.01±2.04 ^a
DT3	21.60±0.02 ^a	20.54±1.21 ^a	21.43±1.00 ^a	2.03±1.01 ^a	112.23±1.12 ^a
DT4	18.16±0.01 ^b	20.26±1.00 ^a	18.66±0.02 ^b	1.76±2.02 ^b	104.37±2.01 ^b
DT5	18.12±0.01 ^b	20.23±1.02 ^a	18.62±1.02 ^b	1.72±0.41 ^b	104.35±1.00 ^b
DT6	21.65±1.23 ^a	20.57±0.24 ^a	21.41±1.23 ^a	1.05±2.01 ^a	106.21±1.23 ^b

Data are presented as mean±standard deviation. Means with the different superscript letters within a column are significantly different ($p<0.05$). DT: dietary treatment, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, LDH: lactate dehydrogenase, DT1: commercial diet (control), DT2: 0% SSM + 100% SBM, DT3: 25% SSM + 75% SBM, DT4: 50% SSM + 50% SBM, DT5: 75% SSM + 25% SBM, DT6: 100% SSM + 0% soya SBM.

The results of the serum chemistry of *C. gariepinus* fed graded levels of *S. indicum* seed meal-based diets are shown in Table 5. Significant differences ($p<0.05$) were confirmed between the levels of serum chemistry indices examined. A significant increase ($p<0.05$) in serum chemistry parameters, but not above the recommended limit was noted in the *C. gariepinus* fed different levels of *S. indicum* seed meal-based diets than those of the control group (DT1). *C. gariepinus* fed DT3 and DT6 showed higher levels of blood glucose (75.33 and 75.32mg/dL), respectively, than others and not differed significantly ($p<0.05$) from each other. This was followed by serum blood glucose levels recorded in *C. gariepinus* fed

DT4 (74.33 mg/dL) and DT5 (74.37mg/dL) while those fed DT1 and DT2 had the lower blood glucose (72.23 and 72.42mg/mg/dL), respectively than others, but significantly ($p<0.05$) not differed from each other. In addition, higher values of total protein (7.02, 7.01, and 7.04g/dL) were shown in *C. gariepinus* fed DT2, DT3, and DT6, respectively, compared to others, and not differed significantly ($p<0.05$) from each other. While the total protein levels (6.56, 6.54g/dL) recorded in *C. gariepinus* fed DT4 and DT5, respectively, have not differed significantly ($p<0.05$) from each other. Thus, the *C. gariepinus* fed the commercial diet (DT1) recorded significantly lower ($p<0.05$) serum total protein (5.31g/dL) than others.

Similarly, the bilirubin (BIL) levels (1.52, 1.42, 1.46mg/dL) in fish fed DT2, DT3, and DT6, respectively, were higher significant ($p<0.05$) compared to others, but not significantly differed from each other. These were followed by the serum BIL (0.44 and 0.45mg/dL) recorded in *C. gariepinus* fed DT4 and DT5, respectively. However, *C. gariepinus* fed a commercial diet (DT1) had a significant ($p<0.05$) decrease in serum BIL levels compared to others. Likewise, a significant increase ($p<0.05$) in cholesterol (CHL) levels was shown in *C. gariepinus* fed DT2, DT3, and DT6 than others. This was

followed by *C. gariepinus* fed DT4 and DT5, while *C. gariepinus* fed DT1 gave a lower significant level ($p<0.05$) of CHL (63.01mg/dL) Higher significant ($p<0.05$) values of albumin (ALB) and creatinine (CTN) were noted in *C. gariepinus* fed DT2, DT3 and DT6 than others, followed by that of those fed DT4 and DT5. Significant decreases ($p<0.05$) in both ALB and CTN were observed in *C. gariepinus* fed a commercial diet (DT1) than others (Table 4).

Table 4: Serum Chemistry Levels of *C. gariepinus* fed Varying levels of *S. indicum* inclusion

DT (%)	GLU (mg/dL)	TPN (g/dL)	BIL (mg/dL)	CHL (mg/dL)	ALB (g/dL)	CTN (mg/dL)
DT1	72.23±0.01 ^c	5.31±0.01 ^c	0.32±1.20 ^c	63.01±1.20 ^c	1.47±0.01 ^c	0.04±2.01 ^c
DT2	72.42±1.00 ^c	7.02±0.01 ^a	1.52±1.02 ^a	82.03±0.01 ^a	3.65±0.01 ^a	0.23±2.10 ^a
DT3	75.34±0.02 ^a	7.01±0.01 ^a	1.46±1.00 ^a	80.24±0.01 ^a	3.54±0.01 ^a	0.21±1.23 ^a
DT4	74.33±0.01 ^b	6.56±1.00 ^b	0.44±2.02 ^b	77.26±0.02 ^b	2.46±0.01 ^b	0.16±1.02 ^b
DT5	74.37±0.01 ^b	6.54±1.00 ^b	0.45±2.02 ^b	77.23±0.01 ^b	2.46±1.00 ^b	0.16±2.01 ^b
DT6	75.32±2.01 ^a	7.04±1.01 ^a	1.46±2.13 ^a	81.22±1.27 ^a	3.56±1.34 ^a	0.21±1.24 ^a

Data are presented as mean±standard deviation. Means with the different superscript letters within a column are significantly different ($p<0.05$). DT: dietary treatment, GLU: glucose, TPN: total protein, BIL: bilirubin, CHL: cholesterol, ALB: albumin, CTN: creatinine, DT1: commercial diet (control), DT2: 0% SSM + 100% SBM, DT3: 25% SSM + 75% SBM, DT4: 50% SSM + 50% SBM, DT5: 75% SSM + 25% SBM, DT6: 100% SSM + 0% soya SBM.

The results of indices of immuno-stimulatory activities in *C. gariepinus* fed graded levels of *S. indicum* seed meal-based diets are presented in Table 6. It reveals that significant differences ($p<0.05$) were observed between the levels of immuno-stimulatory indices determined. Significant increases ($p<0.05$) in the numerical values of immuno-stimulatory indices were noted in the *C. gariepinus* fed different levels of *S. indicum* seed meal-based diets than those of the control group (DT1). No significant difference in increased values of Hb (11.14, 11.18, 11.15, and 11.14g/dL) was observed in *C. gariepinus* fed DT3, DT4, DT5, and DT6, respectively, than others. This was followed by that of *C. gariepinus* fed DT2 (10.21g/dL) while *C. gariepinus* fed a commercial diet had lower Hb (09.01g/dL)

compared to others. In addition, *C. gariepinus* fed DT3, DT4, and DT5 had increased levels of PCV (18.01, 18.12, and 18.15%) and WBC (12.23, 12.05, 12.04×10⁹/L), respectively which are similar significant compared to others. These were followed by the levels of PCV (17.20 and 17.03%) and WBC (11.67 and 11.21×10⁹/L) observed in *C. gariepinus* fed DT2 and DT6, respectively. Lower significant values of PCV (16.67%) and WBC (10.51×10⁹/L) were noted in *C. gariepinus* fed the commercial diet (DT1), respectively than others. Moreover, *C. gariepinus* fed DT2, DT3, DT4, DT5, and DT6 recorded similar significantly increase levels of the LYM (38.48, 30.10, 32.01, 32.10, and 30.11%), NEU (32.01, 32.34, 32.67, 32.65, and 32.32%), and MON

(3.83, 3.12, 3.67, 3.65, and 3.13%), respectively, than others. While *C. gariepinus* fed DT1 gave lower significant values of the LYM (28.24%), NEU (21.32%), and MON (1.45%), respectively, compared to others (Table 6). The increased levels of the EOS and BAS recorded in *C. gariepinus* fed DT2, DT3, DT4, and DT5 were not differed

significantly compared to others, followed by EOS (10.45%) and BAS (2.21%) levels in *C. gariepinus* fed DT6, respectively. *C. gariepinus* fed commercial diet gave lower significant values of EOS (09.12%) and BAS (1.34%) than others (Table 5).

Table 5: Immuno-stimulatory potential of the dietary treatments in *C. gariepinus*

DT (%)	Blood sample							
	Hb (g/dL)	PCV (%)	WBC ($\times 10^9/L$)	LYM (%)	NEU (%)	MON (%)	EOS (%)	BAS (%)
DT1	9.01 \pm 1.0 ^c	16.67 \pm 0.2 ^c	10.51 \pm 1.0 ^c	28.24 \pm 0.3 ^b	21.32 \pm 1.2 ^b	1.45 \pm 0.5 ^b	9.12 \pm 0.5 ^c	1.34 \pm 0.3 ^c
DT2	10.21 \pm 0.2 ^b	17.20 \pm 0.1 ^b	11.67 \pm 0.5 ^b	38.45 \pm 0.4 ^a	32.01 \pm 0.3 ^a	3.83 \pm 0.3 ^a	11.02 \pm 0.3 ^a	3.76 \pm 0.6 ^a
DT3	11.14 \pm 0.4 ^a	18.01 \pm 0.4 ^a	12.23 \pm 0.4 ^b	30.10 \pm 0.5 ^a	32.34 \pm 0.4 ^a	3.12 \pm 0.5 ^a	11.32 \pm 0.5 ^a	3.11 \pm 0.1 ^a
DT4	11.18 \pm 0.3 ^a	18.12 \pm 0.3 ^a	12.05 \pm 0.4 ^a	32.01 \pm 0.6 ^a	32.67 \pm 0.5 ^a	3.67 \pm 0.4 ^a	11.64 \pm 0.2 ^a	3.65 \pm 0.4 ^a
DT5	11.15 \pm 0.2 ^a	18.15 \pm 0.2 ^a	12.04 \pm 0.2 ^a	32.10 \pm 0.3 ^a	32.65 \pm 0.4 ^a	3.65 \pm 0.2 ^a	11.36 \pm 0.3 ^a	3.01 \pm 0.3 ^a
DT6	11.14 \pm 0.5 ^a	17.03 \pm 0.4 ^b	11.21 \pm 0.5 ^b	30.11 \pm 0.2 ^a	32.32 \pm 0.5 ^a	3.13 \pm 0.3 ^a	10.45 \pm 1.0 ^b	2.21 \pm 0.4 ^b

The data are presented in mean \pm SD (average of three readings). Means with the different superscript letters within a column are significantly different from control at $p < 0.05$ by ANOVA and Dunnett's test. **DT:** dietary treatment, **Hb:** haemoglobin, **PVC:** packed cell volume, **WBC:** white blood cells, **LYM:** lymphocytes, **NEU:** neutrophils, **MON:** monocytes, **EOS:** eosinophil, **BAS:** basophils. DT1: commercial diet (control), DT2: 0% SSM + 100% SBM, DT3: 25% SSM + 75% SBM, DT4: 50% SSM + 50% SBM, DT5: 75% SSM + 25% SBM, DT6: 100% SSM + 0% soya SBM.

DISCUSSION

A significant increase in ALT and AST) in the plasma of all *C. gariepinus* fed graded levels of *S. indicum* seed meal-based diets as compared to the control diet (DT1) may be attributed to the dietary amino acid utilization by fish for growth or high demand for energy against the stress-related agents. Also, the rise in ALP levels in the plasma of *C. gariepinus* fed graded levels of *S. indicum* seed meal-based diets as compared to the control diet (DT1) signify the high rate of phosphorylation and transport of substances across the cell membrane that may be leading to elevated kidney detoxification effects (Usese et al. 2018). The elevations of these enzymes may reflect an alteration in the pathway ways of biosynthesis and mixed-function oxidase (Achilike et al. 2019). The analyses of these enzymes give proper situations on the status

of hepatocytes and diseases, which could be damaging to the liver caused by the reactive oxygen species generated (Marigoudar *et al.* 2016; Ndatsu *et al.*, 2020_a). Although, all the values of enzymes evaluated were within the normal range, which signify the sound healthy status of the fish. That is graded levels of *S. indicum* seed meal-based diets exhibited a protective effect against hepatocyte damages caused by abnormal conditions of poor quality of water. The protective ability of graded levels of *S. indicum* seed meal-based diets could be due to the high antioxidant properties of varying levels of *S. indicum* meal embedded in the diets. High antioxidant contents have been reported in *S. indicum* (Adeniyani et al. 2013; Jeong et al. 2004). Phenols are good antioxidant compounds with several effects on health improvement. They are bioactive compounds that have stable radical, potential to donate an electron, and

preventing the oxidation of food ingredients. The ability of phenolic compounds to prevent oxidation of food ingredients reflected their redox potential, which has a significant role to play in neutralizing free radical species (Ndatsu et al. 2013; Adeniyani et al. 2013; (Ndatsu et al., 2020_a). Besides, the elevation of GGT and LDH levels were observed in the plasma of *C. gariepinus* fed graded levels of *S. indicum* seed meal-based diets compared to the control diet (DT1), but the values obtained were not above the normal range for GGT (0-25 U/L) and LHD (140-280U/L). Also, the values of GGT and LDH activities, which were maintained within the normal range, could be attributed to the diet's capacity to inhibit/reduce their rate of synthesis in the liver. High values of GGT obtained, which were not above the normal range in the experimental *C. gariepinus* compared to those fed control diets (DT1) signify the formulated diet's protective effect against cholestatic disorder. GGT is a serum marker of cholestasis, a condition of bile slow flow or stoppage from the liver that obstructs the biliary system (Docas et al. 2014). All experimental diets exhibited hepatocyte protection against stressful-related agents. The mechanism of antioxidants does involve in hepatic tissue protections by the diets over reactive oxygen species (ROS) produced upon exposure to stress-related agents (Jeong et al. 2004). High contents of antioxidant property of *S. indicum* have been documented (Adeniyani et al. 2013, Jeong et al. 2004), which are good quencher/neutralizer of ROS generated in the hepatic cells. Stuff foods are richer in polyphenol compounds (phenols and flavonoids). The flavonoids' antioxidant capacity is stronger than those of vitamin C and E (Alia et al. 2003). High values of LDH, but within the normal range in the treated *C. gariepinus* fed graded levels of *S. indicum* seed meal-based diets could signify protection against hemolysis caused by free radical

generation upon exposure to stressful conditions.

LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis (Ndatsu et al. 2013). The hepatic toxicity caused by stress-related conditions can affect the liver microsomal membrane through altered polyunsaturated fatty acids (Ndatsu et al. 2013). Elevated LDH values were observed in lead-exposed fish and the increased levels of enzyme experienced might suggest the ROS production caused by stressful conditions (Elarabany and Bahnasawy, 2019). LDH is a metabolic enzyme used as a biomarker in the oxidatively stressed fish and its rise that was within the normal range (100-250 U/L) suggested tissue breakdown preventive mechanism in stressed *C. gariepinus* (Elarabany and Bahnasawy 2019, Saliu and Bawa-Allah 2012), it also confirms the observation of Malachy et al. (2015).

All *C. gariepinus* fed *S. indicum* seed meal-based diets had their serum glucose, bilirubin, cholesterol, and creatinine levels significantly rise ($p < 0.05$) than those fed control diet (DT1). Though the values obtained were all not above the acceptable range. This could be attributed to the ability of *C. gariepinus* to generate energy from the available means against the oxidative stress caused by stressful conditions. That is enough energy must be acquired by fish from the available sources to fight against the ROS generated. It might also suggest potent hepatoprotection by *S. indicum* seed meal-based diets against ROS generated by oxidative stress. The hepatoprotection exhibited by formulated diets may signify their ability to trigger insulin secretion from pancreatic cells to enhance the process of glycogenesis. It was reported that an increase production of insulin from the pancreatic tissue in exposed fish improves the

glycogenesis processes (Gbora et al. 2006). Invariably, the results of this study are relatively lower than the values reported by Adesina, (Adesina 2017, Anene et al. 2014. Adesina (2017) have reported that adequate energy liberated from the available sources is required by stressed fish to survive. High demand for glycogen mobilization may signify an increased level of blood glucose and depletion of glycogen in the liver and muscle upon anti-dietary exposure (Adesina 2017).

Furthermore, increased levels of serum bilirubin in *C. gariepinus* fed *S. indicum* seed meal-based diets may suggest a compensatory process against biliary gland damage due to cellular peroxidative changes. The bilirubin *in vivo* plays a vital role as a potent ROS neutralizer, antimutagen, and a protector of endogenous tissue (Pratibha et al. 2004). Its rise levels recorded in fish fed *S. indicum* seed meal-based diets, which was not above the normal range might suggest their potential hepatocyte protections. The synthesis of bile acid depends on the stability of the function of the biliary cell and endoplasmic reticulum (Sureshkumar and Mishra 2002).

Besides, the normal levels of protein and albumin recorded in *C. gariepinus* fed *S. indicum* seed meal-based diets could be due to the no inhibition of protein synthesis in the liver. That is all *S. indicum* seed meal-based diets exhibited potent hepatic damage repair by normalizing the process of protein synthesis. This indicated that all *S. indicum* seed meal-based diets possess hepatoprotective property against stressful conditions. The high antioxidant properties of these diets could be attributed to their hepatic protection mechanism against ROS generated. The antioxidant potentials exhibited by the formulated diets may suggest the presence of phenols and flavonoids (Lin et al. 2016, Okoronkwo et al. 2014). Ndatsu et al. (2013)

and Adesina (2017) have reported that normal levels of protein and albumin in animals (fish) exposed to a toxic agent might suggest the hydration state and water equilibrium changes or inhibition of protein synthesis in the liver. Proteins are one of the major energy sources in fish that monitor blood glucose maintenance (Ndatsu et al. 2013).

Lastly, the normal levels of serum total cholesterol and creatinine recorded in *C. gariepinus* fed *S. indicum* seed meal-based diets compared to those fed the control diet (DT1) could be associated with the hepatoprotective potential of all diets against generated ROS by the stressful situations. Normal levels of serum cholesterol recorded in all fish fed *S. indicum* seed meal-based diets suggest protective property against hypercholesterolemia and atherosclerosis. This could be probably due to the high antioxidant potential of all *S. indicum* seed meal-based diets. Lin et al. 2016) have reported on the highest antioxidant potential of *S. indicum*. It was reported that phenols and flavonoids could protect membrane lipids from oxidation, and a major source of flavonoids is foodstuff (Okoronkwo et al. 2014). Normal levels of creatinine observed in fish served *S. indicum* seed meal-based diets signify their potential nephroprotective properties against ROS induced by a stressful situation. The high values of phenols and flavonoids in *S. indicum* could be probably responsible for the kidney damage repair (Okoronkwo et al. 2014). As the kidneys become impaired for any reason, the creatinine level in the blood will rise above the normal range due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys (Adesina 2017).

Interestingly, increased in the numerical values of immuno-stimulatory indices shown

in the *C. gariepinus* fed different levels of *S. indicum* seed meal-based diets than those fed control group (DT1) signify the ability of *S. indicum* seed meal-based diets to stimulate the immune system. The corresponding increased values of Hb noted in fish fed *S. indicum* seed meal-based diets as compared to those fed control diets (DT1) could reflect the high demand for oxygen in the blood of fish and capacity of experimental diets to fight against anaemia. A high level of Hb signifies high oxygen demand in the blood and its low level means 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). Yakubu et al. (2020) have demonstrated that *S. indicum* seed meal-based diets are excellent protein diets and that those protein molecules are excellent nutrients block and blood builders. Effiong et al. (2014) have reported that high protein diets favoured blood indices. High levels of packed cell volume (PCV) observed in fish fed *S. indicum* seed meal-based diets than the control groups (DT1) are reflections of erythrocytosis and protective against anaemia. PCV is used to measure the mass of red blood cells. An increase in red blood cell mass is equivalent to erythrocytosis and a decrease indicates anaemia (Yakubu et al. 2020).

The corresponding increased values of White blood cell count and differential leukocyte count (lymphocytes, neutrophils, monocytes, eosinophil, and basophils) in fish fed *S. indicum* seed meal-based diets as compared to those fed control diets (DT1) could be probably due to the ability of *S. indicum* seed meal-based diets to enhance the cellular immunity system in the *C. gariepinus*. It could also explain the capacity of *S. indicum* seed meal-based diets to promote good health conditions for the *C. gariepinus*. That is a sign that the high protein content of the experimental diets favored the indices.

Effiong et al. (2014) have demonstrated that high protein diets favored blood indices. These results supported what Ibukun (2012) and Yakubu et al. (2020) have reported that *S. indicum* seed is used in blood cleansing, high in protein content, and can be served as a blood builder. That is the elevated immune systems and the ability of *C. gariepinus* to protect themselves over any vulnerable diseases is determined by the levels of total leukocyte count (Effiong et al. 2014). It could also signify that all the *C. gariepinus* didn't indicate any sign of adverse health defect. The White Blood Cells (WBC) and its components are termed defensive cells of the system and their levels in the system could determine the immune system the capacity of the animals to protect over infection (Adedeji and Adegbile 2011). The animals with elevated levels of leukocyte count circulating in the body can protect themselves against infectious diseases through cell/humoral-mediated responses (William et al. 2016; Adedeji and Adegbile 2011).

CONCLUSION

It could be concluded that all fish fed diets with graded levels of *S. indicum* seed meal had significantly elevated values (but still within the recommended levels) of serum enzyme markers, serum chemistry and Immuno-stimulatory potential than those fed the control diet (DT1). Based on the results of this study, there was not much stress placed on the health of *C. gariepinus* fed even at a higher level of *S. indicum* seed meal inclusion (100%). It could therefore be saved and of more promising in terms of health benefit upon inclusion in the diets of *C. gariepinus*.

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