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RESEARCH PAPER

# Effects of Roselle (*Hibiscus sabdariffa)* Seeds as a Substitute for Soya Bean on Growth and Nutrient Utilization of *Clarias gariepinus* (Burchell, 1822)

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## Abstract

Effects of replacing soybean meal (SBM) with *Hibiscus sabdariffa* (HSSM) seed meal as protein sources in the diet of *Clarias gariepinus* fingerlings (Burchell, 1822) was investigated. *Hibiscus sabdariffa* seed was processed by soaking for 24 hours, sprouting for 48 hours and fermenting for 72 hours. The processing methods that gave higher crude protein level, and lower anti-nutritional factors were used for the replacement of soya bean. The result shows that the crude protein values (CP) recorded for raw, soaked, sprouted and fermented *Hibiscus sabdariffa* were 25.49, 25.19, 24.25 and 25.68%, respectively. In view of this, fermented *Hibiscus sabdariffa* seed meal (FHSSM) was used. Different replacement levels of the soya bean with fermented *Hibiscus sabdariffa* seed meal (0 control, 25, 50, 75 and 100 %) were allotted to five groups of *C. gariepinus* fingerlings (4.0 - 4.4 g) randomly stocked at 10 fish per unit in 1 m x 1 m x 1.2 m hapa installed in a polythene lined pond (7 x 5 x 1.2 m) in completely randomized design with an average rainfall of 550 mm and fed for 150 days. The haematological indices of *C. gariepinus* fed different replacement levels of soya bean with FHSSM were also studied. The haematological parameters indicate that there was no significant difference (P>0.05) among the treatments in most of the parameters. The ANFs in FHSSM are within the tolerable level by fish. This informed the basis for the selection of FHSSM for the feeding trials. Feeding trials conducted indicates no significant difference (P>0.05) in condition factor, survival and protein efficiency ratio (PER) for the various treatments. Better weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) are recorded in fish fed 25% FHSSM. This implies that 25% of soya bean meal in *C. gariepinus* could be substituted with fermented *Hibiscus sabdariffa* seed meal without deleterious effect on growth and utilization performance.

## Introduction

Aquaculture activity is considered as one of the alternatives for the development and improvement of Fisheries (Okechi, 2014). Fish production has continued to be the source of hope toward solving global problem of malnutrition due to its richness in nutritive values above other animal sources of protein FAO (2018). The expansion and intensification of aquaculture production has been recommended towards ensuring increase in fish production in order to meet up with the global demand since capture fisheries have continued to be on the decline over decades (FAO, 2020).

Over the past decade aquaculture has grown in response to an increasing demand for fish as a source of protein globally (Hua *et al.,* 2019). This is because production from captured fisheries has reached its maximum potential possible, as the catch is dwindling with each passing days (Watkiss *et al.,* 2019). According to FAO (2020), fish supplies from capture fisheries will therefore, be unable to meet the growing global demand for aquatic food. The present requirement of fish was put at 3.2 million metric tonnes according to National Biotechnology Development Agency. It further puts our production at 1.1 million metric tonnes leaving a deficit of a little over 2 million metric tonnes (Premiumtimes, 2016). Therefore, there is the need for a viable alternative fish production that can sufficiently meet this demand, and aquaculture fits exactly into this role. As aquaculture production becomes more intensive in Nigeria, fish feed is a significant factor in increasing the productivity and profitability of aquaculture (Hua *et al.,* 2019).

Fish feeds constitutes 40-60% of the total cost of aquaculture production which is expensive and led to extensive studies on replacing a costly protein especially soya bean in fish diets (Prabu *et al.,* 2017). Similarly, Salih *et al.* (2021) observed that fish feed constitutes 60-70% of the operational cost in the intensive and semi-intensive aquaculture system. The need to minimize feed cost through the use of new and cheaper sources of feed ingredients is currently receiving the attention of nutritionists and the selection of feed ingredients for use as fish feed will play a major role in matching its ultimate nutritional requirement in addition to economic success Tiamiyu *et al.* (2015). There is high competition for foodstuffs between human and domestic animals. For economic and practical reasons, fish feed should be prepared from locally available protein source, preferably from those unsuitable for human consumption (Opiyo *et al.,* 2019). Fish is an important source of animal proteins, as more than 500 million people in developing countries depend on fisheries and aquaculture for their livelihood (FDI, 2013). *Clarias gariepinus* is one of the most cultured fish in Nigeria and indeed Africa (Dauda *et al.,* 2018). Its hardiness, ability to tolerate adverse condition, high fecundity and mass artificial seed technique also ease its culture (Dauda *et al.,* 2018).

Soya bean meal is the most commonly used plant protein in feeds for omnivorous fish species commonly used in African aquaculture, such as tilapias and catfishes *(Clarias gariepinus)* (Dawood and Najim, 2022). It is palatable and available at a high cost characterized by a high protein content of 43 to 53% and a low crude fibre less than 3% for the dehulled soya bean meal (Salih *et al.,* 2021). Soya bean meal is considered as the most nutritive plant ingredients widely used in fish, pig and poultry feed. Among plant protein ingredients, soya bean meal has well-balanced amino acid profile. Soya bean meal has the advantage of being resistant to oxidation and spoilage and is naturally clean from organisms such as fungi, viruses and bacteria that are harmful to fish (Orire and Ozoadibe, 2015). The authors further revealed that growth depressive effect of soya bean meal at high inclusion levels may be related to the anti-nutritional components present in Soya bean. Anti-nutritional components in Soya bean meal, such as trypsin inhibitor, antigens, lectins, saponins and oligosaccharides, have negative effect on digestibility of nutrients and growth of fish. However, the high cost of its procurement makes the production of fish with soya bean meal to be expensive. Many studies have shown considerable success in partially or total replacement of soya bean with other unconventional feed ingredients.

Roselle (*Hibiscus sabdariffa*) is an annual dicotyledonous, erect, herbaceous tropical plant. The plant is cultivated majorly in the northern part of Nigeria as edible vegetable and considered to be medicinal (Ijeomah *et al.,* 2012). *Hibiscus sabdariffa* known as Roselle (English), *Yakuwa* (Hausa) and *karkade* (Arabic) is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as feed for fish production. In China the seeds are used for their oil and the plant is used for its medicinal properties, while in West Africa the leaves and powdered seeds are used in meals. It is used in the pharmaceutical and food industries (Islam, 2019). The unprocessed seed contains tannin, hibiscin and hydroxyl flavone as major antinutritional factors (Kwari *et al.,* 2011). However, the most common anti-nutritional factors are phenols, tannins and phytic acid, which have detrimental effects on the health and growth performance of fish (Singh *et al.,* 2017). *H. sabdariffa* is a versatile plant similar to coconut tree, it can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Sudan, Egypt and Mexico (Angbulu *et al.* 2019). The origin of *H. sabdariffa* is uncertain, while others believe that its home country is India and Saudi Arabia (Tetteh *et al.,* 2019). This study will explore the potentials of Roselle seed meal as a replacement for soya bean meal in the diets of *Clarias gariepinus*.

The use of cost effective fish diets has been a challenge globally due to the high cost and competition on the plant protein source among livestock, fish, and humans. The major source of plant source protein in aqua feed is soya bean which is very expensive. This has resulted to sourcing for alternative plant protein source feed ingredients. Roselle (*H. sabdariffa)* seeds have been reported to contain high amount of protein and essential amino acids. However, due to the presence of anti-nutritional factors such as trypsin inhibitors, polyphenols, saponins and tanins, their utilization as a protein source particularly in fish feed is poorly studied. Fermentation of (*H. sabdariffa)* seed gave better crude protein level and lower anti-nutritional factors.

## Materials and Methods

### Experimental Site

The study was carried out at Teaching and Research Fish Farm of the Department of Fisheries, University of Maiduguri, Borno State, Nigeria. The study area falls within the semi-arid zone of North Eastern

Nigeria, located between latitude $11^0$ 48' and $16^0$ North and longitude $13^0$ 12'and $12^0$ East. The mean monthly temperature is 40.2°C prior to the onset of the rain in June and the lowest 31.3°C during the peak of the rainy period of August. The area has an average annual rainfall of about 550 mm (Shettima, 2018)

**Collection and Processing of *Hibiscus sabdariffa* Seed**

*Hibiscus sabdariffa* seeds were procured from Gamboru market, Maiduguri. They were winnowed to remove debris and stones were removed manually and kept in bags until required.

**Processing Methods of *Hibiscus Sabdariffa* Seed Meal**

Fermentation: Five hundred (500) grams of *Hibiscus Sabdariffa* seeds were boiled at 1:3 seed to water for 30 minutes at $100^0$C, the water was drained and allowed to cool. The boiled seed were kept in an air tight sack for 72 hours to ferment (solid fermentation). The processed seeds of *H. sabdariffa* were sun-dried for three days, ground and labeled as raw, soaked, sprouted and fermented.

**Proximate Composition of *Hibiscus sabdariffa* Seed Meal**

Proximate composition of raw and differently processed *Hibiscus sabdariffa* seed were carried out according to Near Infrared Reflectance (NIR) methods. The moisture, crude protein, crude fibre, ether extract, ash nitrogen-free extract of each sample were determined in triplicates using Near Infrared Reflectance (NIR) Multicheck Analyser.

**Determination of the Anti-nutritional Factors**

The anti-nutritional factors were determined using the methods described by AOAC (2000). Each of the samples were determine in triplicates.

**Experimental Diets**

Five iso-nitrogenous diets with 40 % crude proteins were formulated by incorporating 0, 25, 50, 75, and 100% of fermented *Hibiscus sabdariffa* seed meal as a replacement for soya bean meal. These were balanced with other major co-ingredients, using Pearson's Square method along the least cost formulae and labelled as 1 (Control), 2, 3, 4 and 5, respectively. The ingredients were ground, and measured using weighing scale balance as commonly practiced for fish feed preparation. The ingredients were mixed thoroughly and pelleted using (Pelletizing machine), dried, labeled accordingly and stored at room temperature in the laboratory until its ready for use. All ingredients were finely ground and incorporated in the diet homogeneously. The composition and calculated analysis of the experimental diets are shown in Table 1.

**Experimental Design**

The processing methods that gave higher crude protein level, and have better anti-nutritional factors were used for the replacement of soya bean. In view of

**Table 1.** Composition and calculated analysis of the Experimental Diets Ingredients (%)

| ingredient (%) | Levels of SBM replaced with HSSM (%) | | | | |
|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 |
| FHSSM | 0.00 | 10.37 | 20.75 | 31.12 | 41.49 |
| Soya Bean Meal | 41.49 | 31.12 | 20.75 | 10.37 | 0.00 |
| Fish Meal | 41.49 | 41.49 | 41.49 | 41.49 | 41.49 |
| Maize | 13.03 | 13.03 | 13.03 | 13.03 | 13.03 |
| Premix | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Methionine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Lysine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Ascorbic Acid | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vegetable Oil | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Binder (starch) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt (NaCl) | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Di-calcium Phosphate | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated analysis | | | | | |
| Crude protein | 42.91 | 41.41 | 39.93 | 38.42 | 37.24 |
| Crude fibre | 3.16 | 3.34 | 3.55 | 3.76 | 3.99 |
| Ether Extract | 3.87 | 3.80 | 3.77 | 3.71 | 3.69 |
| Ash | 8.15 | 8.21 | 8.30 | 8.35 | 8.42 |
| NFE | 37.75 | 37.58 | 37.13 | 37.29 | 37.12 |
| ME (Kcal/kg) | 3349 | 3284 | 3319 | 3356 | 3356 |

***Min-vitamins premix supplies the following per kg of feed:**
Vitamin A=3400000IU; vitamin D3=600 000IU; vitamin E=4 000 mg; vitamin K3=600 mg; vitamin B1=640 mg; vitamin B2=1 600 mg; pantothenic=2000 mg; vitamin B6=600 mg; vitamin B12=4 mg; folic acid=200 mg; biotin H2=300 mg; choline chloride=70 000 mg; cobalt=80 mg; copper=1200 mg; iodine=400mg; iron=8000mg; manganese=16 000 mg; selenium=80 mg; zinc=12000 mg and anti-oxidant=500 mg.
Keys: FHSSM=Fermented Hibiscus sabdariffa seed meal; ME=Metabolisable energy; NFE=Nitrogen-free extract; SBM=soya bean meal. ME (Kcal/kg) is calculated according to the formula of Pauzenga (1985) as: 37 x %CP + 35.5 X %NFE

that fermented *Hibiscus sabdariffa* seed were used. Different replacement levels of the soya bean with fermented *Hibiscus sabdariffa* seed meal; (0 control, 25, 50, 75 and 100%) were allotted to five groups of *C. gariepinus* fingerlings (4.0 - 4.4g) randomly stocked at 10 fish per unit in 1 m x 1 m x 1.2 m hapa installed in a polythene lined pond (7 x 5 x 1.2 m) in completely randomized design. The treatments were in triplicates. The *Clarias gariepinus* were weighed every four weeks to adjust the feed rate accordingly. The experiment lasted for 150 days.

**Data Collection of Growth Parameters**

At the end of the rearing period the following data were recorded; final weight (g), standard length (mm), total feed intake (g) and mortality for each treatment. Growth parameters were estimated for each treatment using the following formulae:

Feed intake=feed supplied to the fish (1)

Weight gain (WG)=final weight (FW) –initial weight (IW) (2)
Busacker *et al.* (2012)

$$\text{Daily weight gain (DWG)}=\frac{W2-W1}{t} \quad (3)$$
Busacker *et al.* (2012)
t=rearing periods in days, where: $W_2$=final weight, $W_1$=initial weight

Relative growth rate (RGR)=$LnW_2 - LnW_1/t$ (4)
Ahmed *et al.* (2012)
Where: In=natural logarithm, $W_2$=final weight of the fish in grams, $W_1$=initial weight of the fish in grams, t=rearing periods in days.

$$\text{Feed conversion ratio(FCR)}=\frac{\text{Dry weight of feed consumed (g)}}{\text{weight gain (g)}} \quad (5)$$
Ayoola *et al.* (2012)

$$\text{Specific growth rate (SGR)}=\frac{LnW2 - LnW1}{t}\ X\ 100 \quad (6)$$
Ahmed *et al.* (2012)
Where: $LnW_2$=logarithm of final weight of fish, $LnW_1$=logarithm of initial weight of fish, t=rearing periods in days.

$$\text{Protein efficiency ratio (PER)}=\frac{\text{final weight - initial weight}}{\text{protein intake}} \quad (7)$$
Ayoola *et al.* (2012)

$$\text{Net protein utilization (NPU)}=\frac{\text{protein gained}}{\text{protein consumed}} \quad (8)$$
Ayoola *et al.* (2012)

$$\text{Condition factor (K)}=\frac{100W}{L3} \quad (9)$$
Ayoola *et al.* (2012)
Where; W= weight of fish (g), L = length of the fish (cm).

$$\text{Survival (\%)}=\frac{No-Ne}{No}\ X\ 100 \quad (10)$$
Ayoola *et al.* (2012)
No=Initial total number of fingerlings, Ne=Total number of mortality at the end of feeding trial.

**Haematological Analysis**

The haematological parameters were determined using Haematocrit method, White blood cell (WBC) count was performed with WBC detector block using Direct Current (DC) detection method. Red blood cell (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV) and Platelets (PL) were determined using Haematocrit and micro haematocrit centrifuge. The following blood indices for each of the treatment were estimated using the following formulae:

$$\text{Mean corpuscular volume MCV (fl)} = \frac{PCV}{RBC}\ X\ 10\ \text{(Miale, 1982)}$$

$$\text{Mean corpuscular haemoglobin (MCH pg)}= \frac{Hb}{RBC}\ X\ 10\ \text{(Miale, 1982)}$$

$$\text{Mean corpuscular haemoglobin concentration (MCHC)}= \frac{Hb}{pcv}\ X\ 100\ \text{(Miale, 1982}$$

**Statistical Analysis**

Data obtained from the study were subjected to One-way Analysis of Variance (ANOVA). Differences between the means were determined using Least Significant Difference (LSD) at 95% confidence level (P= 0.05) with the aid of Statistix 10.0, a statistical package.

## Results

**Proximate Composition of Raw and Processed *Hibiscus sabdariffa* Seed Meal**

Proximate composition of *Hibiscus sabdariffa* seeds is shown in Table 2. Dry matter values ranged from 93.91 to 94.39% with the highest (94.39%) recorded in HSSM processed by soaking for 24 hours, followed by those processed via sprouting with (94.29%) while fermented HSSM had (94.11%) and the least (93.91%) was observed in control (raw) HSSM. All the four treatments significantly (P<0.05) differ from one another. Crude protein of *Hibiscus sabdariffa* seed meal ranged from 24.25 to 25.68% with the highest (25.68%) in fermented HSSM followed by (25.49%) recorded in control (raw) and 25.19% observed in soaked HSSM, while the least 24.25% was observed in sprouted HSSM. All the treatment differs significantly (P<0.05) from one another. Crude fibre of *Hibiscus sabdariffa* Seed Meal ranged from 17.50 to 18.45% with the highest (18.45%) in fermented HSSM, followed by (18.00%) in soaked HSSM while 17.95% was observed in control (raw) HSSM. The lowest 17.50% was observed in soaked HSSM. There were significant (P<0.05) difference among the treatment of *Hibiscus sabdariffa* Seed. Ether extract of *Hibiscus sabdariffa* Seed Meal ranged from 16.80 to 19.38%. The highest (19.38%) ether extract was recorded in fermented HSSM, followed by (19.00%) in control (raw) HSSM, while (17.44%) was recorded in soaked HSSM and the least (16.80%) was observed in sprouted HSSM. There were significant (P<0.05) difference in the ether extract among the treatments.

Ash content of *Hibiscus sabdariffa* Seed Meal ranged from 5.02 to 6.01%; the highest (6.01%) ash content was recorded in the raw HSSM, followed by 5.75% ash content recorded in fermented HSSM, while the least 5.02% ash content was observed in soaked HSSM. There was significant (P<0.05) difference between the ash content of control (raw) compared to soaked HSSM. However, there was no significant (P>0.05) difference between the ash level (5.61%) of sprouted HSSM and the ash content (5.75%) of fermented HSSM. Nitrogen free extract (NFE) ranged from 24.85 to 29.63% with the highest 29.63% NFE recorded in sprouted HSSM, followed by 29.10% NFE in soaked HSSM. There was no significant (P>0.05) variation between the NFE of sprouted HSSM compared to that of soaked HSSM. The lowest 24.85% was recorded in fermented HSSM. There was no significant (P>0.05) variation among the NFE of fermented compared to control (raw) HSSM.

Tannin in raw HSSM was significantly (P<0.05) higher (2.16 %) compared to the values (0.99 %, 0.93 % and 0.63 %) recorded in the treatments soaked, sprouted and fermented respectively. It was observed that there was reduction in tannin level from 0 % in control to 92.60 in fermented *Hibiscus sabdariffa* seed meal. Similarly, the phytate level was reduced by 82.89% processed by fermenting. However, similar result of 83.12 % reduction in phytate, 93.42 % reduction in tannin after soaking *Bauchinia monandra* for 96 hours were reported by Balogun (2013). Lower values of 41.02 % reduction in tannin, 48.79 % trypsin inhibitor and 14.22% phytate acid reduction were recorded by Gemede (2014).

**Proximate Composition of Experimental Diets**

Proximate composition of the experimental diets is presented in Table 3. Dry matter values were between 93.40 and 93.80% the highest (93.80%) dry matter was recorded in 100% inclusion level of FHSSM diet followed by 93.56% in 50% inclusion level of FHSSM diet and the lowest (93.40%) was observed in 75% inclusion of FHSSM diet. There were no significant (P>0.05) difference in the dry matter values among the entire treatments (0, 25, 50, 75 and 100 % FHSSM). Crude protein values were between 30.05 and 40.93%; the highest (40.93%) CP was recorded in 100% inclusion of

**Table 2.** Mean Proximate Composition of Raw and Processed *Hibicus sabdariffa* Seed Meal

| Constituent (%) | Processing Methods | | | | SEM |
|---|---|---|---|---|---|
| | Raw | Soaked | Sprouted | Fermented | |
| Dry Matter | $93.91^d$ | $94.39^a$ | $94.29^b$ | $94.11^c$ | 0.01* |
| Crude Protein | $25.49^b$ | $25.19^c$ | $24.25^d$ | $25.68^a$ | 0.01* |
| Crude Fibre | $17.95^c$ | $17.50^d$ | $18.00^b$ | $18.45^a$ | 0.01* |
| Ether Extract | $19.00^b$ | $17.44^c$ | $16.80^d$ | $19.38^a$ | 0.07* |
| Ash | $6.01^a$ | $5.02^c$ | $5.61^b$ | $5.75^b$ | 0.07* |
| NFE | $24.94^b$ | $29.10^a$ | $29.63^a$ | $24.85^b$ | 0.39* |
| Moisture content | $6.09^a$ | $5.61^d$ | $5.71^c$ | $5.89^c$ | 0.02* |
| Saponins (%) | $2.20^a$ | $1.80^b$ | $1.00^c$ | $0.63^d$ | 0.02* |
| % reduction | 0.00 | 18.19 | 54.55 | 71.37 | - |
| Tannins (%) | $2.16^a$ | $0.99^b$ | $0.93^c$ | $0.16^d$ | 0.02* |
| % reduction | 0.00 | 54.17 | 56.95 | 92.60 | - |
| Phytate mg/100g | $5.90^a$ | $1.02^c$ | $2.00^b$ | $1.01^c$ | 0.04* |
| % reduction | 0.00 | 82.72 | 66.11 | 82.89 | - |
| TI (TUI/mg) | $3.23^a$ | $1.55^b$ | $0.00^c$ | $0.00^c$ | 0.09* |
| % reduction | 0.00 | 42.73 | 100.00 | 100.00 | - |

Means within the same row with different superscripts differ significantly (P< 0.05).
*= Significant (P<0.05), NFE= Nitrogen-free extract, SEM= standard error of means, TI = Trypsin inhibitor

**Table 3.** Mean Composition of Fermented *Hibiscus sabdariffa* seed Meal

| Parameters (%) | Levels of SBM replaced with FHSSM (%) | | | | | SEM |
|---|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 | |
| Dry Matter | $93.55^a$ | $93.55^a$ | $93.56^a$ | $93.40^a$ | $93.80^a$ | $0.12^{NS}$ |
| Crude Protein | $30.05^e$ | $33.71^d$ | $36.34^c$ | $38.95^b$ | $40.93^a$ | 0.01* |
| Crude Fibre | $4.90^a$ | $4.21^b$ | $3.85^c$ | $3.75^d$ | $3.47^e$ | 7.07* |
| Ether Extract | $8.00^e$ | $8.33^d$ | $8.55^c$ | $9.21^b$ | $9.78^a$ | 7.74* |
| Ash | $6.45^c$ | $8.40^b$ | $8.70^b$ | $3.75^d$ | $9.85^a$ | 0.13* |
| NFE | $44.30^a$ | $38.39^b$ | $36.11^c$ | $32.94^d$ | $29.77^e$ | 0.02* |
| Moisture content | 6.45a | 6.45a | 6.44a | 6.60a | 6.20a | $0.06^{NS}$ |

Means within the same row with different superscripts differ significantly (P<0.05).
Keys: SEM = Standard error of Means, FHSSM = Fermented *Hibiscus sabdariffa* seed meal, NFE = Nitrogen free extract, *= Significant (P<0.05), NS= Not significant

FHSSM diets, followed by (38.95%) CP in 75% inclusion level of FHSSM diet. The lowest (30.05%) CP was recorded in 0% FHSSM inclusion level (control). However, 50% inclusion level of FHSSM diet was (36.34%) which is better than (33.71%) CP in 25% inclusion of FHSSM diet. There was significant (P<0.05) difference among the entire treatments. Crude fibre values were between 3.47 and 4.90%; the highest (4.90%) CF was recorded in 0% (control) inclusion level of FHSSM diet, followed by (4.21%) CF values in 25% inclusion level of FHSSM diet and the least (3.47%) was observed in 100% inclusion of FHSSM diet. There was significant (P<0.05) difference among the entire treatments. Ether extract values (EE) were between 8.00 and 9.78%. The highest (9.78%) EE was recorded in 100% FHSSM diet followed by (9.21%) in 75% FHSSM diet and the lowest (8.00%) was recorded in the 0% (control) FHSSM diet however, 8.33 was recorded in 25% FHSSM diet which is lower than 8.55% recorded in 50% FHSSM diet. there were significant (P<0.05) difference in the ether extract among the entire treatments (0, 25, 50, 75 and 100% FHSSM).

Ash content value of FHSSM diet were between 3.75 and 9.85%. the highest (9.85%) ash was recorded in 100% FHSSM diet, followed by (8.70%) in 50% FHSSM diet and the lowest (3.75%) ash content was recorded in 75% HSSM diet. There was no significant (P<0.05) difference in 8.40% recorded in 25% FHSSM diet compared to (8.70%) recorded in 50% FHSSM diet. Nitrogen free extract (NFE) values ranged between 29.77% and 44.30%, the highest (44.30%) NFE was recorded in 0% (control), followed by (38.39%) in 25% FHSSM diet and the lowest (29.77%) was observed in 100% FHSSM diet. There was significant (P<0.05) difference among the entire treatments group.

Growth performance and Feed Utilization of *Clarias gariepinus* fed varying replacement levels of fermented *Hibiscus sabdariffa* seed meal (FHSSM) with soya bean meal are shown in Table 4. Final weight (151.33 g to 253.77g) of *Clarias gariepinus* fed replacement of SBM with FHSSM. The highest (253.77 g) final weight was observed in fish fed 25% FHSSM diet replacing soya bean meal, followed by (177.00 g) in fish fed 100% replacement of SBM with FHSSM. The lowest (151.33 g) final weight was recorded in fish fed 75 % replacement of SBM with FHSSM. Significant variation (P<0.05) were observed in final weight among between 25% replacement of SBM with FHSSM compared to other treatments. However, there were no significant (P>0.05) difference among fish fed 0, 50, 75, and 100% replacement of SBM with FHSSM diet. Weight gain in fish fed 25% was higher (249.04 g) than the other treatments fed replacement of SBM with FHSSM, followed by (172.60 g) in fish fed 100% replacement of SBM with FHSSM. the lowest (146.63 g) WG was recorded in fish fed 75% replacement of SBM with FHSSM diets. There was significant (P<0.05) difference in the weight gain among the treatments. However, weight gain in fish fed 0, 50, 75 and 100% FHSSM are statistically similar. Daily weight gain ranged between 0.97 to 1.66 g. significant (P<0.05) difference exist among the treatments groups fed replacement of SBM with FHSSM. The highest (1.66 g) daily weight gain was observed in fish fed 25% replacement of SBM with FHSSM diets and the lowest (0.97 g) daily weight gain is recorded in fish fed 75% replacement of SBM with FHSSM diets. The value for specific growth rate (SGR) ranged between (0.93 to 1.15%/day). The fish fed 25% replacement of SBM with FHSSM showed higher (1.15%/day) SGR value than the fish fed other replacement of SBM with FHSSM diets, while the lowest (0.93%/day) SGR was recorded in fish reared with 75% replacement of SBM with FHSSM. There was significant (P<0.05) difference in the SGR values among the treatments. There was no significant difference (P>0.05) in the SGR value among 0, 25, 50 and 100% replacement of SBM with FHSSM diets. Condition factor were observed to be between (1.24 to 1.42), with the highest (1.42) in fish fed 0% (control) replacement of SBM with FHSSM diets and the lowest (1.24) was observed in fish fed 100 % replacement of SBM with FHSSM diets. There were no significant (P>0.05) differences of condition factors among the entire treatment values of replacement of SBM with FHSSM diets. Feed conversion

**Table 4.** Mean Growth Performance of *Clarias gariepinus* Fed Diet with FHSSM

| Parameters | Levels of SBM replaced with FHSSM (%) | | | | | SEM |
|---|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 | |
| Initial weight (g) | 4.13$^{a}$ | 4.70$^{a}$ | 4.63$^{a}$ | 4.70$^{a}$ | 4.40$^{a}$ | 0.46$^{NS}$ |
| Final weight (g) | 157.00$^{b}$ | 253.77$^{a}$ | 168.00$^{b}$ | 151.33$^{b}$ | 177.00$^{b}$ | 31.02* |
| Weight gain (g) | 152.87$^{b}$ | 249.04$^{a}$ | 163.37$^{b}$ | 146.63$^{b}$ | 172.60$^{b}$ | 31.04* |
| Feed intake (g) | 181.20$^{c}$ | 261.68$^{a}$ | 207.71$^{bc}$ | 234.53$^{ab}$ | 228.61$^{ab}$ | 13.46* |
| Daily weight gain g | 1.02$^{b}$ | 1.66$^{a}$ | 1.09$^{b}$ | 0.97$^{b}$ | 1.15$^{b}$ | 0.21* |
| FCR | 1.23$^{ab}$ | 1.07$^{b}$ | 1.27$^{b}$ | 1.63$^{a}$ | 1.41$^{ab}$ | 0.23* |
| PER | 2.01$^{a}$ | 2.29$^{a}$ | 1.88$^{a}$ | 1.53$^{a}$ | 1.81$^{a}$ | 0.34$^{NS}$ |
| NPU | 0.11$^{c}$ | 0.13$^{bc}$ | 0.15$^{b}$ | 0.19$^{a}$ | 0.20$^{a}$ | 0.01* |
| SGR | 1.04$^{ab}$ | 1.15$^{a}$ | 1.04$^{ab}$ | 0.93$^{b}$ | 1.06$^{a}$ | 0.05* |
| Condition factor | 1.42$^{a}$ | 1.31$^{a}$ | 1.26$^{a}$ | 1.25$^{a}$ | 1.24$^{a}$ | 0.09$^{NS}$ |
| Survival rate (%) | 73.34$^{a}$ | 73.34$^{a}$ | 73.33$^{a}$ | 70.00$^{a}$ | 70.00$^{a}$ | 3.65$^{NS}$ |

Means within the same row with different superscripts differ significantly (P<0.05)
keys: * = Significant (P<0.05), NS=Not Significant (P>0.05), SEM=Standard error of means, SBM=soya bean meal, FHSSM=Fermented *Hibiscus sabdariffa* seed meal, FCR=feed conversion ratio, PER=protein efficiency ratio, SGR=specific growth rate, NPU=Net protein utilization

ratio (FCR) ranged from 1.07 in fish fed 25% replacement of SBM with FHSSM to 1.63 in fish fed 75% replacement of SBM with FHSSM. The poorest (1.63) FCR was observed in fish fed 75% replacement of SBM with FHSSM, while the best (1.07) was recorded in fish fed 25% replacement of SBM with FHSSM diets. There were no significant (P>0.05) difference in FCR value among the treatment. Feed conversion ratio of (1.63) in fish fed 75% replacement of SBM with FHSSM was significantly (P>0.05) poorer than (1.07) in fish fed 25% replacement of SBM with FHSSM diets. There were no significant (P>0.05) difference in the protein efficiency ratio (PER) among the entire treatments. Protein efficiency ratio in fish fed 0, 25, 50, 75, and 100% are significantly (P>0.05) similar. Net protein utilization (NPU) ranged from 0.11 to 0.20. the highest (0.20) NPU was obtained in fish fed 100% replacement of SBM with FHSSM, while the lowest (0.11) NPU value was recorded in fish fed 0% replacement of SBM with FHSSM. There were no significant differences among values in fish fed 0, and 25% replacement of SBM with FHSSM. However, fish fed 25%, 50% and 75% showed significant (P<0.05) difference among the treatments group. While fish fed 25% show no significant (P>0.05) differences compared to fish fed 50% replacement of SBM with FHSSM. There were no significant (P>0.05) difference in the survival rate (SR) among the entire treatments.

**Haematological Indices of *Clarias gariepinus* Fed Fermented *Hibiscus sabdariffa* Seed Meal as a Replacement for Soya Bean Meal**

Haematological indices of *Clarias gariepinus* fed replacement of SBM with FHSSM are presented in the Table 5 packed cell volume (PCV) were 29.33%, 27.00%, 31.33%, 37.67% and 31,33% in the five respective diets. There was no significant (P>0.05) difference in PCV values among the treatments. The highest (37.67%) PCV was recorded in fish fed 75% replacement of SBM with FHSSM while, the lowest (27.00%) PCV was recorded in fish fed 25% replacement of SBM with FHSSM diets.

The haemoglobin (Hb) values ranged between 8.93 and 13.60g/dl. They were variation in the Hb values of *Clarias gariepinus* fed varying replacement levels of FHSSM. However, Hb in fish fed 25% and 50% replacement of SBM with FHSSM are similar to the Hb of fish fed 0% (control) replacement of SBM with FHSSM, while the highest (13.60g/dl) Hb was recorded in fish fed 75% replacement of SBM with FHSSM, followed by (12.85g/dl) Hb in fish fed 100% replacement of SBM with FHSSM. The lowest (8.93 g/dl) was observed in fish fed 25% replacement of SBM with FHSSM. There was no variation (P<0.05) in fish fed 75% and 100% replacement of SBM with FHSSM.

Red blood cell (RBC) values of fish fed varying levels of FHSSM as a replacement for SBM ranged from 2.28 to 2.57 x$10^6$/mm$^3$. The highest (2.57 x$10^6$/mm$^3$) RBC was recorded in fish fed 50% and 75% replacement of SBM with FHSSM, while the lowest (2.28 x$10^6$/mm$^3$) RBC was recorded in fish fed 25% replacement of SBM with FHSSM. There were no significant (P<0.05) difference among the entire treatments group.

White blood cell (WBC) value of fish fed varying level of HSSM ranged from (4.15 to 4.38 x$10^3$/mm$^3$). The highest (4.38 x$10^3$/mm$^3$) WBC was record in fish 50% replacement of SBM with FHSSM, followed by (4.37 x$10^3$/mm$^3$) WBC in fish fed 75% replacement of SBM with FHSSM. The lowest (4.15 x$10^3$/mm$^3$) was observed in fish fed 25% replacement of SBM with FHSSM diet. The were no significant (P<0.05) difference among the entire treatment.

MCV value of fish fed varying levels of FHSSM ranged from (11.73 to 14.71fl) with the highest (14.71) in fish fed 75% replacement of SBM with FHSSM, followed by (12.40fl) in fish fed 100% replacement of SBM with FHSSM and the least (11.73fl) MCV was recorded in fish fed 0% (control) and 25% replacement of SBM with FHSSM diet. There were no significant

**Table 5.** Haematological Indices of *Clarias gariepinus* Fed Fermented *Hibiscus sabdariffa* Seed Meal Replacing Soya Bean Meal

| Parameters | Levels of SBM replaced with HSSM (%) | | | | | SEM |
|---|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 | |
| Packed Cell Volume (%) | 29.33$^{ab}$ | 27.00$^{b}$ | 31.33$^{ab}$ | 37.67$^{ab}$ | 31.33$^{ab}$ | 3.97* |
| Haemoglobin Conc. (g/dl) | 10.96$^{ab}$ | 8.93$^{b}$ | 10.80$^{ab}$ | 13.60$^{a}$ | 12.86$^{a}$ | 1.62* |
| Red Blood Cell (x$10^6$/mm$^3$) | 2.52$^{a}$ | 2.28$^{a}$ | 2.57$^{a}$ | 2.57$^{a}$ | 2.55$^{a}$ | 0.15$^{NS}$ |
| White Blood Cell (x$10^3$/mm$^3$) | 4.23$^{a}$ | 4.15$^{a}$ | 4.38$^{a}$ | 4.37$^{a}$ | 4.32$^{a}$ | 0.11$^{NS}$ |
| MCV (fl) | 11.73$^{a}$ | 11.73$^{a}$ | 12.24$^{a}$ | 14.71$^{a}$ | 12.40$^{a}$ | 1.97$^{NS}$ |
| MCH (pg) | 43.93$^{a}$ | 39.60$^{a}$ | 41.97$^{a}$ | 52.81$^{a}$ | 50.32$^{a}$ | 6.60$^{NS}$ |
| MCHC (%) | 37.37$^{ab}$ | 37.17$^{ab}$ | 34.33$^{b}$ | 36.60$^{ab}$ | 47.83$^{a}$ | 5.36* |
| Differential Count | | | | | | |
| Neutrophils (%) | 14.33$^{a}$ | 14.67$^{a}$ | 14.00$^{a}$ | 14.33$^{a}$ | 16.00$^{a}$ | 2.78$^{NS}$ |
| Lymphocytes (%) | 76.00$^{a}$ | 74.33$^{a}$ | 70.00$^{a}$ | 70.33$^{a}$ | 72.66$^{a}$ | 3.81$^{NS}$ |
| Basophils (%) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00$^{NS}$ |
| Monocytes (%) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00$^{NS}$ |
| Esinophils (%) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00$^{NS}$ |

Means with the same superscripts in the same row are not significantly different (P>0.05)

Key: SEM=Standard Error of Mean, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, HSSM=*Hibiscus sabdariffa* seed meal, SBM=Soya bean Meal, *=Significant (P<0.05), NS=Not Significant

(P<0.05) difference among the entire treatments.

MCH value was highest (52.81 pg) in fish fed 75% replacement of SBM with FHSSM diets, followed by (50.32 pg) in fish fed 100% replacement of SBM with FHSSM while the lowest (39.60 pg) MCH was recorded in fish fed 25% replacement of SBM with FHSSM diets. The were no significant (P<0.05) difference among the treatment groups.

MCHC value of fish fed replacement of SBM with FHSSM ranged from 34.33 to 47.83 %. the highest (47.83 %) MCHC was recorded in fish fed 100% replacement of SBM with FHSSM diets, followed by (37.37 %) in fish fed 0% (control). The lowest (34.33 %) MCHC value was recorded in fish fed 50% replacement of SBM with FHSSM. There was no significant difference (P<0.05) in fish fed 0, 25, 50 and 75% replacement of SBM with FHSSM diets. However, fish fed 100% replacement of SBM with FHSSM differed (P>0.05) with fish fed 50% replacement of SBM with FHSSM diets.

The leucocyte differential count (LDC) parameters (neutrophils, eosinophils, lymphocytes, monocytes and basophils) recorded in fish fed replacement of SBM with FHSSM showed no significant variation (P>0.05) between the fish group fed 0% (control) replacement of SBM with FHSSM and those groups fed diets containing 25, 50, 75 and 100% replacement of SBM with FHSSM. Monocytes, basophils and eosinophils however were not detected in the blood samples collected from the various groups fed diet with graded levels of FHSSM as a replacement for SBM.

## Discussions

### Proximate Composition of *Hibiscus sabdariffa* Seed Meal under Different Processing Methods

The crude protein (CP) of the raw (control) HSSM recorded in this study was higher (25.49%) compared to the value (21.94%) reported by Ari *et al.* (2015). The CP level obtained in this study for fermented HSSM was (25.65%) which is also higher than the results (23.20%) obtained by Ari *et al.* (2015). The results of the crude protein value of *Hibiscus sabdariffa* seeds were similar with the reports of Igwebuike *et al.* (2010). Ari (2014) observed variations in the proximate composition of *H. sabdariffa* seeds subjected to different processing methods. The crude protein values in this study shows lower CP value (25.19%) in soaked HSSM compared to result reported by Taofik *et al.* (2018) with (27.88%) in soaked HSSM but the raw (control) HSSM in this study showed better result (25.49%) compared with the result of Taofik *et al.* (2018). The variation maybe as a result of differences in the time of processing or processing methods.

The crude fibre (CF) value of the raw and differently processed *H. sabdariffa* seed recorded in this study were higher (17.95, 17.50, 18.00 and 18.45%) compared to the result (15.84 and 15.86%) reported by Igwebuike *et al.* (2010). However, Angbulu *et al.* (2019) reported lower (6.0%) CF value in raw (control) and (6.28%) for soaked *H. sabdariffa* seed while Taofik *et al.* (2018) reported CF level of (27.26%) and (26.22%) in raw (control) and processed FHSSM which is higher than the value reported in this study. However, Hainida *et al.* (2008) reported CF level of (17.89%) and (18.35%) which are close to the CF value recorded in this study. The ether extract (EE) value obtained in this study (19.00, 17.44, 16.80 and 18.45%) for unprocessed and processed *Hibiscus sabdariffa* seed. However, lower (5.27%) EE value and 5.73% were reported for raw and soak *Hibiscus sabdariffa* seed by Igwebuike *et al.* (2010). Taofik *et al.* (2018) reported lower (15.92 and 9.22%) EE value for raw and processed HSSM than the value reported in this study. Angbulu *et al.* (2019) reported higher (26.79 and 22.22%) for raw and processed HSSM compared to the value reported in this study. However, Hainida *et al.* (2008) reported values (19.20%) and (21.00%) for raw and processed seed of *Hibiscus sabdariffa* seed which is close to the value reported in this study. The nitrogen-free extract (NFE) value obtain in this study (24.94 - 29.63%) is lower than the value (52.10 and 41.76%) reported by Igwebuike *et al.* (2010). Angbula *et al.* (2019) reported higher value 39.19% and 31.61% compared to the value reported in this study. However, Taofik *et al.* (2018) and Hainida *et al.* (2008) reported (21.40-29.12%), (23-29.21%) value, respectively which are close to the values reported in this study. Higher NFE level of the processed *Hibiscus sabdariffa* compared to the raw may be due to leaching of some toxic and other chemical components of the seeds by the water during soaking.

### Proximate Composition of the Experimental Diets

The crude protein (CP) of the diets in this study, were within the ranges recommended (30-40% CP) in *Clarias gariepinus* formulated diets as being optimum for growth in *C. gariepinus* culture Adewole *et al.* (2014). However, Wilson & Moreau (1996) suggested 35% CP for *C. gariepinus* better growth. Ether extract (EE) in this study 8.00 – 9.78% is considered to be similar to the EE values (10-20%) in fish diets which generally supports optimal growth rate without producing an excessively fatty carcass Tibbetts and Lall (2013). The values however exceeded 4.15-5.05% as reported by Dienye and Olumuji (2014) for *Moringa oleifera* leaf meal based diets.

The values of ash and crude fibre contents obtained in this study favourably aligned with 8-12% recommended for optimal fish growth Condey, (2012). Higher ash and crude fibre contents reduce the digestibility of other feed ingredients in the diet and result in high waste output which may cause water pollution and poor growth Adesina and Agbatan (2021). Values of moisture observed in the experimental diets compared favourably with 6.31- 8.10% reported by Dienye and Olumiji (2014) for *M. oleifera* based diet while the value recorded for nitrogen-free extract in this study were similar 30.01-38% with the value reported by Dienye and Olumuji (2014). Adewumi (2019) recorded 35.51- 43% NFE level of experimental diet for *C. gariepinus.* The differences between the observed proximate values in this study and other related studies could be due to the influence of environmental factors on the seeds, morphological differences in plants species, processing methods and variations in ingredients combinations Akajiaku *et al.* (2014).

**Growth Performance of *Clarias gariepinus* Fed Fermented *Hibiscus sabdariffa* Seed Meal as a Replacement for Soya Bean Meal**

The mean weight gain recorded in this study was higher (11.73 g) compared to the weight gain obtained by Adesina and Agbatan (2021) who fed *C. gariepinus* 40 % sundried Flamboyant leaf meal for 70 days. Similarly, Asuwaju *et al.* (2015) reported lower (7.89 g) weight gain value when *C. gariepinus* was fed 35 % CP diet. Lower (102.9 g) weight gain was also reported by Adewole *et al.* (2014) who fed *C. gariepinus* 20% fermented locust bean meal. However, Fagbenro (2005) reported higher (267 g) weight gain when *C. gariepinus* was fed 20% *H. sabdariffa* seed meal.

The feed conversion ratio (FCR) in this study was best (1.07) in fish fed 25% FHSSM compared to other treatments groups. Lower FCR value indicate better feed utilization by the fish. Similar FCR value (1.06) was reported by Fagbohun *et al.* (2019) who fed *C. gariepinus* juvenile with 20% *Hibiscus sabdariffa* leaf meal. Adewole (2014) reported better FCR for *C. gariepinus* fed *H. sabdariffa* supplemented diet when compared to the control. However, Aderolu *et al.,* (2018) reported higher (3.95) FCR for *C. gariepinus* juvenile fed 35% CP conventional diet. Fagbenro (2005) reported FCR of (1.83,1.85, 1.88, 1.92, 1.72 and 2.07) for *C. gariepinus* fed 0, 20, 40, 60, 80 and 100% *Hibiscus sabdariffa* leaf meal respectively. On the other hand, Ogueji *et al.,* (2017) reported (0.99) FCR for *C. gariepinus* juvenile fed 4.0g/100g ginger supplemented diet.

The specific growth rate (SGR) of the fish fed 25% FHSSM in this study was similar to the SGR (1.63) reported by Fagbenro (2005), when *C. gariepinus* was fed 100% *Hibiscus sabdariffa* leaf meal. However, Ogueji *et al.,* (2017) reported higher (2.73 %/day) SGR for *C. gariepinus* fed 4.0g/100g *H. sabdariffa* leaf meal. The author further reported (3.13% /day) SGR for *C. gariepinus* fed 4.0g/100g ginger supplemented diet. The difference maybe as a result of variations in the level of inclusion or protein content in the diets. The protein efficiency ratio PER obtained in this study suggested superior dietary protein utilization compared to (1.54 – 1.98) reported for *Heteroclarias* fed cassava root meal based diets as reported by (Abu *et al.,* 2010). Similar values (1.67 – 2.48) PER was recorded by Oyelere *et al.* (2016) for *C. gariepinus* fed commercial diets. However, Adesina and Agbatan (2021) reported higher (4.96 – 5.57) PER in fish fed Sundried Flamboyant leaf meal for *C. gariepinus* fingerlings. Aderolu *et al.* (2018) reported similar (1.62 – 2.16) PER in *C. gariepinus* fed 35% CP diet. According to Davis (2004) PER is a measure of how effectively the protein source in the diet can supply the needed essential amino acids in the fish fed with such a diet. The similarities of PER in this study with those of other authors who fed fish with unconventional legumes may suggest a closely related nutritional, chemical composition and feeding values.

The condition factor (K) recorded in this study are insignificant among the treatment groups. It has better K value compared to what Ogueji *et al.,* (2017) reported (0.39 – 0.45) in *C. gariepinus* fed *H. sabdariffa* meal 2.0 g and 4.0 g respectively. Ogueji *et al.* (2017) reported (0.6 – 0.71) for *C. gariepinus* fed 2.0 g/100 g and 4.0 g/100 g ginger supplemented diets. The K means the fish administered different treatment diets have done well despite some minor stress as a result of the cumulative effect of the residual anti-nutritional factors in the diet. The survival rate recorded in this study are similar to the value (70.00 – 75.50%) as reported by (Adesina and Agbatan 2021). However, Fagbenro (2005) reported higher rate (93.3 – 96.7%) for *C. gariepinus* fed HSSM. Survival rate recorded in this study were similar in all the treatments and could not be attributed to the test ingredients as a result of the random nature of mortalities observed in the study.

**Haematological indices of *C. gariepinus* fed HSSM Replacing SBM**

The packed cell volume (PCV) values recorded in this study were higher than the value (20.01 – 20.16%) reported by Gabriel *et al.* (2011). Similar values of PCV (26.33-36.33%) were reported by (Aderolu *et al.,* 2018). The results (25.23-33.23%) obtained by Fagbohun *et al.* (2019) were also close to the results (27.00 – 37.67%) recorded in this study. However, all the values (20.00 to 37.67s) are within the range for healthy fish as reported by Akinrotomi *et al.* (2011).

The haemoglobin concentration (Hb) value in this study were within the range (8.6-10.58 g/dl) reported by Fagbohun *et al.* (2019). Similar value of (Hb) (7.98-10.87 g/dl) were reported by Onimisi *et al.* (2015). Fagbohun *et al.* (2019) observed Hb value (8.6 – 10.58 g/dl) for *C. gariepinus* fed vary level of roselle leaf meal, While Aderolu *et al.,* (2018) reported values of (8.76-10.13 g/dl) which are close to the values reported in this study. High value (Hb) 15.31 g/dl were documented by Kori-soakpere and Ubogu (2008) for juvenile hybrid as well as 13.00 g/dl recorded for *C. gariepinus* by Ogueji *et al.* (2017) which are similar to 13.60 g/dl (Hb) recorded in this study. This variation may be due to differences in the species, environment and the diets.

Similarly, the red blood cell (RBC) values (2.28-2.57x10$^6$/mm$^3$) in this study are similar to the value of *Heteroclarias* (2.46x10$^6$/mm$^3$) as reported by Diyaware *et al.* (2010). Higher value of (RBC) 3.53 – 4.02 were reported by Fagbohun *et al.* (2019) for fish fed roselle leaf meal. Aderolu *et al.* (2018) reported higher value ($3.48 – 3.75 x10^6/mm^3$) (RBC) in *C. gariepinus* fed 35% CP diet. Lower value of ($1.90 x10^6/mm^3$) (RBC) was reported by Onimisi *et al.* (2015) for *C. gariepinus* fed 100% fermented *Senna obtusifolia* seed meal. White blood cell value 4.15 – 4.38 reported in this study are close to the WBC value 4.25 – 4.87 reported by Onimisi *et al.* (2015). However, lower value ($2.09x10^3/mm^3$) in *C. gariepinus* fed *Lagenaria vulgaris* as reported by Mamman *et al.* (2013). Report by Omitiyin (2006) who observed WBC decreased from 9.2 to $5.0 \times 10^3/mm^3$ in *C. gariepinus* fed poultry litters. WBC are higher in adult fish than juvenile as observed by Adewole *et al.* (2014). Higher value ($98.23x10^3/mm^3$) WBC was reported by Diyaware *et al.,* (2010) for *C. anguillaris* juveniles.

MCV values recorded in this study ranged from (117.3 - 147.1 fl) which are similar to the values (117.68 – 138.07 fl) reported by Diyaware *et al.* (2010) for *C. anguillaris* juvenile and *H. bidorsalis* respectively. Similar values 126.0 – 147 fl were reported by Onisimi *et al.* (2015) for *C. gariepinus* fed fermented 0 and 75% *Senna obtusifolia* seed meal. Lower value of (74.96 – 82.10 fl) MCV were reported by Aderolu *et al.* (2018) for *C. gariepinus* fed 35% CP diet. MCH value in this study were similar to the value (41.30 pg) reported for *Clariabranchus* by Diyaware *et al.* (2010). Lower values 24.93- 27.30 were reported by Aderolu *et al.,* (2018) for *C. gariepinus* fed 35% CP diet. The lymphocytes values reported in this study ranged from 70.33 – 76.00%. higher value by various authors 90.00 – 98.00% Onimisi *et al.* (2015), 98.23%, 98.03% and 98.30% values were reported by Diyaware *et al.* (2010) for *C. anguillaris, Clariabranchus* and *Heteroclarias,* respectively.

## Conclusion

The study revealed that fermented *Hibiscus sabdariffa* seed meal contained crude protein (CP) up to 25.68% but low in essential amino acid particularly leucine. The seeds of *H. sabdariffa* contained anti-nutritional factor (ANFs) such as trypsin inhibitors, tannin, saponin and phytic acid fermenting of HSSM proved to be the best processing methods as it led to reduction in tannins, trypsin inhibitors, saponin and phytic acid respectively. Fish fed 25% FHSSM replacing soya bean meal had better FCR, PER and weight gain compared to other treatments group. The FHSSM had no deleterious effects on the fish as evidenced by the haematological parameters which were within the normal range for healthy fish. It is evident that 25% replacement of FHSSM for soya bean meal served as an ideal rate for better fish growth with no deleterious effects on the fish as evidenced by the haematological parameters which were within the normal range for healthy fish

## Ethical Statement

Not applicable.

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## Author Contribution

U.U.: conceived the work, designed the experiment and carried out the experiment. H.M.S.: Analysis the Raw data, M.Y.D. and M.Z.H.: supervised the work and also read and approved the manuscript.

## Conflict of Interest

The authors declare that they have no conflict of interest in this paper.

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