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Haematological Response of *Clarias gariepinus* Fed *Ocimum gratissimum* Additive Diets

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Abstract

Health of fish during culture is paramount to sustainable aquaculture, it is based on this that *Clarias gariepinus* were fed experimental diets containing *Ocimum gratissimum* additive for twelve weeks. 600g of fresh *O. gratissimum* leaves were collected from Sabon gar;'i market Girei LGA, Adamawa state and divided into three equal parts and processed using air-drying, fermentation and refrigeration for 5days after which they were milled and were subjected to phytochemical and proximate analyses in the Laboratory of Department of Fisheries, Modibbo Adama University of Technology, Yola, Adamawa state Nigeria. 40% crude protein diet was formulated with inclusions of fermented *O. gratissimum* at 0%, 1%, 2%, 3% and 4% and coded D_0, D_1, D_2, D_3 and D_4 respectively. Each diet was fed to *Clarias gariepinus* fingerlings at 5% body weight and stocked at 10 fish per 50L circular plastic tanks for twelve weeks in the wet laboratory of the department of fisheries. The phytochemical qualitative results showed nine chemicals present while the quantitative ranked steroid having highest value and tannin with phytate with lowest quantities. The proximate showed fermented leaves having highest protein of 21.15%. White blood cell (WBC) was highest in D_4 ($49.7 \times 10^9 L^{-1}$) and lower in D_1 ($30.4 \times 10^9 L^{-1}$), red blood cell (RBC) was higher in D_4 ($2.36 \times 10^{12} L^{-1}$) and lowest in D_0 ($0.14 \times 10^{12} L^{-1}$), haemoglobin (Hb) recorded highest in D_4 (13.3 g/dl) and lowest in D_0 (2.2 g/dl) and Packed cell volume (PCV) was highest in D_4 (28%) and lowest in D_0 (1.60%).

Keywords: *Phytochemicals; Haematology; Fermentation; Additive; Clarias gariepinus*

Introduction

Interest in fin fish and shellfish nutrition has increased markedly over the past two decades due to the global increase in aquaculture production. National Research Council [1] has documented nutrient requirement for both cold and warm water fish species which forms the basis for their feed formulations. Nutrient bioavailability considered as one of the important factors in determining fish disease resistance [2]. Ugoala., *et al.* [3] documented that disease outbreak in fish can occur when there is stress which could include poor feed and feeding; hence there is need to improve aquatic animals' nutrition for prevention of disease and improved health status. In the most severe cases, diets that are inadequate with respect to essential nutrients (protein, amino acid, essential fatty acids, vitamins and minerals) lead to gross malnutrition and high disease susceptibility; through feed additives can be used to upgrade the nutritional utilization of such diets [4]. In general, gross malnutrition is no longer a problem, however, we are now faced with the more challenging task of determining the subtler effects that micronutrients, and their interactions with other dietary components have on the immune system of fish. Several literature reports on nutrition and fish responses to feed lack complete information on the quantitative nutrient requirements of most fish species [5,6].

Lack of readily available nutritive fish feed ingredients have continued to be a major constraint to the survival of aquaculture in the competitive global food production system [7]. Consequently, fish nutritionists have considered the recruitment of alternative protein feed ingredients necessary for inclusion in fish diet [8]. Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity [9-12]. However, in the compounding of fish ration with plant protein sources, caution needs to be exercised as to their inclusion levels in fish diets as well as ensuring their proper processing for effective utilization [13,14]. Fish culture had been reported as expensive and the break-even output reducing due to increase in the cost of ingredients and wastages of some of the feeds administered to fish. Even in intensive aquaculture system, feed consumption has been reported to be on high side [15]. The cost of sustaining healthy culture of fish is increasing as some farmers have to include antimicrobials (antibiotics and antifungal) into their feeds for control of diseases that can easily wipe-off their fish [14].

Some of these chemicals are injurious to man because fish don't digest them and if on high concentrations could accumulate in the tissues and pass to man through the food chain. Furthermore, the use of chemicals and hormones to stimulate growth in fishes are not justifiable on financial ground as they seem to be costly and could not be afforded by local fish farmer. For this reason and other operational factors some practicing farmers are getting discouraged, hence there is an urgent need to research into additives that will enhanced feed utilization and promote fish health. There are large numbers of feed additives available to improve fish growth performance. Some of the additives used in feed production are synthetic products especially hormones and antibiotics which use have been criticized in aquaculture and feed industries by government regulatory agencies. The usage of natural products such as medicinal herbs and plants in aquaculture to minimize or substitute for synthetic products has been advocated for by World Health Organization [3]. Several attempts have been made to incorporate medicinal plants as feed additives to enhance feed utilization and animal productive performances (Levic., *et al.* 2008). Platel., *et al.* (2002) also reported highest stimulatory influence on digestion, bile secretion and pancreatic enzymes activity when medicinal herbs were incorporated in animal diets.

Immunostimulants are valuable for the prevention and control of fish diseases in aquaculture as they represent an alternative and supplementary treatment to vaccination. They also have additional effects such as growth enhancement and increase in the survival rates of the fishes under stress. Certain medicinal plants are believed to promote positive health and maintain organic resistance against infection by re-establishing body equilibrium and conditioning the body tissues. It has been documented by Gayatri and Rajani [16] that *Ocimum gratissimum* extract has been used as immunostimulant, growth and survival rate enhancer.

Blood is a vital physiological, pathological and nutritional body factor because it is highly susceptible to internal and external fluctuations [17]. Physiological and morphological changes in blood indicate the quality of environment and therefore, blood parameters are important in diagnosing the health and functional status of an animal in relation to substance they are exposed to. Blood is important as it transports nutrients, gases and endocrine factors coupled with its function as reservoir of metabolic products. Thus, an alteration in blood parameters often reflects the overall impact of environmental constituent and the animal nutritional intake. Hence, there is need to research into the effect of incorporating *O. gratissium* feed additive in the blood and well-being of *Clarias gariepinus*.

Materials and Methods

Experimental Procedures

Collection of African Basil leaves

1.20 kg Fresh Africa basil leaves were bought from Sabon Gari market, transported to the dry laboratory of Department of Fisheries, washed and divided into 400g each for processing

Sample preparation

The first part of 400g was packed into a polythene bag, air-tighten and kept in a refrigerator for 120 hours while the second part was fermented following the Solid-state fermentation procedure of Sogbesan [18] which lasted for 120 hours and the other 400g was air-dried under laboratory condition for 120 hours for five days. At the end of the 120 hours, all the processed Basil leaves were separately milled using pestle and mortar in the laboratory, sieved to get extract using distilled water. Each of the extract was kept in bottle with corked for quantitative and qualitative phytochemical and while the remaining leaves were used for proximate analyses.

Feed Formulation

40% Isoproteic diet was formulated using maize, fish meal, soybean meal and five experimental diets were prepared by including 0g, 1g, 2g, 3g, and 4g of Basil leaves. Each inclusion was deducted from source of energy (maize) as presented on table 1.

Phytochemical Screening of African Basil Leaf extract

The processed African Basil leaves extract were screened for phytochemical compositions by adopting the methods of Ramesha and Srinivas (2014), Prabavathy and Valli [19], Savita and Rita [20].

Qualitative Phytochemical screening methods

Test for Alkaloids: A drop of ethyl acetate extract was spotted on the pre-coated TLC plate followed by spraying with Dragendorffs reagent. The orange red or brown color confirms the presence of alkaloids.

Test for Tannins: 3 ml of ethyl acetate extract and 2 ml of 10% alcoholic ferric chloride solution were added to extract from each of the processed leaf. A dark blue or greenish grey coloured solution confirms the presence of tannins.

Ingredients composition	Experimental Diets				
	D_0	D_1	D_2	D_3	D_4
Maize	30.00	29.00	28.00	27.00	26.00
Fish meal	33.00	33.0	33.00	33.00	33.00
Soybean	32.00	32.00	32.00	32.00	32.00
FOGLM	0	1.00	2.00	3.00	4.00
Palm oil	1.00	1.00	1.00	1.00	1.00
Bone meal	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Iondized salt	0.50	0.50	0.50	0.50	0.50
Cassava starch	0.50	0.50	0.50	0.50	0.50
Lysine	1,00	1.00	1.00	1.00	1.00
Methionine	1.00	1.00	1.00	1.00	1.00
Total	100g	100g	100.g	100g	100g
Calculated protein	40.50	40.58	40.68	40.80	40.90
Feed cost	39.50	39.40	39.20	39.10	38.90
Calculated Gross energy kcal/J	410.40	409.40	408.35	407.29	406.24
Calculated Digestible energy kcal/J	365.93	365.29	364.64	364.01	363.36
Gross energy: protein	10.13	10.08	10.04	10.00	9.93
Digestible energy: protein	9.03	9.01	8.96	8.93	8.88

Table 1: *Percentage composition of Dry matter (g/100g) of Experimental Diet.*

FOGLM: Fermented Ocimum gratissimum leaf meal.

GE = Lipid (value) x lipid GE (value) + CP (value) x CP GE + NFE (value) x NFE GE (estimate).

DE = Lipid (value) x lipid DE (value) + CP (value) x CP DE + NFE (value) x NFE DE (estimate).

Test for Phenols: A drop of ethyl acetate extract was dropped on the filter paper, then drop of phosphomolybdic acid was added; a blue color indicates presence of phenols.

Test for Saponins: 2 ml of the extract was shaken with 5 ml of distilled water, presence of frothing indicates the presence of saponins.

Test for Flavonoids: To 2 ml of ethyl acetate extract, a piece of magnesium strips and 1 ml of concentrated hydrochloric acid were added. A pink red or red colored solution shows the presence of flavonoids.

Test for Steroids: To 1ml of ethyl acetate extract; 1ml of chloroform, 2 - 3 ml of acetic anhydride and 2 drops of concentrated tetraoxosulphate VI acid were added. A dark green colored solution shows the presence of steroids.

Test for Terpenoids: 1 ml of ethyl acetate extract, 1ml of chloroform, 2 - 3 ml of acetic anhydride and 2 drops of concentrated tetraoxosulphate VI acid were added. A dark pink or reddish colored solution shows the presence of terpenoids.

Test for Glycosides: Each extract was hydrolyzed with Hydrochloric acid and neutralized with Sodium hydroxide solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

Quantitative Analysis

Determination of Alkaloid

This was determined following adopted methods of Harnobe [21], Obdoni and Ochuko [22] and Ifemeje., et al. [23] in which Five (5g) of each processed Africa Basil leaves were put in a 250 ml beaker and 200 ml of 10% acetic acid (CH3CO2H) in ethanol (C2H5OH) was added. The mixture was covered and allowed to stay for 4 hours at 250C. It was then filtered, and the filtrate concentrated on a water bath until it reaches a quarter of its original volume. Concentrated NH4OH was then added drop by drop until precipitation was complete. The mixture was allowed to settle, and the precipitate collected on a weighed filter paper and washed with dilute NH4OH. The precipitate was dried and weighed. The percentage alkaloid was calculated as shown in equation (i)

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W_t \, Sample} \times 100 \quad \text{..... equation (i)}$$

Where, W_1 = Weight of empty filter paper; W_2 = Weight of filter paper + Alkaloid

Determination of Saponin

Saponin was estimated following the methods of Odboni and Ochuko [22] and Ifemeje., *et al.* [23] in which Five (5g) of each processed Africa Basil leaves put into 20% acetic acid in ethanol separately and allowed to stand in a water bath at 500C for 24 hours. This was filtered, and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated NH_4OH was then added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle, and the precipitate was collected by filtration and weighed. The saponin content was calculated in percentage as shown in equation (ii):

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W_t \, Sample} \times 100 \quad \text{..... equation (ii)}$$

Where, W_1 = Weight of filter paper; W_2 = Weight of filter paper + residue

Determination of Terpenoids

The protocol of Ladan., *et al.* (2014) was adopted in which 2g of each processed Africa Basil leaves were soaked in 50ml of 95% ethanol for 24hrs. The extract was filtered, and the filtrate extracted with petroleum ether (60 – 80°C) then concentrated to dryness.

$$\% \text{ Terpenoids} = \frac{(\text{Wt of beaker + Dried filtrate}) - (\text{Wt of empty beaker})}{W_t \, Sample} \times 100 \quad \text{..... equation (iii)}$$

Haematocrit (PCV)

This was measured after centrifugation at 15000 rpm using an MSE micro centrifuge.

Haemoglobin concentration (Hb)

This was analysed using NIHON KOHDEN Automated Hematology Analyzer (Celtics and Japan).

Leucocyte count (WBC)

The haemocytometer was used for LC determination with 0.8cm objective of the microscope and large squares (area = $1mm^2$, depth = 0.1 mm) the volume was $0.1mm^3$ and the dilution factor was 20. Four squares were used and the total count per mm^3 was:

20 x 1 x L cells ÷ 0.4 = 50 x L cells ..... equation (iv)

Where L = number of leucocytes that was counted

Erythrocytes (RBC)

These were determined in haperinized blood diluted by Haymen solution at a ratio of 1:200. Neubauer improved haemocytometer placed on a compound microscope stage was used to count/estimate the erythrocyte population. The number of cells counted, R (average of two fields) was multiplied by the dilution factor and the volume of $1/4000mm^3$ (area = 1/4000 mm^3, depth = 1/10 mm^3) and counting was done in 80squares with the sum total volume if 1/50 mm^3 the dilution factors is 200.

200 x 500 x R cells = 10,000 x R ..... equation (v)

Mean Cell Volume (MCV)

It is expressed in picogrammes

MCV = PCV ÷ RBCs

Mean Corpuscular Haemoglobin (MCH)

This was calculated using the equation:

MCH (pg) = Hb ÷ RBCs x 10

Results

The phytochemical analyses of the three processed methods of *Ocimum gratissimum* in this study have shown the presence of tannins, phenol, flavonoids, steroids, alkaloids, terpenoid, glycosides, saponins and phytate in qualitative for fresh, air-dried and fermented as shown in table 2. For quantitative, tannin (2.40%), phenol (7.01%), flavonoid (1.76%), steroid (0.30%), alkaloid (10.2%), terpenoid (4.79%) and phytate (5.51%) were recorded highest in wet sample and lowest in fermented sample in which tannin (0.488%), phenol (5.793%), flavonoid (1.112%), alkaloid (5.81%), terpenoid (3.08%) and phytate (0.5%1) except for steroid (17.717%), glycoside (5.67%), saponin (3.95%) that were recorded highest in fermented and lowest in wet sample steroid (0.30%) and glycoside (2.30%) as indicated in table 3.

Africa Basil Leaves Treatments			
Phytochemicals	**Refrigerator**	**Air dried**	**Fermented**
Tannin	+	+	+
Saponin	+	+	+
Steroid	+	+	+
Flavonoid	+	+	+
Alkaloid	+	+	+
Terpenoid	+	+	+
Glycoside	+	+	+
Phenol	+	+	+
Phytate	+	+	+

Table 2: *Qualitative Screening of Ocimum gratissimum leaves water extract.*

Sample	Wet sample	Air-dried	Fermented
Tannin (mg/g)	2.40^{a}	1.80^{ab}	0.51^{b}
Phenol (mg/g)	7.01^{a}	5.91^{ab}	5.79^{a}
Flavonoid (mg/g)	1.76^{a}	1.43^{a}	1.11^{a}
Steroid (mg/100g)	0.30^{c}	5.76^{b}	17.72^{a}
Alkaloid (%)	10.2^{a}	7.32^{ab}	5.81^{b}
Terpenoid (%)	4.79^{a}	3.70^{a}	3.08^{a}
Glycoside (%)	2.30^{a}	2.67^{a}	5.67^{b}
Saponin (%)	3.61^{a}	3.30^{a}	3.95^{a}
Phytate (%)	5.51^{a}	2.09^{b}	0.51^{c}

Table 3: *Quantitative Analysis on Fresh, Air-dried and Fermented of Ocimum gratissimum.*
Mean of data on the same row with different superscripts are significantly different ($p < 0.05$).

The proximate composition of *O. gratissimum* has shown the presence and quantity of the following moisture, ash, fat, fibre, protein and carbohydrate in which the three processed methods had the moisture content were as followed wet (75.06%), air-dried (8.39%) and fermented (8.87%), the ash content for wet (6.31%), for air-dried (14.25%) and for fermented (13.12%), the fat content for wet (1.50%), for air-dried (2.83%) and for fermented (2.79%), the fiber content for wet (4.36%), for air-dried (8.18%) and for fermented (8.01%), the protein for wet (7.65%), for air-dried (18.43%) and for fermented (21.15%) and the carbohydrate content for wet, air-dried and fermented are 7.82, 47.92 and 46.07 respectively (Table 4).

In table 5, the result of some haematological indices of this study showed that the white blood cell, red blood cell, haemoglobin, packed cell volume and mean cell volume followed the same trend in sense that white blood cell in D_5 was recorded highest with value of 49.7 x $109L^{-1}$ and 30.4 x $109L^{-1}$ which was the lowest was recorded in D2, and the red blood cell was recorded highest in D_5 with value of 2.36 x $1012L^{-1}$ and lowest value was recorded in D_0 with value of 0.14 x $1012L^{-1}$, the haemoglobin was also highest in D_4 with value of 13.30 g/dL and lowest value of 2.20 g/dL was recorded in T_1, the packed cell volume has the values of 28.00% which was highest in D_4 and D_0 has the lowest value of 2.20%, the mean cell volume of 1.25fl was recorded highest in D_1 and D_2 was recorded lowest which has the value of 1.13fl.

Parameters	Wet sample	Air-dried	Fermented
Moisture (%)	75.06a	8.39b	8.87b
Ash (%)	6.31b	14.25a	13.12a
Fat (%)	1.50b	2.83a	2.79a
Fibre (%)	4.36b	8.18a	8.01a
Protein (%)	7.65b	18.43a	21.15a
Carbohydrate	7.82b	47.92a	46.07a

Table 4: *Proximate Composition of Ocimum gratissimum.*

Mean of data on the same row with different superscripts are significantly different ($p < 0.05$).

Haematology	D_0 (0%)	D_1 (1%)	D_2 (2%)	D_3 (3%)	D_4 (4%)
White Blood Cell(L^{-1})	42.23×10^{9c}	30.4×10^{9ab}	39.6×10^{9b}	42.9×10^{9a}	49.7×10^{9a}
Red Blood Cell (L^{-1})	0.14×10^{12b}	0.39×10^{12b}	0.48×10^{12b}	2.07×10^{12a}	2.36×10^{12a}
Haemoglobin (g/dl)	2.2^{b}	3.9^{b}	4.0^{b}	12.3^{a}	13.3^{a}
Packed Cell Volume (%)	1.60^{c}	4.4^{b}	6.0^{b}	24^{a}	28^{a}
MCV (10^{-11})	1.14^{a}	1.13^{a}	1.25^{a}	1.16^{a}	1.18^{a}
Lymphocyte (%)	93^{a}	25^{c}	35^{c}	67^{b}	74^{b}
Monocyte (%)	5^{b}	3^{b}	5^{b}	23^{a}	23^{a}

Table 5: *Some Haematology of Experimental Fish Fed on Ocimum gratissimum Diet.*

Mean of data on the same row with different superscripts are significantly different ($p < 0.05$).

Discussion and Conclusion

The phytochemical analysis of *O. gratissimum* in this study have shown the presence of tannins, phenol, flavonoids, steroids, alkaloids, terpenoid, glycosides, saponins and phytate and this qualitatively agreed with Abdullahi [24] and Abu [25] who reported the same phytochemicals in *O. gratissimum* leaves and quantitatively fermentation had showed a reductions in some phytochemical substances except for steroid, glycoside and saponin, this result corroborate with that of Ray and Roy [26] who reported reduction of phytochemicals in sesame seed meal and increase in the nutritive values after fermentation. Sogbesan., *et al.* [17] also reported reduction of phytochemicals in maize cob as fermentation time increases. Akpomedaya and Gabriel [27] and Sogbesan., *et al.* [28] reported that boiling, soaking, sun-drying, cooking, toasting, germination and fermentation reduces phytochemicals.

The proximate composition of this work showed that moisture content not above the recommended quantity for fresh ingredients as was postulated by Crickenberger and Carawan [29]. The air-dried and fermented gave the best proximate value which is in line with report of [29].

The crude fibre content on the average should not exceed 30% to 36% of plant biomass [30,31] for plant feedstuff. The results obtained in this study fibre content were lowered and falled within the range. Also, an ingredient in protein content in the fermented leaves agreed with the Sogbesan., *et al.* [18] who reported that solid state fermentation technique increases the nutrient contents by adding single cell protein usable carbohydrates reduces the anti-nutritional factors.

The result of crude protein content of *O. gratissimum* in this study for the three processed methods are as followed for wets sample (7.65%), air-dried (18.76) and fermented (21.15%). This result was supported by work of Sogbesan., *et al.* [28] who subjected duckweed to different processed methods which are raw duckweed, soaked in potash, sundried and blanched and these affected the crude protein values, which are 29.28%, 36.25%, 28.62% and 30.04% respectively. The crude protein value of 18.76% obtained in this research was is within the range recommended by Ugwoke., *et al.* [3] who reported 12 - 18% for air-dried scent leaf.

Similarly, Lateef., *et al.* [32] reported on the effect of solid- state fermented agro-wastes using fungus *Rhizopus stolonifer* that the crude protein contents of the substrates increased significantly up to 94.8%. Yang., *et al.* (1986) reported an increase in crude protein of residual substrate up to 60.9% after harvest of mushrooms. This increase in protein might be due to the action of some fungi which are single cell protein. The increase in the protein of *O. gratissimum* might be due to the fact that single cell proteins grew in the leaves which were fermented for the period of 5 days and this agrees with the report of Adu [33] and Alemawor., *et al.* [34] and Sogbesan [18]. These authors also noted that optimum fermentation period occurs after complete colonization of substrate by the organism which is within 5 days. Iyayai [35] and Sogbesan and Ekundayo [8] and Michael and Sogbesan [28] have documented that during fermentation, the incorporation of fungal protein or the bioconversion of carbohydrates through the colonized substrates might also result to increase in protein.

The red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb) and packed cell volume (PCV) followed the same trend in which they were recorded highest in D_4 and lowest in D_0. This research agreed with the study of Iruthayam*., et al*. [36] who reported increase in white blood cell, red blood cell and haemoglobin in fish fed diets containing *Ocimum tenuiflorum, Zingerber officinale* and *Allium cepa* at 0.5g each for Indian catfish fingerlings (*Mystus montanus*) compared with the control. Similar report has been made by Gopalakannan and Arul [37] that WBC and RBC counts were higher in *Labeo rohita* fingerlings fed with *Magnifera indica* kernel when compared to control. Bello., *et al*. [38] reported increase in RBC, PCV and Hb in fish fed diets containing onion bulb and walnut leaf extract over control and that the inclusion of onion bulb and walnut leaf residues does not result into anaemia and could support its use in aquaculture as immunostimulants. Also, Akinmutimi [39] reported that both biochemical and haematological blood components are influenced by the quality and quantity of feed and also the concentration of phytochemicals presents in the feed. Sahu., *et al*. [40] reported increase in red blood cell count of *Labeo rohita* fingerlings fed *Magnifera indica kamel* and postulated that this increase is an indication of enhanced cellular immunity. The increase in red blood values in the treated groups may be due to the ability of *O. gratissimum* to trigger erythropoiesis and decrease the rate of oxidant induced haemolysis, due to the presence of anti-oxidants present in the plant extract as reported by Sheeja., *et al*. [41]. This improvement on haemotological profile of the *C. gariepinus* used for this study might due to the presence of phytochemicals compositions of *O. gratissimum* which can that act as anti-bacteria and anti- fungi; and similar observation was made by Ephraim., *et al*. [42] that white blood cell count level significantly increased in the test groups when compared to the control group reemphasize the anti-bacteria and anti-fungi properties of plants and justify the use of the plant by traditional medicine practitioners. This research is recommending fermentation and air-drying of *O. gratissimum* as better processing or treatment to have reduced phytochemicals and increased nutrient values; also, its inclusion at 3% in fish diet is better for haematological profile improvement in *C. gariepinus* [43-54].

Bibliography

1. NRC (National Research Council). "Nutrient Requirements of Fish". *National Academy Press*. *Washington DC* (1993): 72.

2. Sogbesan OA and Mansur DA. "Contextual Aquaculture and Fisheries Digest". *Paracellete Publisher Adamawa* (2016): 212.

3. Ugoalan E., *et al*. "Natural products and aquaculture Development. IOSR Journal of Pharmacy and Biological Science (2014): 70-82.

4. De silva SS and Anderson TA. "Fish Nutrition in Aquaculture". *Champman Hall London New York Tokyo* (1995): 305.

5. Lall SP and Olivier G. "Role of micronutrients in immune response and disease resistance in fish". *International Fish Nutrition in Practice* (1993): 101-118.

6. Waagbo R. "The impact of nutritional factors on the immune system in Atlantic salmon, SalmosalarL: A review". *Aquaculture and Fisheries Management* 25.2 (1997): 175-197.

7. Ogunji JO., *et al*. "Effects of different processing methods.of pigeon pea (Cajamuscajan) on the haematology of African catfish (Clariasgariepinus) larvae". *Conference on International Agricultural Research for Development. Deuscher Tropentage, Stuttgart-Hohenheim* (2005).

8. Sogbesan OA and Ekundayo TM. "Cost benefits of Fermented Groundnut Shell Meal as Supplemented Feed in The Diets of Clarias gariepinus fingerlings". *Nigeria Journal of Fisheries and Aquaculture* 2 (2014): 35-46

9. Nwanna LC., *et al*. "Water hyacinth (Eichhornia crassipes) (Mart Solms): Pigeon pea (Cajanus cajan) on the haematolog of African Catfish (Clarias gariepinus) larvae. Conference on International Agricultural Research for Development, Deuscher Tropentage, Stuttgart-Hohenheim (2008).

10. Ahmed YM., *et al*. "Potential Sex Reversal and Growth Performances in Clariasgariepinus (Burchell, 1822) Fry Fed Dietary Genistein under Laboratory Condition". *Journal of Agriculture and Food Technology* 5.5 (2015): 1-4.

11. Idowu TA., *et al*. "Effect of varying levels of fermented sorghum (Sorghum bicolor) on growth and haematology profile of Clarias gariepinus (Burchell, 1822) fingerlings". *Journal of Agricultural and Environmental* 12.1(2016): 89-96.

12. Sogbesan OA and Oyebamiji DO. "Performance of Oreochromisniloticus(Linn) Fed Moringa Leaf Meal Supplemented Diet. *Journal of Aquaculture Resources Development* 7 (2016): 11.

13. Francis G., *et al*. "Antinutritional factors present in plant -derived alternative fish feed ingredients and their effects in fish". *Aquaculture* 199.3-4 (2001): 197-228.

14. Sogbesan OA., *et al*. "Culture and Utilization of Earthworm as Animal Protein Supplement in the Diet of Heterobranchus. Longifilisfingerlings". *Journal of Fisheries and Aquaculture Science* 2.6 (2007): 375386.

15. Ibiyo LMO., *et al*. "Performance of Clariasgariepinus fed National Institute for Freshwater Fisheries Research (NIFFR) feed supplement with Nutrase Xylanase". *Nigeria Journal of Fisheries* 12.1 (2015).

16. Gayatri N and Rajani KS. "Immunomodulatory Effect of Ocimum gratissimumlinn. Leaf Extract on a Common Fish Clariasbatrachus". *International Journal of Drug Delivery* 6.3 (2004): 268-278

17. Muhammed NO., *et al*. Nutritional Evaluation of Fungi Treated ocoa Bean Shell Nigerian". *International Journal of Pure and Applied Sciences* 5 (2000): 1059-1064.

18. Sogbesan OA., *et al*. "Maize cob: A Reliable Co-energy source in Fish Feed". *In: National Workshop on Application of solid state Fermentation Technology in Aquaculture* (2012): 118-128.

19. Prabavathy D and Valli-Nachiyar C. "Cytotoxic Potential and Phytochemical Screening of Justicia Beddomei And Its Endophytic Aspergillus Spp". *Asian Journal of Pharmaceutical and Clinical Research* 6.5 (2013): 43-47

20. Savitha D., *et al*. "Phytochemical Analysis, And Anti-Tumor Screening of Endophytes Of Tinospora cordifolia". *International Journal Pharmacology and Bioscience* 3.4 (2012): 533-540.

21. Harborne JB. "Phytochemical methods". *Chapman and Hall, Ltd, London* (1973): 49-188.

22. Obdoni B and Ochuko P. "Phytochemical Studies and Comparative Efficacy of the Crude Extracts of some Homostatic Plants In Edo and Delta States of Nigeria". *Global Journal of Pure and Applied Science* 8.2 (2001): 203-208.

23. Ifemeje JC., *et al*. "Determination of the Anti-nutrient Composition of Ocimumgratissimum, Corchorusolitorius, Murrayakoenigii Spreng and Cucurbita maxima". *International Journal of Innovation and Scientific Research* 3.2 (2014): 127-133.

24. Abdullahi M. "Phytochemical constituents and antimicrobial and Grain protectant Activities of Clove Basil (Ocimum gratissimum L) Grown in Nigeria". *International Journal of Plant Research* 2.1 (2012): 51-58.

25. Abu NE., *et al*. "Phytochemical, Proximate and Anti-nutrient compositions of four leafy vegetables used in South Eastern Nigeria". *Africa Journal of Biotechnology* 13.50 (2014): 4541-4546.

26. Ray AK., *et al*. "Improvement of nutritive value of Sesame (Sesame indicum) oilseed meal in formulated diets for Rohu (Labeorohita) fingerlings after fermentation with two phytase-producing bacterial strains isolated from fish gut. National Workshop on Application of Solid state Fermentation in Aquaculture.1st Edition (2009): 38.

27. Akpomedaya DE and Gabriel -Ajobiewe RAO. "Impact of boiling chemical composition of maize cobs using three fungi". *Fermentation Technology and Bioengineering 2* (2011): 30-42.

28. Sogbesan OA., *et al*. "Utilization of Treated Duckweed Meal (Lemna pausicostata) as Plant Protein Supplement in African Mud Catfish (Clarias gariepinus) Juvenile Diet". *Journal of Fisheries and Aquaculture* 6 (2015):141.

29. Crickenberger RG and Carawan RE. "Using food processing by products for animal fed" (1996): 53.

30. Koji T., *et al*. "Studied on the utilization of rice straw X. Estimation of nutritive value by method of artificial rumen". *Journal of Japan Grassland Science* 28.2 (1982): 225-233.

31. Anon A. "Chanese Feeding Standard for Aquatic Animals". *Bejing: China Agricultural University Press* (2000): 149.

32. Lateef A., *et al*. "Improving the quality of agro-wastes by solid-state fermentation; enhanced anti-oxidant activities nutritional qualities". *Biotechnology Group Microbiology Unit, Department of Pure and Applied Biology, LadokeAkintola University of Technology, Ogbomoso, Nigeria* 24.10 (2008): 2369-2374.

33. Adu AD. "Enrichment of cassava peels through pleurrotusos treatus fermentation for use as animal feed". MSc. Project, Department of Biochemistry, Kwame Nkrumah University of Science and Technology - Kumasi, Ghana (2009): 54.

34. Alemawor F., *et al*. "Effect of pleurotusa streatus fermentation on cocoa pod husk composition: influence of fermentation period and Mn2+ supplementation on the fermentation process". *African Journal of Biotechnology* 8.9 (2009): 1950-1958.

35. Iyayi EA. "Changes in the cellulose, sugar and crude protein content of agro-industrial by-products fermented with Aspergillus niger, Aspergillus flavus and penicillium sp". *African Journal of Biotechnology* 3.3 (2004): 186-188.

36. Iruthayam VK., *et al*. "Medicinal plants as immunostimulants for health management in Indian catfish". *Journal of Coastal Life Medicine* 2.6 (2014): 426-430.

37. Gopalakannan A and Arul V. "Immunodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of-Cyprinuscarpio and control Aeromonanshydrophila infection in ponds". *Aquaculture* 225.1-4 (2006): 179-187.

38. Bello OS., *et al*. "Haematological and Blood Biochemical Changes in African catfish (Clariasgariepinus) Fed Walnut (Tetracarpidiumconophorum Mull Arg) Leaf and Onion (Allium cepa Linn) bulb Supplemented Diets". *America Journal of Experimental Agriculture* 4.12 (2014): 1593-1603.

39. Akinmutimi AH. "Evaluation of Sword bean (canavania gladiate) as an Alternative Feed Resources for Broiler Chickens. PhD. Thesis, Department of non-ruminant animal production Michael Okpara University of Agriculture Umudike (2004).

40. Sahu S., *et al*. "Effect of magniferaindical kernel as a feed additive on immunity and resistants to Aeromonashydrophila in labeorohita fingerlings". *Fish and Selfish Immunology* 23.1 (2007): 109-118.

41. Sheeja K. *et al*. "Anti-oxidant and anti-inflammatory activities of the plant and rographis paniculata NEES". *Journal of Immonopharmacology and Immunotoxicology* 28.1 (2006): 129-140.

42. Ephraim KD., *et al*. "Effect of aqueous leaf extract of ocimum gratissimumon haematological and biochemical parameters in rabbit". *Africa Journal of Biomedical Research* 3 (2000): 175-179.

43. Blazer VS. "Nutrition and disease resistance in fish". *Annual Revision of Fish Disease* 2.1 (1992): 309-323.

44. Daramandy EM and Davenport SGJ. "Haematological Techniques 2nd Edition Janda Churchill London (1985): 56-59.

45. Haruna AB and Adikwu IT. "Haematological responses to familiar diets on Clarias gariepinus". *Journal of Arid Zone Fisheries* 1 (2001): 12-22.

46. Idowu TA., *et al*. "Fish Disease and Health Management in Aquaculture Production". *International Journal of Environment and Agriculture Science* 1 (2017): 002.

47. Joseph IB. "Survival and growth response of Jatropha curcas L. to three Restoration Techniques on Degraded Soil Burkina Faso". *Annals of Forest Research* 54.2 (2011): 17184.

48. Micheal KG and Sogbesan OA. "Beneficial usage of Maggot meal (Musca domestica), single cell protein (Agarius bisporus) and fish meal in the diet of Clarias gariepinus fingerlings (Burchell, 1822)". *Fisheries and Aquaculture Journal* 6.3 (2015):133.

49. Muhammed NO., *et al*. "Deleterious Effect of Defatted Terminaliacatappa Seed Meal-Based Diet on Haematological and Urinary Parameters of Albino Rats". *NISEP Journal* 4.2 (2004):51-57

50. Oboh G., *et al*. "Nutrient and anti-nutrient content of Aspargillusniger fermented cassava products (flour and gari)". *Journal of Food Compound and Analysis* 15.5 (2002): 617-622.

51. Oboh O J., *et al*. "Nutritional and Antimicrobial Properties of OcimumgratissimumLeaves". *Journal Biological Science* 9.4 (2009): 377-380.

52. Orafidiya LO., *et al*. "The effect of honey and surfactant type on the antibacterial properties of the leaf essential oil of Ocimumgratissimum Linn. against common wound-infecting organisms". *International Journal of Aromatherapy* 16.2 (2006): 57-62.

53. Solomon SG and Ochume J. "Growth Performance and Haematological Parameters of Clariasgariepinus Fingerlings Fed Varied Level of Bitter Cola (Garcinia kola seed) Meal". *Journal of Fisheries International* 8.3-6 (2013): 63-68.

54. Svobodova Z., *et al*. "Unified Methods of Haematological Examination of Fish". Research unit of fish culture and hydrology, Vodal, Czecho Slovakia (1991): 31.

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