The International Journal of Engineering and Science (IJES)
|| Volume || 9 || Issue || 01 || Series I || Pages || PP 37-44|| 2020 ||
ISSN (e): 2319-1813 ISSN (p): 23-19-1805

Response of African Catfish to Bitter Leafmeal (Vernonia Amygdalina): A Sperm Quality Enhancer and Cholesterol Reducer

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----------***ABSTRACT***----------
Bitter leaf (Vernoniaamygdalina)plantis a shrub or small tree that grows throughout tropical Africa. It beenknown for the maintenance of health and treatment of various diseases. However,the aim of this study is to determine the effect of Vernoniaamygdalinaleaf on the growth performance, serum indices and sperm quality of juvenile African catfish (Clariasgariepinus).
Five isonitrogenous diets(35%CP) were formulated with different inclusion levels of Bitter leaf meal such that diet1 -D1 (control) has 0%, while D2, D3, D4 and D5 has 5%, 10%, 15% and 20% respectively. A total of Fifty (50) healthy male juvenile Clarias gariepinus were randomly distributed into plastic tanks (150L) at stocking rate of five (5) fish per tank and replicated twice. The fish were fed 3% of their body weight twice daily at 8:00am and 4:00pm for a period of 70 days. At the end of the feeding trials, blood samples were collected to determine serum biochemical indices andsperm sac were excised from the fish samples in all the treatments. Data were collected on growth performance - Mean weigh gain (MWG), percentage weight gain (PWG), specific growth rate (SGR), feed conversion ratio (FCR),protein efficiency ratio (PER);serum biochemical indices-ALP, AST, ALT,TP and sperm quality - percentage motility, percentage livability and death percentage were subjected to one way analysis of Variance (ANOVA) using Completely Randomized Design (CRD).
Significant differences were observed in all the growth parameters of Clariasgariepinus fed bitter leaf meal. The fish fed control (T1) diet had the highest mean weight gain (MWG), percentage weight gain (PWG) and specific growth rate (SGR) with value 240.81g, 187.27% 0.65grespectively while the fish fed 15% BLM (T4) had the least MWG (122.71g), PWG (95.43%) and SGR (0.42g). Even though, the least FCR was recorded in T1 (1.31) while the highest value for FCR (1.61) was obtained in fish fed 10%BLM(T3). The result of serum biochemical indices revealed that all parameters were significant ($p<0.05$). However, fish fed 10% bitter leaf meal (T3) had highest percentage motility(78.50%), percentage livability (182.49%) and lower dead percentage (48.51%)while the least values(50.08%, 102.75%. 102.75%) respectively were obtained in fish fed 20% BLM(T5). In the same vein, Highest ALP(36.91u/L), AST(167.98u/L), ALT (59.00u/L) and TP(63.87g/L) were obtained at T3 (10% BLM) while the least values ALP(16.68u/L), ALT(19.04u/L) and TP(35.12g/L) were recorded at T4 (15% BLM). The least AST was obtained at T5(20% BLM). Although, the least Cholesterol (77.28%) value was obtained at T3(10%). The results from this studyhave revealed that although bitter leaf meal reduces growth and fat but a little quantity is enough to improve the sperm quality of Clariasgariepinus, therefore10%BLM can be includedin the diet of African cat fish to improve sperm quality and as well reduced the level of fat in the fishwithout any residual effect on the organism.
KEYWORDS; *Clarias gariepinus, Growth performance, serum, Sperm quality.*

Date of Submission: 01-01-2020 Date of Acceptance: 16-01-2020

I. INTRODUCTION

Globally, aquaculture is still the only growing industry which can close up the wide gap created between fish production and consumption. Also ,in Nigeria, aquaculture remain the only option that may ensure the maintenance of the current level of per capital supply of fish at 6.6kg/year particularly despite the declining situation of capture fisheries (Ojitiku, 2008) therefore the availability of gametes throughout the year is important to ensure a constant supply of fish (Oteme *et al.*,1996).This availability is dependent on gonadal development and fecundity, which are subsequently affected by dietary nutrients (Izquierdo *et al.*,2001). The quality of sperm is highly variable and dependent on feeding regime, feed quality and the rearing temperature of the fish (Adewumi *et al.*,2005). Other factors include stress, age of broodstocks, breeding season, diseases, hormonal induction and spermiation (Azlina *et al*., 2011).Androgen synthesis and sperm quality are positively impacted by dietary lipid and fatty acids(Nyina-Wamwiza*et al*.,2012). In the rainbow trout, ascorbic acid deficiency reduced both sperm concentration and motility, and thus fertility (Ciereszko and Dabrowski, 1995).

Insufficient intake of energy and protein has been associated with sub-optimal reproductive performance (Bindari *et al.,* 2013). Hence, the need for some compounds to boost the level of lipids, and ascorbic acid in fish to improve the production and quality of sperm thereby enhancing fertility. Today in aquaculture the use of plants as fertility enhancers is now receiving attention in aquaculture (Dada *et al*., 2010). V. amygdalina commonly known as Bitter leafis a perennial shrub belonging to the family Asteraceae (Ijeh andEjike,2011). Bitter leaf is an indigenous edible vegetable leaves and rich sources of proteins, carbohydrates, minerals, vitamins and fibres. It also contain betacarotene, a precursor of vitamin A (Aregheore, 2012) and ascorbic acid (Ucheck, 2004). Anthraquinones, tannins, flavonoids, alkaloids, saponins, glycosides and terpenoids are phytochemicals present in the plant (Audu *et al.,* 2012). The vitamins A and C, and the phytochemicals present in bitter leaves are responsible for its antioxidant properties (Hamzahet al., 2013) and the bitter taste of V. amygdalina are due to lots of saponins present (Ijeh and Ejike, 2011). Apart from its dietary value, the leaf is also anti-parasitic (Ucheck, 2004), hypoglycaemic and hypolipidaemic (Arhoghro *et al*., 2009), hepatoprotective (Farombi and Owoeye, 2011), nephroprotective (Ebong, 2011), antidiarrhoeic (Hassan *et al.,* 2007), anti-cancer (Hamzah *et al.*, 2013), anti-febrile (Areghore, 2012) and aids healing (Ucheck, 2004). In humans the plant has been used as fertility inducer in infertile women (Areghore, 2012).While in South Eastern Nigeria it hastening parturition or causing abortion if used in preterm pregnancy (Ijeh *et al.,* 2011). There are a lots of reports by researchers on the use of bitter leaf for different animals. Oyeyemiet al.(2008) revealed that uncontrolled use of Vernoniaamygdalina has an adverse effect on the spermiogram and spermatozoamorphology of the intact male Wistar rats. Also, a decrease in testosterone levels and sperm motility of treated rats has been observed bymaking use of methanolic extract of the plant, thus having a deleterious effect on the reproductive functions in malealbino rats(Oyedeji *et al.,* 2013). the year (Oteme *et al.,* 1996) which is consequently hinged on feeding fish with essential nutrients (Izquierdo *et al.*, 2001). The cost of procuring conventional fish feed is high; it therefore becomes imperative to look into ways of improving fish production at affordable cost using locally available vegetables. Bitter leaf is known to be abundance in some of the nutrients essential for fish reproduction–carbohydrates, lipids, vitamins A and C. therefore V. amygdalina can be used as a non-conventional feed ingredient in production of fish feed with a huge reduction in price and ensuring high profit. The study therefore evaluate the nutritive value of Bitter leaf and also investigate its effect on the growth performance, serum indices and sperm quality of male Clariasgariepinus .

II. MATERIAL AND METHODS

Experimental site

The experiment was carried out at the Fishery Unit of the Teaching and Research Farm of LadokeAkintola University of Technology, Ogbomoso, Oyo State. Ogbomoso is located in the latitude 807^{o}N and 812^{o}N and longitude 404^{o} and 415^{o}e, the main annual rainfall is 1247mm with a relative humidity is between 75 and 95%. It is situated at about 500m above sea level with a main annual temperature of 26.2^{o}c (Oguntoyinbo, 1979).

Experimental fish and management

A total number of fifty (50) healthymale juvenile African catfish were purchased from a reputable farm at Sunsun, Ogbomoso, Oyo state, Nigeria. The fish were acclimatized for two (2) weeks in tanks containing aerated water and they were fed floating feed to empty their gut of all other feed so as to maintain a uniform stomach condition in preparation for the experiment. The fish were randomly distributed in replicate into ten plastic tanks at stocking density of 5fish per tank. The fish were fed twice daily, both in the morning and evening (8:00am and 4:00pm respectively) and they were weighed every two weeks, at the same time the daily feed rate of 3% of the total body weight were adjusted in like manner.

Collection and processing of test ingredient

Bitter leaves were harvested from a reliable source at Oke-afin, Ogbomoso, Oyo State, Nigeria. It was processed by removing the leaves from the stem, and air dried under normal temperature, the leaves were grinded to a fine powdered form and the sample was taken to laboratory for proximate analysis.

Experimental diets and formulation

The feed ingredients were purchased from a reputable feed mill in ogbomoso, Oyo State, Nigeria. The ingredients includes; maize, fish meal, soya bean meal, groundnut cake, wheat offal, bonemeal, lysine, methionine, premix, oil, oyster shelland salt

Five experimental diets were formulated from conventional feed ingredients and and varying levels (0%, 5%, 10%, 15% and 20%) of bitter leaf (Vernonia amygdalina) were included such that Diet 1, serves as control diet (without bitter leaf) and other diets D2,D3, D4, D5 containing varying levels of bitter leaf

respectively. All formulated diets were mixed, pelletized in a pelletizing machine, and the pelletized feeds were sundried to a constant weight and packed into an air tight container to avoid mould.

Table 1 Gross Composition of Experimental Diet (g/kg)

Ingredients and %CP	T1 (0% BLM)	T2 (5% BLM)	T3 (10%BLM)	T4 (15%BLM)	T5 (20% BLM)
Maize (10%)	20.60	20.14	19.67	19.20	18.73
Wheat offal (17%)	10.30	10.04	9.83	9.60	9.37
Groundnut cake (45%)	22.20	22.69	23.20	23.80	24.20
Soybean meal (42%)	33.30	31.63	29.97	28.30	26.64
Fishmeal (65%)	11.10	11.30	11.50	11.60	11.90
Bitter leaf (23%)	_	1.67	3.33	5.00	6.66
Oyster shell	0.50	0.50	0.50	0.50	0.50
Bone meal	0.50	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25
Vegetable oil	0.25	0.25	0.25	0.25	0.25

D- Diets, BLM-Bitter leaf meal

Data Collection

Fish weight and feed intake data were collected during experimental period, in which the following parameters such as mean weight, percentage weight gain, specific growth rate, feed conversion ratio, protein intake and protein efficiency ratio were calculated.

Mean weight gain (MWG) = $\frac{W2-W1}{n}$ Where n = number of day, W_2 = final weight, W_1 = initial weight

Percentage weight gain (PWG) % = $\frac{\text{mean weight (g)}}{\text{initial mean weight (g)}} \times 100$

Specific growth rate (SGR) = $\frac{\text{Log } W2 - \text{Log } W2}{T2 - T1} \times 100$

Where W_1 = initial weight (g), W_2= final weight (g), $T_2 - T_1$ = time interval between initial and final weight (days). Log = natural log to base

Feed conversion ratio (FCR)= $\frac{\text{feedintake}}{\text{Netweightgain}}$

Protein efficiency ratio (PER) = $\frac{\text{Netweightgain}}{\text{Amountofproteinfed}}$

The blood samples were collected from the experimented fish randomly selected from each treatment, by cutting the caudal peduncle of the fish. The blood samples for serum biochemical test was dispensed into a bottle without EDTA after which were taken to a reputable laboratory for analysis and the serum biological indices parameter determined include; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Serum albumin (ALB), Total protein (TP), Globulin (GLO), Creatine, Cholesterol (CHOL) and glucose.

Male fishes were randomly selected from all the treatments for milt collection. The male fishes were sacrificed and the testes were removed, then the sperm were examined and snapped and viewed under the computer aided sperm analysis microscopic system .Concentration of sperm was determined by counting the numbers of spermatozoa in sample diluted with distilled water (100^X) in a Burkerhaaemocytometer under 400x magnification(Rainis *et al*,2003).

Percentage motility was determined by the following: Each sample was estimated using large microscope at 400xmagnification, immediately after addition of 20ul distilled water as an activating solution. During spermatozoa activation, immotile sperm cell (ISC) was counted, when the activation stopped, whole sperm cell (WSC) was counted; motile sperm cell (MSC) was calculated Conyurt *et al*. (2008).

MC=WSC-ISC

MC%=MC/WSC x100

Chemical analysis

Proximate composition of test ingredient (bitter leaf), fish sample and experimental diets were determined according to the method of AOAC, (2000).

Statistic analysis

All data collected during experimental period were subjected to one-way analysis of variance (ANOVA) using completely randomized design in accordance with SPSS (2000) and duncan's multiple range test was employed to reveal significant differences among the treatments mean.

III. RESULTS

The proximate composition of the processed bitter leaf is as shown in Table2. The processed bitter leaf contained 23.56%Crude protein, 25.20% crude fibre, 2.20%ether extract, 10.80% moisture and 12.61% ash.

The growth performance and nutrient utilization of juvenile African catfish is shown in Table3. The survival rate was recorded to be 100%. It was revealed from the table that treatment 1(T1) has the highest FMW(g), MWG(g), PWG(g), TFI(g), AFI(g), SGR(g) and PI with the values 369.40g, 240.81g, 187.27%, 1582.32g, 316.46g, 0.65g and 110.71g respectively while the least were observed in treatment 4(T4) with values 251.38g, 122.71g, 95.43%, 924.88g, 184.98g, 0.42g and 64.74g respectively. The highest FCR was observed in treatment 3(T3) (1.66) and the least in treatment 1(T1). The highest PER value was obtained in T5(4.67)and T3 had the lowest(1.72). The highest AFI and WG value was obtained in T1 with values (316.46g, 1204.05g) respectively while T4 had the least (184.98g, 613.05g) respectively.

The initial and final carcass composition of juvenile African catfish fed bitter leaf meal is as shown in table 4. The highest CP was obtained in T1 (48.13%) and the least was observed in T3 (41.13%), the highest CF was obtained in T2 (9.23%) while the least was obtained in T5 (4.20%). The highest EE was observed in T4 (29.00%) while the least was obtained in T1 (20.41%). The highest moisture was obtained in T3 (17.01%) while the least was obtained in T1 and T5 (4.02%), the highest Ash was observed in T1(12.80%), however T2 had the least (4.65%). The least NFE was observed in T3(10.83%) while T2 had the least (1.92%).

Table 5. reveals the effect of bitter leaf meal on the sperm quality of juvenile African catfish. The highest milt volume was obtained it T2 (4.50ml) while the least was obtained in T5 (1.50ml), PH of T1, T3, T5 were significantly similar (7.25ml) which was lower than the PH observed in T2 (7.75ml). The highest spermatocrit and sperm alive values were recorded in T3 with values 48.00% and 182.49% respectively while the least were observed in T5 with values 27.50% and 102.75% respectively. Motility was observed to be high in T3 (48.75%) and low in T5 (50.08%). The highest sperm density value was recorded in T1 (250.50g/ml) while the least was obtained in T5(205.58g/ml).

Table 2: The Proximate Composition of Bitter leaf meal (Test ingredient)

Parameters (0%)	Crude protein	Crude Fibre	Ether extract	Moisture	Ash
	23.56	25.20	2.20	10.80	12.61

Table 3: **Growth Performance and Nutrient Utilization of Juvenile African Catfish(Clariasgariepinus) Fed Varying Inclusion Levels Of Bitter leaf Meal**

Parameters	T1 (0%BLM)	T2 (5%BLM)	T3 (10%BLM)	T4 (15%BLM)	T5 (20%BLM)	SEM
IMW(g)	128.59	128.59	128.59	128.59	128.59	0.01
FMW(g)	369.40^{a}	307.20^{b}	296.00^{c}	251.30^{e}	278.00^{d}	7.31
WG(g)	1204.05^{a}	883.72^{b}	836.87^{c}	613.05^{e}	747.05^{d}	36.54
MWG(g)	240.81^{a}	178.61^{b}	167.41^{c}	122.71^{e}	149.41^{d}	7.31
ADWG(g)	3.44^{a}	3.05^{ab}	2.39^{bc}	1.75^{c}	2.13^{c}	0.15
PWG(%)	187.27^{a}	139.73^{b}	130.19^{c}	95.43^{e}	116.19^{d}	5.69
SGR	0.65^{a}	0.52^{b}	0.52^{b}	0.42^{c}	0.48^{bc}	0.02
TFI(g)	1582.32^{a}	1227.88^{c}	1387.72^{b}	924.88^{e}	1200.36^{d}	40.43
AFI(g)	316.46^{a}	245.58^{c}	277.54^{b}	184.98^{e}	240.05^{d}	8.09
FCR	1.31^{c}	1.37^{c}	1.66^{a}	1.51^{b}	1.61^{a}	0.03
PI	110.71^{a}	85.95^{c}	97.14^{b}	64.74^{e}	84.02^{d}	2.83
PER	2.17^{b}	2.08^{c}	1.72^{e}	1.92^{d}	4.67^{a}	0.20

a,ab,b,c,d and e superscripts. Means in the same row with the same superscript are not significantly different (P > 0.05).

T- Treatment, BLM- Bitter leaf meal, IMW- Initial mean weight, FMW-Final mean weight, MWG-Mean weight gain, SGR-Specific growth rate, WG –Weight gain, PWG- Percentage weight gain, ADWG-Average daily weight gain, TFI- Total feed intake, AFI- Average feed intake, FCR-Feed conversion ratio, PI-Protein intake, PER- Protein efficiency ratio, T-Treatment, SEM- standard error of mean

Table 4.Initial and Final Carcass Composition of Juvenile African Catfish Fed Bitter Leaf Meal

PARAMETER	INITIAL	T1 (0%BLM)	T2 (5%BLM)	T3 (10%BLM)	T4 (15%BLM)	T5 (20%BLM)	SEM
CP	50.73^{a}	48.13^{a}	46.38^{c}	41.13^{d}	47.25^{b}	47.26^{b}	0.47

CF	4.62[d]	6.01[c]	9.23[a]	4.42[d]	8.02[b]	4.20[e]	0.37
EE	21.40[e]	20.41[e]	24.02[b]	21.67[d]	29.00[a]	23.88[c]	0.55
MOISTURE	3.61[e]	4.02[e]	13.80[b]	17.01[a]	5.41[d]	4.02[e]	0.91
ASH	12.20[a]	12.80[a]	4.65[e]	4.93[d]	7.84[c]	11.41[b]	0.61
NFE	-	8.63[b]	1.92[e]	10.83[a]	2.48[d]	3.84[c]	0.66

[a, b, c, d, e] indicates that mean values with different superscripts along the same row are significantly different ($p<0.05$).

BLM – bitterleaf meal, CP- Crude protein, CF- Crude fibre, EE- Ether extract,NFE- Nitrogen free extract ,SEM- standard error of mean

Table 5. Effect of Bitter leaf meal on the Milt/Sperm quality of Juvenile African Catfish (Clariasgariepinus)

Parameter	T1	T2	T3	T4	T5	SEM
PH	7.25[b]	7.75[a]	7.25[b]	7.50[ab]	7.25[b]	0.06
Volume (ml)	2.50[c]	4.50[a]	3.42[b]	3.50[b]	1.50[d]	0.15
Spermatocrit (%)	37.50[c]	39.00[bc]	48.00[a]	42.50[b]	27.50[d]	1.11
Motility (%)	65.00[b]	65.00[b]	78.50[a]	65.00[b]	50.08[c]	1.43
Sperm density(g/ml)	250.50[a]	250.49[a]	231.00[ab]	213.50[bc]	205.58[c]	4.20
%live sperm	163.10[ab]	168.60[ab]	182.49[a]	139.00[b]	102.75[c]	139.74
%dead sperm	87.40[b]	81.90[bc]	48.51[d]	74.50[c]	102.75[a]	73.09

a,ab,b,c,d and e superscripts. Means in the same row with the same superscript are not significantly different (P > 0.05).

T- Treatment

Serum biochemical indices of juvenile African catfish fed bitter leaf meal is as shown in table 6. The highest ALP value was observed in T3 (36.91u/l) while the least was observed in T4 (16.68u/l). There is no significant difference in the ALT and AST value of T1, T2, T4 and T5 which were lower compared to T3. There is no significant difference between the TP, ALB, and GLOB, value of T1, T2, T4 and T5 while T3 recorded the highest value 63.87g/l, 24.73g/l and 39.14g/l respectively. The CHOL value observed to be high in T2 (125.57mg/dl) than other treatments. However, there is no significant difference between the UREA level observed in T1, T2, T4 and T5 while T3 recorded the highest value. It was revealed from the table that the highest Creatinine level were observed in T3 (0.41mg/dl) and the least was observed in T1, T4, and T5 which were significantly similar (0.20mg/dl)

Table 6.Serum Biochemical Indices of Juvenile African Catfish Fed Bitter Leaf Meal

Parameter	**T1 (0%BLM**	**T2(5%BLM)**	**T3(10%BLM)**	**T4(15%BLM)**	**T5(20%BLM)**	**SEM**
ALP(U/L)	26.46[ab]	19.35[b]	36.91[a]	16.68[b]	26.83[ab]	1.88
AST(U/L)	95.02[b]	73.57[b]	167.98[a]	66.19[b]	60.90[b]	8.19
ALT(U/L)	27.42[b]	21.21[b]	59.00[a]	19.04[b]	20.21[b]	3.08
TP(g/L)	42.89[b]	46.91[b]	63.87[a]	35.12[b]	39.03[b]	2.30
ALB(g/L)	15.77[b]	17.80[b]	24.73[a]	13.95[b]	14.54[b]	0.90
GLOB(g/L)	27.12[b]	29.11[b]	39.14[a]	21.17[b]	24.49[b]	1.42
CHOL(mg/dl)	104.55[b]	125.57[a]	77.28[c]	98.87[b]	105.12[b]	2.22
UREA(mg/dl)	8.12[b]	7.20[b]	16.74[a]	7.87[b]	8.71[b]	0.80
CREA(mg/dl)	0.20[c]	0.30[b]	0.41[a]	0.20[c]	0.20[c]	0.17

[a, b, c] indicates that mean values with different superscripts along the same row are significantly different ($p<0.05$). ALP- Alkaline phosphate, ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, TP- Total protein, ALB- Albumin, GLOB- Globulin, CHOL- Cholesterol, CREA- Creatine.

IV. DISCUSSION

The major interest of the fish farmer involved in hatchery business is to maximize profit hence, the need for substance that will enhance the production of good quality fish sperm under cultured environment. The use of medicinal plants as fertility enhancer in aquaculture has been receiving some attention. Adeparusi *et al* (2010) has used Kigella Africana fruit to enhance sperm production in *Clarias gariepinus*. This present study; therefore investigate the effect of bitter leaf on the growth, serum biochemical indices and the sperm quality of male African cat fish. The results in this study revealed the final mean weight, mean weight gain, total feed intake, percentage weight gain, specific growth rate and average feed intake observed to be significantly decreased as the inclusion levels of bitter leaf increased. The highest growth values were obtained from fish fed control diet (0% bitter leaf meal) for twelve weeks. This agrees with the findings of Bello *et al.,* (2012) who recorded similar decrease in weight gain of fish when fed diets supplemented with walnut leaf and onion bulb

residue. Also, the best feed conversion ratio (least FCR) was observed in Treatment 1 (T1) while the highest FCR was recorded for T3. This is similar to the work of Bello et al., (2012) who reported that inclusion of 1.5% of walnut leaf increase feed conversion ratio (FCR) in the supplemented groups than the control. The highest protein efficiency ratio (PER) was recorded in treatment 5(T5) while treatment 3(T3) recorded the lowest. This agrees with the findings of AbdelRahman(2009)who observed that the addition of propolis-ethanolic extract and crude propolis increase the feed conversion ratio (FCR) and protein efficiency ratio (PER) in the supplemented groups when compared with the control. Treatment 1(T1) had the highest average feed intake and weight gain while treatment 4 (T4) had the lowest. This findings agree with the report of Karangiya *et al.*,(2016) who reported that supplementation of garlic improves the growth rate(weight gain) performance of broilers when added at the rate of 1% and can be an alternative to antibiotic growth promoter in the feeding of broiler chicken. High level of ALP indicates bile duct obstruction, liver disease and low level indicates starvation or malnutrition. The AST and ALT levels of blood serum of fish at T2 (5%BLM) and T3 (10%BLM) than T4's (15%BLM). The higher values of alanine aminotransferase suggested that the blood serum enzyme in the fish efficiently utilized amino acids for metabolic purposes confirming the observation of Adesina (2008). Increased levels of transaminase in the blood serum of the fish are usually associated with dying or damaged liver cells while a decrease could suggest leakage of enzyme into the serum (Yilmaz *et al*, 2006; Ozovere,2013). However both the AST and ALT levels in this present study were within the normal range which agrees with the findings ofEnethLalhathangi and RamjatButagohain (2018).Proteins are among the main energy sources which play an important role in the maintenance blood glucose in fish (Shwetha *et al*, 2012). The observed decrease in total protein content of fish at T4 (15%BLM) and T5 (20%BLM) could be linked with the level of antinutrients present in the diets which were similar with the report of Adesina (2017) on juvenile African catfish fed graded levels of MESSM. A reduction in serum total protein content in *Channa punctatus* induced with stem bark extract of Croton tigliumwas also reported by Yadev *et al.*, (2003). However, Ajani (2006) related such significant decrease in total protein level to impaired water quality. GLOB and ALB content also exhibit the same pattern with total protein which decreases at T4 and T5. According to Adesina (2017), the slight reduction in the serum TP,GLOB and ALB levels in fish blood samples in some treatments might be due to the degradation and utilization for metabolic purposes. Jee *et al* (2005) stated that the decrease in TP,ALB and GLOB may be due to impaired synthesis of protein or enhanced loss of proteins through excretion and may also indicate some problems in the kidney. The highest level of UREA and CREA were observed at T3 (10%), Adham *et al.,* (2002) stated that the high levels of blood urea and creatine result either from increased synthesis or decreased urinary clearance by the kidney or decreased degradration of these compounds. High serum urea has been reported as an indicator of muscular wastage in animals (Adesehinwa, 2008). Low level of serum cholesterol indicates liver disease, starvation, kidney disease, pancreatitis, diabetics and higher level is associated with seizes (EnethLalhuthangi and RajatBurahgohain, 2018).

In an attempt to determine sperm quality in various mammals including fish, different parameters such as spermatozoa motility, percentage motile sperm, sperm volume and sperm density are usually measured. Spermatozoa motility is the most commonly used criterion to evaluate semen quality according to Bozkurt *et al*, (2006). Even though, spermatozoa motility varies within species of male depending on the ripeness, age and time of sampling.

This result also showed that sperm motility was significantly affected by the dietary treatment as the highest value was observed in Treatment 3 (T3) although treatment 5 (T5) had the lowest, this shows that bitter leaf had significant effect on sperm motility. This result agrees with the findings of Ajala and Owoyemi (2015) who reported that sperm motility value increased parallel to the dietary content of Vernonia amygdalina and that of Francis *et al* (2013) who also reported that motility increases with inclusiuon level of *Vernonia amygdalina* in the diet of giant African catfish *(Heterobranchus bidorsalis*) brood stock. Although, this study is in contrary to the report of Orlu and Ogbalu (2011) that the exposure of *Clarias gariepinus* to increasing extract concentrations of L. alopecuriodes (vahl) caused a decrease in sperm motility. The increased sperm motility of fish fed diet 10g/kg inclusion level may be due to carbohydrate, lipid and vitamin content of the leaves. Vitamin C deficient diet is known to decrease sperm motility Cierezko and Dabrowski (1995). Percentage livability (% live sperm) and sperm density were proportionally decreasing with an increased inclusion level of bitter leaf. This report is in agreement with the findings of Oyeyemi *et al* (2011) who observed that male *Clarias gariepinus* treated with Aloe vera gel extract had decreased sperm liviability. The higher livability percentage obtained in group fed 10g/kg bitter leaf may be associated with the supplementary lipid, vitamin A, vitamin C derived from *Vernionia amygdalina* in their diets.

V. CONCLUSION

Observation from this study showed that 10% bitter leaf inclusion in the diet of juvenile *Clarias gariepinus* resulted to highest sperm motility, percentage livability and sperm density as well reducing the level

of fat in the fish. However, the highest growth performance was recorded in fish fed control (0%BLM) diet, this implies that Bitter leaf does not support the growth performance but enhanced sperm quality as well as reducing the fatin juvenile African catfish (*Clarias gariepinus*).

VI. RECOMMENDATION

Farmers whose their main objective is to improve the sperm quality of their fish for hatchery business is thereby advice to include little quantity of bitter leaf meal not more than 10% in the diets of their fish for a better result. Bitter leaf meal can also be used to reduce the level of fat in the fish particularly for those customers that have preference for catfish but they are irritated by the high level of fat.

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