Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.134258 2025, 24(5) 1141-1157

Research Article

Dietary effect of siam weed (*Chromolaena odorata*) leaves on the growth performance, reproductive indices, intestinal morphology, and blood profile of *Clarias gariepinus* broodstocks

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Keywords

Siam weed,
Chromolaena odorata,
Blood profiles,
Growth performance,
Reproductive indices,
Intestinal morphology

Article info

Received: November 2024
Accepted: February 2025
Published: September 2025

Abstract

This study aimed to evaluate the therapeutic potential of siam weed (*Chromolaena odorata*) leaves meal (SWLM) on the growth performance, reproductive indices, intestinal morphology, and blood profiles of African sharptooth catfish (*Clarias gariepinus*) broodstocks. One hundred and twenty male and female broodstocks with an average body weight of 450±0.01g were obtained for the experiment and allocated into 12 experimental fiber tanks at 10 fish per tank (5 males and 5 females). Six diets were formulated (40% crude protein) with different inclusion levels of SWLM; 0, 15, 30, 45, 60, and 90% denoted as the control, $SWLM_2$, $SWLM_3$, $SWLM_4$, $SWLM_5$, and $SWLM_6$ diets, respectively. The fish were fed twice daily at 3% body weight with replicated twice, for 56 days. The results showed that the fish fed with experimental diets significantly improved growth performance, such as weight gain, specific growth rate, and feed conversion ratio. The reproductive indices were significantly higher (p<0.05) in the fish fed with SWLM compared to the control group. The dietary groups treated with SWLM at 30%, 60%, and 90% improved growth performance, intestinal morphology, reproductive indices, and blood profiles of *C. gariepinus*. The results of the intestinal morphology also showed that SWLM had a significant effect (p<0.05) on the broodstocks. In conclusion, incorporating SWLM in the diets can improve growth performance, blood profiles, intestinal morphology, and reproductive indices in the catfish broodstocks.

Introduction

The need to meet the growing animal-source of protein demand by teeming global population has culminated in global aquaculture expansion (Okon *et al*., 2022). Fish serves as a major source of protein for humans, providing a significant portion of nutrients to a large proportion of people, particularly in the developing world (Nwokoye *et al*., 2007). The global production of fish and other aquatic animals for human use occurs either by commercial fishing or through aquaculture and farming techniques. Globally, aquaculture contributed up to 49.9 percent of global aquatic animal production in 2021 (FAO, 2023). According to FAO (2010), the world production of fish in 2005 consisted of 93.2 million tons captured by commercial fishing in the wild plus 48.1 million tonnes produced by fish farms, which has increased significantly over the years. Total fisheries and aquaculture production of aquatic animals reached a record of 182 million tonnes in 2021. Nigeria, the largest producer in sub-Saharan Africa, has experienced a declining trend since 2016 worsened in 2020 with a sharp decrease of 9.6 percent (FAO, 2022). In Nigeria, the most common fish reared are tilapia and catfish because they are mostly found in freshwater habitats. Additionally, both tilapia and catfish are resilient species that can tolerate a wide range of environmental conditions, making them ideal choices for aquaculture in Nigeria.

To increase fish production under controlled conditions, attempts are made to obtain sperm from the fish with high-quality seeds, although, several factors affect fish seed quality, such as different strains, genetics, nutrition, content of feed and deposition of organic matter, chemical fertilizer into water used for cultured medium and hatchery purposes. To fulfill the increased demands for *C. gariepinus*, the availability of *C. gariepinus* must be increased by improving the reproductivity and productivity of the broodstocks. The growth and development of embryos and larva is determined by the availability of nutrients and materials in the egg to support the optimum growth and development of embryos and larvae. Therefore, survivals of fish in their early life are strongly influenced by the egg quality. Nutritional deficiencies in eggs can result in the inhibition or cessation of embryogenesis activities that can cause deaths in the new organisms before being hatched or the occurrence of abnormal growth of the larvae produced (Rawung *et al*., 2020). The adequacy of nutrition and good health of broodstock during the process of vitellogenesis will affect the quality of eggs produced (Olusola *et al*., 2021).

The use of medicinal plants to improve reproduction in animals, including fish, poultry, and livestock, is an area of growing interest and research. Siam weed (*Chromolaena odorata*) is a major perennial shrub that belongs to the family Asteraceae and grows up to 7 m tall and has white to mauve flowers (Ekenyem *et al*., 2010). The Siam weed is used as an herbal medicine and produces a characteristic smell when crushed (Ling *et al*., 2007). *Chromolaena odorata* has a complex mixture of flavonoid compounds, including aurone, flavones, and flavonol (Vital, 2008). *Chromolaena odorata* is a source of high-quality protein, which could serve as a potential source of protein supplement, and this could explain its folkloric use in animal nutrition. Therefore, considering the nutritive composition of *C. odorata*, there is scant information about its

utilization in aquaculture, most especially indigenous fish species like *C. gariepinus*, which command high economic value in Nigeria. This study was therefore carried out to investigate the therapeutic potential of *C. odorata* leaves meal on the reproductive performance, intestinal morphology, and blood profiles of *C. gariepinus* broodstocks.

Materials and methods

Experimental location

The experiment was carried out at the Teaching and Research Farm of Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The experiment lasted for eight weeks (56 days) between April-May, 2023.

Source of the plant and identification

Siam weed was collected from Igodan town in Okitipupa, Nigeria, and identified in the Department of Biological Sciences (Botany programme) of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria. After collection, the leaves were air dried for two weeks to maintain the nutrients, ground into fine powder and kept in an air-tight container until required.

Experimental design, source, and acclimatization of experimental fish

Water was sourced from the Research and Teaching Farm borehole of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria and 12 fiber holding tanks were filled with water to a depth of 1.2 m each. One hundred and twenty *Clarias gariepinus* broodstocks were stocked into the 12 fiber holding tanks (1.5 m x 1.5 m x 1.2 m) containing ten (10) *C. gariepinus* broodstocks (5 males and 5 females) per treatment tank (mean weight 598±0.80g) were procured from a reputable fish farm in Okitipupa, Ondo State, Nigeria. The broodstocks were acclimatized for three weeks in fibre holding tanks. During this period, they were fed with commercial diets (blue crown, 6 mm) of 40% crude protein twice daily at 3% body weight. The diets were assigned to the tanks as designated and were fed at 3% body weight in two equal portions at 08:00 hours–09:00 hours and 17:00 hours–18:00 hours for 56 days. All fish were removed from each fiber holding tank every fourteen days and batch weighed, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly. At the end of the experiment, the growth performance and reproductive indices were determined.

Formulation of experimental diets

The ground *C. odorata* at different inclusion levels of 0, 15, 30, 45, 60, 90% was added to feed ingredients such as fishmeal, yellow maize, millet, soybean, groundnut cake, di-calcium phosphate, vitamin premix, vegetable oil, starch and salt to formulate 40% crude protein diet. Each diet mixture was treated separately and extruded through a 6 mm mincer pelleting machine to form a noodle-like size suitable for the adult *Clarias gariepinus*. The pelleted diets were sun-dried, packed in labeled polythene bags, and stored until required (Table 1).

Table 1: Gross composition of experimental diets (g/ 100g).

Ingredients	Control (0%)	$COLM_2$ (15%)	$COLM_3$ (30%)	$COLM_4$ (45%)	$COLM_5$ (60%)	$COLM_6$ (90%)
Fish meal	13.33	13.33	13.33	13.33	13.33	13.33
Soybean	26.66	26.66	26.66	26.66	26.66	26.66
Groundnut cake	26.66	26.66	26.66	26.66	26.66	26.66
Yellow Maize	8.45	8.45	8.45	8.45	8.45	8.45
Millet	16.90	14.36	11.83	9.29	6.76	1.69
DCP	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Vegetable oil	2.00	2.00	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00	1.00	1.00
Starch	1.00	1.00	1.00	1.00	1.00	1.00
C. odorata	-	2.54	5.70	7.61	10.14	15.21
Total	100	100	100	100	100	100

COLM = *Chromolaena odorata* Leaf meal; Vitamin/mineral premix: Vit. A: 1,000,000 IU; Vit. B_1: 250mg; Vit B_2: 1750mg; Vit B_6: 875mg; Vit. B_{12}: 2500mg; Vit. C: 12,500mg; Vit D_3: 600,000 IU; Vit. E: 12,000 IU; Vit. K3: 15mg; Calcium D-pantothenate: 5000mg; Nicotinic acid: 3750 mg; Folic acid: 250mg; Cobalt: 24,999 mg; Copper: 1999 mg; Iron: 11,249mg; Selenium (Na_2SeO_3. $5H_2O$): 75mg; Iodine (Potassium iodide): 106mg; Anti-oxidant: 250mg

Biological evaluation:

Weight gain = Final body weight – initial body weight

$$\text{Weight gain (\%)} = \frac{\text{(Final body weight – initial body weight)}}{\text{Initial body weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{100\ (\log \text{final body weight} - \log \text{initial body weight})}{\text{Time (days)}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight gain (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Initial Number of Fish Stocked – Mortality}}{\text{Initial Number of Fish stocked}} \times 100$$

$$\text{Nitrogen metabolism} = \frac{(0.549)\ (a+b)\ h}{2}$$

Where, a = Initial mean weight of fish; b= final mean weight of fish; h= experimental periods in days (Olusola and Olorunfemi, 2017).

Evaluation of reproductive indices

At the end of the feeding trials, males and females (n=3) were randomly selected per dietary treatment for reproductive indices such as milt volume, motility duration, sperm counts, egg size estimation, relative fecundity, percentage fertilization, percentage hatchability, gonado-somatic index (GSI), the weight of ovary and belly diameter were calculated as described by Olusola *et al*. (2021).

Milt volume

A small incision was made into the lobe of the testes, the milt was squeezed out into a petri dish. This was measured with plastic syringe in mL.

Motility duration

One (1ul) of milt from a male was placed on a Weubauer hemocytometer, a drop of distilled water was added and covered with a slip. The sperm activity was viewed under an Olympus microscope at 100×

magnification to see when all the sperm stopped.

Sperm count

The concentration of sperm was determined by counting the number of spermatozoa in a sample diluted with distilled water (100x) in a bird hemocytometer, under 400x magnification.

Egg size estimation

Female broodstocks (3) were randomly collected from each treatment and used for egg size estimation and percentage fertilization. Five (5) eggs were randomly collected from females in each treatment and viewed under a light microscope filled with a calibrated ocular meter rule/micrometer to calculate the egg size as mean egg size (mm):

$$\text{Egg size} = \frac{\text{Length of long axis+length of short axis}}{2}$$

$$\text{Relative fecundity} = \frac{\text{Total number of eggs}}{\text{Body weight}}$$

$$\text{Fertilization rate} = \frac{\text{No of fertilized egg in sample}}{\text{Total No. of egg in sample}} \times 100$$

$$\text{Hatching rate} = \frac{\text{No of hatched egg in a sample}}{\text{Total No. of egg in a sample}} \times 100$$

Hatching time

The time of first hatching was observed across the treatments and recorded.

Gonadosomatic index (GSI):

$$\text{GSI} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

Weight of the ovary

Female *C. gariepinus* (3) were euthanized and dissected, the ovary was carefully removed by using a dissecting set and batch-weighed using a digital weighing balance. (5. Mettle, max = 500g, d= 0.01g).

Belly diameter

Female broodstocks (3) were randomly collected from each treatment and their belly diameter was determined as follows:

$$\text{Belly diameter} = \frac{\text{Length of horizontal axis from ventral region of pectoral fin to the vent region + belly width.}}{2}$$

Artificial propagation

Male and female spawners (n=3) from each treatment were taken and used for artificial propagation techniques- hormone administration, stripping, fertilization and incubation as described by Olusola *et al*. (2023).

Hormone injection

Female spawners (3) were injected using a 5 mL graduated syringe intramuscularly with pituitary hormone suspension that was obtained from the male at an angle of 45^0C in the dorsal muscles in the direction of the tail.

Stripping and fertilization

Females (3) from each treatment were stripped of their eggs after the latency period into clean, well-labeled dry bowls and weighed. The milt was evenly

distributed over the egg mass of the sample of each treatment with a 5 mL saline solution (0.9% NaCl), and proper mixing was done by rapid agitation in clockwise and anti-clockwise direction/ movement and afterwards, some clean water was added.

Incubation

Fertilized eggs from each treatment were evenly distributed in a single layer of a netting substrate ''kakaban'' of 2 mm mesh size and water was allowed to run through the incubation period so as to obtain an adequate oxygen supply. After hatching, siphoning was done and hatchlings were counted and the incubation period was recorded appropriately.

Blood profile assessment

Blood samples (5 mL) were collected by the caudal ablation method from both the control and treated fish before the experiment, and at the end of the experiment (56 days). The blood samples were dispensed into tubes containing ethylene diamine tetra acetate (EDTA) anticoagulant and transported in ice-packed bags to the Microbiological Laboratory unit of Ondo State Specialist Hospital, Okitipupa, Nigeria for hematological analysis. Red blood cells and white blood cells were counted by Neubaeur improved hemocytometer using Hayem's and Turk's solution as diluting fluids, respectively. Hemoglobin (Hb) was estimated by the Cyanomethemoglobin method as described by Kelly (1979). Packed cell volume (PCV), Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) were calculated using the standard formula described by Blaxhall and Daisley (1973).

Pack cell volume (PCV)

This was determined after drawing freshly collected blood samples into microhematocrit tubes (75 mm long, 1.1 – 1.2 mm internal diameter) sealed with plasticine (cristaseal) at one end. The blood was centrifuged for five minutes at 3000 rpm using a Hawksley micro-hematocrit centrifuge, England. The actual value was obtained using hematocrit reader.

Mean Corpuscular Volume (MCV): The diluted blood was centrifuged at 200 rpm for 10 minutes to remove the red cell nuclei that cause turbidity. Calculation, this was the average volume of a single cell exposed in fentolitres (fl) or μm:

$$MCV = \frac{PCV}{RBC} \times 1000$$

Mean Corpuscular Hemoglobin Concentration (MCHC): The diluted blood was centrifuged at 200 rpm for 10 minutes to remove the red cell nuclei that cause turbidity. Calculation, this was referring to the percentage hemoglobin content in g/dl by the PCV and the result expressed as a percentage:

$$MCHC = \frac{\text{Hb content}}{PCV}$$

Mean Corpuscular Hemoglobin (MCH): The diluted blood was centrifuged at 200 rpm for 10 minutes to remove the red cell nuclei that cause turbidity. Calculation, this is expressed as the average hemoglobin (Hb) content in picograms (pg) of a single red blood cell (RBC):

$$MCH = \frac{Hb}{RBC}$$

Intestinal histomorphology

Samples of the experimental fish (n = 2) were dissected dorso-ventrally with the aid of a clean scalpel, and the intestines were taken carefully and placed in a container containing 10% diluted formalin solution and transported to the Histopathology Laboratory Department of Veterinary Pathology, University of Ibadan, Nigeria. Cryptal depth, villi height, villi width, muscular thickness, and goblet cells were measured using a micrometer rule as described by (Agbebi *et al*., 2023). Each treatment sample was analyzed in a slide, recorded, and an average value calculated.

Statistical analysis

Data resulting from the experiment was subjected to one–way analysis of variance (ANOVA) test using the Statistical Package for the Social Sciences (SPSS) software. Determination of significant mean differences among individual means was done at P = 0.05 using Duncan's least significant difference.

Results

Growth performance and nutrient utilization of C. gariepinus

A general increase was observed in the final body weight, weight gain, percentage weight gain, specific growth rate, nitrogen metabolism and production performance index of the fish. The treated groups had higher values compared to the control. The final weight gain was highest in $SWLM_3$ and $SWLM_5$ (760g), respectively. However, the lowest was recorded in $SWLM_4$ (710g). The highest value for percentage weight gain was recorded in $SWLM_3$ and $SWLM_5$ (68.89g), respectively. However, the lowest was recorded in $SWLM_4$ (57.78g). For the feed conversion ratio, $SWLM_2$ (0.76) had the lowest value of feed conversion ratio while $SWLM_4$ (1.46) had the highest value of feed conversion ratio. Also, the treated groups had a better survival rate and production performance index compared to the control (Table 2).

Table 2: Growth Performance and Nutrient Utilization of *Clarias gariepinus* (n=10) fed different inclusion levels of *Chromolaena odorata* leaf meal for 56 days.

Parameters	Control (0%)	$SWLM_2$ (15%)	$SWLM_3$ (30%)	$SWLM_4$ (45%)	$SWLM_5$ (60%)	$SWLM_6$ (90%)
IBW (g)	450±0.01^{a}	450±0.00^{a}	450±0.01^{a}	450±0.01^{a}	450±0.00^{a}	450±0.00^{a}
FBW (g)	750±0.03^{d}	730±0.05^{c}	760±0.08^{e}	710±0.02^{a}	760±0.09^{e}	720±0.01^{b}
WG (%)	300±0.02^{ab}	305±0.00^{b}	310±0.02^{b}	260±0.01^{a}	310±0.04^{b}	270±0.03^{ab}
PWG (%)	64.670.05^{ab}	67.78±0.06^{b}	68.89±0.07^{b}	57.78±0.02^{a}	68.89±0.01^{b}	60.00±0.00^{ab}
SGR	0.40±0.03^{c}	0.38±0.01^{b}	0.41±0.02^{c}	0.36±0.01^{a}	0.41±0.02^{c}	0.38±0.01^{b}
FCR	0.94±0.07^{b}	0.76±0.03^{a}	1.23±0.05^{c}	1.46±0.06^{d}	1.26±0.05^{c}	1.41±0.06^{d}
SR (%)	90±0.01^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}
NM	18446.40±8.24^{a}	11682.72±2.67^{a}	18600.12±3.43^{a}	17831.52±5.67^{a}	18600.12±4.50^{a}	17985.20± 6.35^{a}
PPI	482.15±0.54^{ab}	544.65±1.23^{b}	553.57±1.56^{b}	464.29±0.98^{a}	553.57±1.58^{b}	482.14± 1.76^{ab}

IBW= Initial Body Weight, FBW= Final Body Weight, WG= Weight gain, PWG= Percentage Weight Gain, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, SR = Survival Rate, NM = Nitrogen Metabolism, PPI = Production Performance Index, SWLM = Siam Weed *Chromolaena odorata* Leaves meal. Means (n=2) in each row with similar superscripts are not significantly different (p>0.05).

Hematology

The hematological parameters of *C. gariepinus* treated with *C. odorata* leaves meal at different inclusion levels are shown in Table 3. $SWLM_6$ (36.00 %) recorded the highest value of pack cell volume (PCV) and $COLM_3$ (31.00 %) recorded the lowest value of PCV. $SWLM_6$ (12.00 g/dl) recorded the highest value of hemoglobin, while $SWLM_3$ (10.30 g/dl) recorded the lowest value hemoglobin (HB). The highest value of total protein (TP) was recorded in $SWLM_4$ (71.80 g/dl) and the lowest value was recorded in $SWLM_3$ (64.00 g/dl). $SWLM_4$ recorded the highest value of Globulin (33.50 g/dl) and the lowest value was recorded in $SWLM_3$ (27.30 g/dl). However, there was a significant difference (p<0.05) in all the hematological indices except for Neutrophil, lymphocyte, monocyte, and eosinophil in which there were no significant differences (p>0.05) among the dietary groups.

Table 3: Hematological indices of *Clarias gariepinus* juveniles (n=10) fed different inclusion levels of *Chromolaena odorata* leaf meal for 56 days

Parameters	Before treatment	Control (0%)	$SWLM_2$ (15%)	$SWLM_3$ (30%)	$SWLM_4$ (45%)	$SWLM_5$ (60%)	$SWLM_6$ (90%)
PCV (%)	32.00±0.01^{a}	33.00±0.02^{a}	35.00±0.01^{a}	31.00±0.00^{a}	33.00±0.03^{a}	34.00±0.05^{a}	36.00±0.07^{a}
Hb (g/dl)	10.70±0.03^{a}	11.00±0.01^{a}	11.70±0.03^{a}	10.30±0.01^{a}	11.00±0.02^{a}	11.30±0.02^{a}	12.00±0.06^{a}
Glucose (mg/dl)	5.30±0.09^{a}	5.60±0.03^{a}	6.10±0.04^{a}	5.90±0.06^{a}	4.80±0.05^{a}	5.30±0.06^{a}	4.90±0.08^{a}
Sodium	133.40±0.90^{ab}	130.60±0.06^{acb}	137.00±0.80^{b}	127.80±0.07^{a}	129.40±0.08^{a}	131.60±0.40^{ab}	131.90±0.50^{ab}
Potassium	3.80±0.07^{a}	3.50±0.01^{a}	3.90±0.04^{a}	3.60±0.02^{a}	3.80±0.09^{a}	3.60±0.04^{a}	3.70±0.01^{a}
Chloride	94.70±0.60^{a}	100.10±0.45^{a}	99.70±0.30^{a}	100.40±0.80^{a}	97.80±0.10^{a}	98.00±0.30^{a}	95.00±0.10^{a}
Globulin (g/dl)	35.90±0.40^{c}	31.10±0.70^{abc}	29.20±0.05^{ab}	27.30±0.01$_{a}$	33.50±0.60^{bc}	31.60±0.08^{abc}	33.00±0.10^{bc}
Albumin (g/dl)	39.10±0.20^{a}	38.40±0.01^{a}	40.20±0.09^{a}	36.70±0.03^{a}	38.30±0.02^{a}	39.40±0.04^{a}	36.50±0.02^{a}
Total protein (g/dl)	75.00±0.80^{b}	69.50±0.70^{ab}	69.40±0.10^{ab}	64.00±0.09^{a}	71.80±0.90^{b}	70.90±0.07^{a}b	69.50±0.03^{ab}
Urea (mg/dl)	6.10±0.70^{b}	2.90±0.00^{a}	4.30±0.02^{ab}	3.30±0.01^{a}	3.80±0.04^{ab}	5.30±0.08^{ab}	2.80±0.01^{a}
Creatinine (g/dl)	79.40±0.90^{c}	57.70±0.70^{a}	60.80±0.80^{b}	58.30±0.60^{ab}	58.40±0.50^{ab}	75.80±0.03^{c}	51.60±0.04^{a}
Calcium	2.40±0.02^{a}	2.20±0.01^{a}	2.10±0.01^{a}	2.30±0.02^{a}	2.20±0.01^{a}	2.50±0.01^{a}	2.30±0.00^{a}
Alkaline phosphatase	21.00±0.08^{a}	18.00±0.50^{a}	19.70±0.10^{a}	18.40±0.10^{a}	20.50±0.70^{a}	17.30±0.10^{a}	16.40±0.10^{a}
Cholesterol (mg/dl)	3.70±0.01^{a}	3.80±0.03^{a}	3.60±0.04^{a}	3.70±0.06^{a}	3.30±0.01^{a}	3.90±0.03^{a}	4.20±0.07^{a}
Triglycerides	1.00±0.01^{a}	1.20±0.02^{a}	1.20±0.04^{a}	0.90±0.03^{a}	0.80±0.02^{a}	0.70±0.01^{a}	1.30±0.10^{a}
Neutrophils (%)	67.00±0.30^{ab}	70.00±0.60^{ab}	68.00±0.50^{ab}	73.00±0.80^{b}	71.00±0.30^{ab}	70.00±0.20^{ab}	65.00±0.10^{a}
Lymphocytes (%)	28.00±0.01^{a}	30.00±0.40^{a}	30.00±0.50^{a}	25.00±0.20^{a}	26.00±0.10^{a}	30.00±0.60^{a}	30.00±0.50^{a}
Monocytes (%)	3.00±0.02^{b}	0.00±0.00^{a}	2.00±0.01^{ab}	1.00±0.00^{ab}	2.00±0.01^{ab}	0.00±0.00^{a}	3.00±0.03^{b}
Eosinophils (%)	2.00±0.02^{a}	0.00±0.00a	0.00±0.00a	1.00±0.01^{a}	1.00±0.01^{a}	0.00±0.00^{a}	2.00±0.01^{a}

PCV = Pack cells volume, Hb = Hemoglobin. SWLM = Siam Weed *Chromolaena odorata* Leaves meal.
Mean values with the same superscript along the rows were not significantly different (p>0.05).

Reproductive performance of male and female

The results of male and female *C. gariepinus* fed with *C. odorata* leaf meal in terms of milt volume, sperm counts, weight of testes, weight of eggs, size of eggs, weight of ovary, relative fecundity, hatching time, and latency period increased in the treated groups when compared to the control. These reproductive indices were significantly different (p<0.05) among the dietary groups (Table 4).

Table 4: Reproductive performance of male *Clarias* gariepinus (n= 10) fed different inclusion levels of *C. odorata* leaves meal for 56 days.

Parameters	Control	$SWLM_2$ (15%)	$SWLM_3$ (30%)	$SWLM_4$ (45%)	$SWLM_5$ (60%)	$SWLM_6$ (90%)
Weight of male (g)	750±0.03^{d}	730±0.05^{c}	760±0.08^{e}	710±0.02^{a}	760±0.09^{e}	720±0.01^{b}
Weight of testis (g)	3.34±0.06^{c}	1.74±0.02^{b}	1.30±0.01^{a}	6.47±0.060^{e}	6.39±0.71^{d}	8.89±0.91^{f}
Milt volume (mL)	1.43±0.05^{c}	0.69±0.00^{a}	1.21±0.01^{b}	2.80±0.02^{c}	2.29±0.01^{d}	3.75±0.04^{f}
Motility duration (s)	36.00±0.43^{a}	42.00±0.51^{b}	38.50±0.71^{a}	53.00±0.91^{c}	52.00±0.65^{c}	57.00±0.81^{d}
Sperm counts (10^4 spm/mL)	120,453.63±605^{a}	355,557.54±212^{b}	424,201.45±203^{b}	653,077.02±209^{d}	548,009.06±185^{c}	1,368,464.93±382^{e}

SWLM = Siam Weed *Chromolaena odorata* leaf meal. Means of duplicate data, mean value in each row with similar superscripts are not significantly different (p>0.05).

Intestinal morphology of male and female C. gariepinus

The results of the morphometric indices show that the area of absorption depends on the villi height and the villi width, while the ratio of the villi height to the cryptal depth is also considered. The highest villi height/cryptal depth ratio was observed $SWLM_3$ (4.79), with the lowest being in $SWLM_6$ (3.33). Also, the highest area of absorption was observed in $SWLM_4$ (333157.12) while the lowest was deduced to be in $SWLM_6$ (317405.81) (Tables 5 and 6).

Histopathological parameters of male and female C. gariepinus

Results of histopathological analysis are shown in the photomicrographs (Figs. 1 and 2). The testis of fish in Figure A indicates that there were no observable lesions. Similarly, Figures B, C, and D indicated that the testis of fish have no observable lesions. The fish egg in Figure A indicates that there were no observable lesions. Figures B, C, and D indicate moderate atrophy of follicles. However, no observable lesions were indicated in the egg of the fish in Figure E.

Table 5: Reproductive performance of female *Clarias gariepinus* (n=10) fed different inclusion levels of *C. odorata* leaf meal for 56 days.

Parameters	Control (0%)	$SWLM_2$ (15%)	$SWLM_3$ (30%)	$SWLM_4$ (45%)	$SWLM_5$ (60%)	$SWLM_6$ (90%)
Weight of female (g)	750±0.03^{d}	730±0.05^{c}	760±0.08^{e}	710±0.02^{a}	760±0.09^{e}	720±0.01^{b}
Belly diameter (mm)	11.50±0.00^{a}	12.10±0.03^{a}	11.90±0.02^{a}	11.30±0.04^{a}	11.00±0.01^{a}	11.40±0.02^{a}
Weight of ovary (g)	94.00±0.07^{d}	67.00±0.02^{b}	48.00±0.00^{a}	62.00±0.01^{b}	82.00±0.05^{c}	93.00±0.08^{d}
Weight of eggs (g)	104.34±0.50^{a}	109.53±0.90^{a}	123.01±0.98^{b}	120.76±0.90^{b}	141.76±1.00^{c}	127.24±0.60^{b}
Size of eggs (mm)	1.00±0.00^{b}	1.00±0.01^{b}	0.90±0.01^{a}	1.02±0.04^{b}	1.26±0.09^{c}	1.38±0.07^{d}
Relative fecundity	113.28±0.94^{a}	138.72±0.65^{b}	155.81±1.00^{c}	152.96±0.87^{c}	134.67±0.20^{b}	138.14±0.65^{b}
GSI	13.43±0.04^{c}	11.16±0.06^{b}	8.00±0.08^{a}	10.33±0.09^{b}	10.25±0.05^{b}	13.29±0.08^{c}
Number of eggs	79298.40±5.00^{a}	83235.20±7.20^{b}	79348.60±8.40^{a}	91777.60±6.10^{c}	107737.60±9.00^{e}	96702.40±3.23^{d}
Latency period (hr)	11.30±0.01^{a}	12.00±0.02^{a}	11.30±0.04^{a}	12.00±0.02^{a}	10.30±0.01^{a}	10.25±0.01^{a}
Hatching time (hr)	26.00±0.40^{a}	24.00±0.50^{a}	26.00±0.60^{a}	26.00±0.70^{a}	28.00±0.30^{a}	28.00±0.33^{a}
Hatchability (%)	61.84±0.70^{a}	78.95±0.64^{d}	69.08±0.35^{b}	71.32±0.28^{bc}	76.52±0.21^{cd}	65.79±0.15^{ab}
Fertilization (%)	77.00±0.51^{a}	89.00±0.32^{a}	85.79±0.62^{a}	88.42±0.54^{a}	82.50±0.67^{a}	86.58±0.98^{a}

GSI=Gonado-somatic index**,** SWLM = Siam Weed *Chromolaena odorata* leaf meal. Means of duplicate data, mean value in each row with similar superscripts are not significantly different (p>0.05).

Table 6: Intestinal morphology of male and female *Clarias gariepinus* (n=10) fed different inclusion levels of *C. odorata* leaves meal for 56 days.

	Villi height	Villi width	Crypta l depth	Cryptal width	Muscle thickness	Goblet cells	Area of Absorption	Villi Height/ Cryptal depth ratio
Control	1794.35±3.99^{c}	185.56±2.80^{a}	192.25±0.40^{c}	408.52±11.71^{bc}	265.83±7.98^{b}	21.33±2.67^{c}	332980.19±573.50^{a}	4.40±0.13^{b}
$SWLM_2$	1848.98±24.02^{d}	180.11±0.03^{a}	149.76±6.12^{a}	435.76±8.06^{cd}	279.78±6.21^{b}	1.33±0.67^{a}	333013.77±433.22^{a}	4.25±0.08^{b}
$SWLM_3$	1856.85±7.53^{d}	177.06±0.49^{a}	175.60±2.27^{b}	387.82±2.40^{b}	235.21±13.54^{a}	19.00±1.00^{c}	328773.77±743.87^{a}	4.79±0.01^{c}
$SWLM_4$	1673.91±9.00^{b}	198.73±29.45^{a}	200.13±5.82^{c}	352.79±11.92^{a}	273.18±6.61^{b}	2.67±0.67^{ab}	333157.12±572.25^{a}	4.78±0.15^{c}
$SWLM_5$	1573.18±13.49^{a}	205.63±0.96^{a}	215.34±4.92^{d}	352.93±0.81^{a}	229.02±4.60^{a}	6.67±1.33^{b}	323465.75±174.75^{a}	4.46±0.04^{bc}
$SWLM_6$	1552.50±24.07^{a}	204.42±1.07^{a}	221.19±6.04^{d}	467.01±18.51^{d}	224.64±2.24^{a}	6.67±1.67^{b}	317405.81±744.40^{a}	3.33±0.13^{a}

SWLM = Siam Weed *Chromolaena odorata* leaf meal. Means of duplicate data, mean value in each row with similar superscripts are not significantly different (p>0.05).

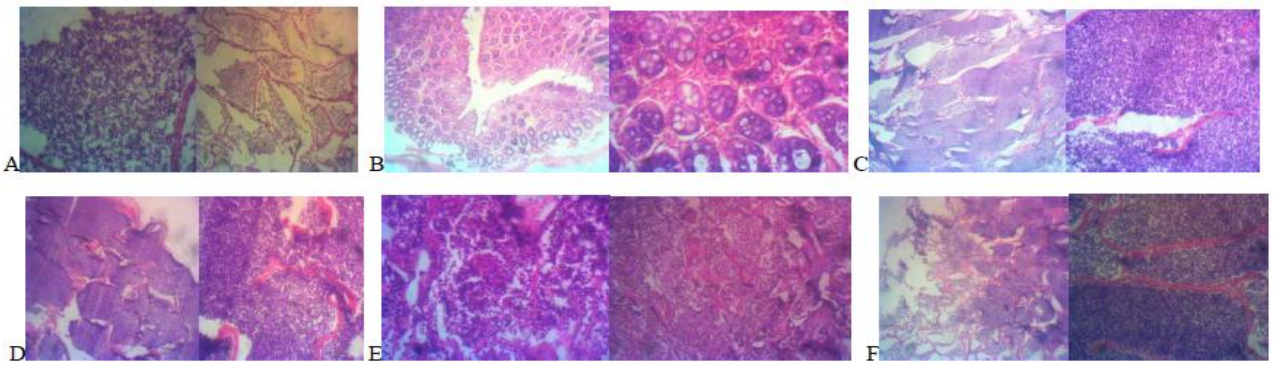

Figure 1: Transverse section of the testis of *Clarias gariepinus* broodstock fed different graded levels of *Chromolaena odorata* leaf meal. (A) Control showed that there were observable lesions. (B) Testis of *Clarias gariepinus* broodstock at 15% SWLM inclusion indicating that there are no observable lesions. (C) Testis of *Clarias gariepinus* broodstock at 30 % SWLM indicating that there is no observable lesions. (D) Testis of *Clarias gariepinus* broodstock at 45% SWLM showing that there is no observable lesion. (E) Testis of *Clarias gariepinus* broodstock at 60% SWLM indicating that there are no observable lesions. (F) Testis of *Clarias gariepinus* broodstock at 90% SWLM indicating that there is no observable lesion. [H & E × 100, 400]. SWLM = Siam Weed *Chromolaena odorata* leaf meal.

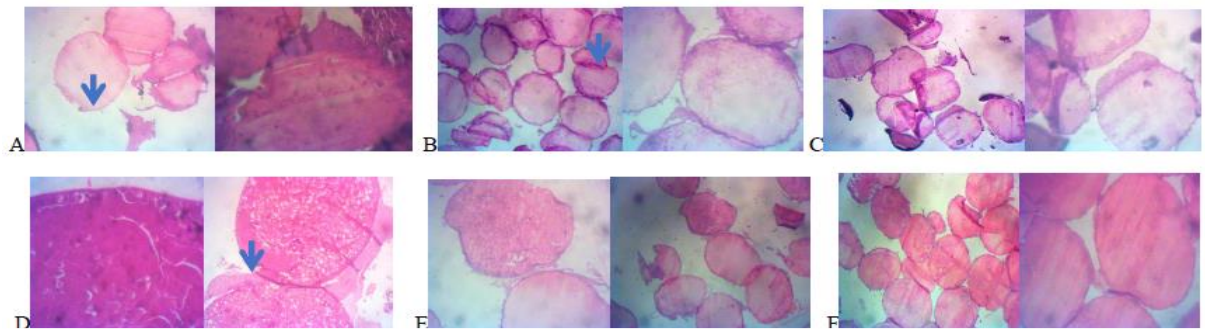

Figure 2: Transverse section of the egg of *Clarias gariepinus* broodstock fed different graded levels of *Chromolaena odorata* leaf meal. (A) Control showed that there were observable lesions and degeneration of follicles (arrows). (B) Egg of *Clarias gariepinus* broodstock at 15% SWLM inclusion indicating that there were observable lesions, moderate atrophy of follicles (arrows) (C) Egg of *Clarias gariepinus* broodstock at 30 % SWLM inclusion indicating that there is no observable lesion. (D) Egg of *Clarias gariepinus* broodstock at 45% SWLM inclusion showing that there was observable lesion, moderate atrophy of follicles (arrows) (E) Egg of *Clarias gariepinus* broodstock at 60% inclusion SWLM indicating that there is no observable lesion. (F) Egg of *Clarias gariepinus* broodstock at 90% SWLM inclusion indicating that there is no observable lesion. [H & E × 100, 400]. SWLM = Siam Weed *Chromolaena odorata* leaf meal.

Discussion

The results show an improvement in the weight of the fish among the dietary groups, but the treated groups showed greater performance when compared to the control group. This is in line with the findings of Amulejoye *et al*. (2020), who observed that *C. gariepinus* administered dietary feed with *Mangifera indica* leaf extract showed an increase in the weight of *C. gariepinus*. The survival rate among the fish fed diets containing *C. odorata* leaves meal was higher than the control diet. The results suggest that dietary *C. odorata* leaves meal in all treated groups promoted the growth of *C. gariepinus* broodstock. The increase in the weight gain of the fish fed the treated groups could be attributed to the presence

of growth-promoting constituents (saponins and flavonoids) present in the diets. These results showed that *C. odorata* leaves meal enhances nutrient utilization, which is reflected in improved weight gain, feed conversion ratio and specific growth rate. These findings are consistent with those of Adekunle *et al*. (2015), who reported improved weight gain, survival rate, and specific growth rate in *C. gariepinus* fed diets containing *C. odorata* leaf meal compared to the control. Similar results were also reported by Olusola *et al*. (2021), who recorded higher values in weight gain, percentage weight gain, specific growth rate, and feed conversion ratio in *C. gariepinus* broodstock fed with thorn apple (*Datura stramonium*) compared to the control diet. The results were also in agreement with Adeparusi *et al*. (2010) who reported an improved performance in weight gain, specific growth rate, and feed conversion ratio in *C. gariepinus* fed with *Kigelia africana* compared to the control diet. The observed improvement in the treated groups when compared to the control diet group could be as a result of the growth-promoting properties of the plants. These properties may enhance digestion and nutrient absorption, leading to increased fish weight in the treated groups.

Previous hematological studies of nutritional effects brought the knowledge that erythrocytes, packed cell volume and hemoglobin are the major and reliable indicators of various sources of stress (Rainza *et al*., 2000), and these parameters decrease in the presence of anti-nutritional factors (Osuigwe, 2007). Oyawoye and Ogunkunle (1998) pointed out that hematological components of blood are valuable in monitoring feed toxicity, especially with feed constituents that affect the formation of blood in culture fisheries. The red blood cell count (RBC), hematocrit (PCV) and hemoglobin (Hb) concentration vary with diet as well as temperature, the season of the year, and the nutritional status of the fish. The hematocrit values (PCV) in this present experiment show that there was no significant difference in the values recorded within the group when compared with the control treatment. The values observed in the treated fish were within the normal range of hematocrit (PCV) for African Catfish as reported by some researchers (Erondu *et al*., 1993; Adeyemo *et al*., 2014). Normal values usually range between 20% to 35% and rarely attain greater than 50%. A general increase was observed in hemoglobin (HB) as compared to the initial value obtained; however, there was no significant difference in hemoglobin. This was in agreement with Babale (2016), who stated that hemoglobin contents and erythrocyte counts tend to increase with the length and age of the fish. In general, there was no significant difference in neutrophils, lymphocytes, monocytes, and eosinophils between the treated groups and the control group.

The biochemical analysis can also be used to detect the health of fish (De–Pedro *et al*., 2005; Martins *et al*., 2008). In this study, the level of total protein observed in all the groups ranged from 64.00±0.09 mg/dl to 71.80±0.90 mg/dl. The treated groups performed better compared to the control in terms of total protein, albumin and globulin values. There was a significant difference in total protein, and globulin among the treated groups. Glucose is used

as an indicator of stress (Islam *et al*., 2019). This study reveals that there was a general increase in the values of glucose in the treated and untreated (control) groups. It was recorded that a higher value in glucose was obtained in the control than in the treated groups, except in treatment $SWLM_4$ (33.50 mg/dl) and SWLM 6 (33.00 mg/dl), respectively. A significantly higher value of creatinine was obtained in the treated groups. The results obtained are similar to those reported by Zapryanoval *et al*. (2021), who recorded a higher creatinine value in *C. gariepinus*. There was no significant difference in the blood urea nitrogen level of the treated groups. This could be attributed to variations in the synthetic capability of the liver and clearance by the kidneys. Total cholesterol level is associated with disease resistance in fish (Maita *et al*., 1998), thus, it is an important diagnostic tool in this species. However, there was no significant variation in the cholesterol concentrations in the treated groups and the control group. This study revealed that there was no significant difference in the value of glucose.

High fecundity values were obtained in the fish fed with dietary *C. odorata* leaves meal at $SWLM_3$ and $SWLM_4$ compared to the control. Similar results were also reported by Olusola *et al.* (2021) on the use of the medicinal herb, cattle stick (*Carpolobia lutea*) leaves as a fertility-enhancing agent for catfish *C. gariepinus*. Adeparusi *et al.* (2010) reported that catfish *C. gariepinus* broodstocks fed with *Kigelia africana* diets exhibited improved reproductive performance than those fed with the control diet. Also, Dada and Ogunduyile (2011) reported that catfish *C. gariepinus* broodstocks fed with *Mucuna prurien*-supplemented diets exhibited improved reproductive performance than those fed with the control diet. The increase in the fecundity of *C. gariepinus*, can be a result of flavonoids, glycosides, and steroids in the plant, which are potent antioxidants capable of increasing the production of estrogen, the major hormone involved in the production and maturation of eggs in the ovaries. The size of eggs was higher in $SWLM_6$ and this has an effect on the fertilization of the eggs. Some authors, however, opined that egg diameter is not a good indicator of egg and larval quality. Dada (2012) reported that fish with lower egg sizes have high fecundity therefore, the importance of egg size has been difficult to ascertain because of conflicting results from various studies and because of the problems in separating the effect of other factors such as age, strain, and nutritional status of the fish. The result of this study shows a significantly higher value in fertilization in the treated groups and there is no significant difference among the dietary groups. $SWLM_2$ revealed better hatching time compared to the other treated groups and the control. The treated groups had better values in milt volume, motility duration, sperm count, weight of eggs, size of eggs, hatchability and relative fecundity compared to the control diet. These results are in agreement with Olusola *et al.* (2021) who reported a higher performance in fertilization, hatchability, and belly diameter in the treated groups of *C. gariepinus* broodstock fed with thorn apple (*Datura stramonium*) seed compared to the control diet. Sharma *et al.* (2009) observed an increase in sperm counts of *C.*

gariepinus broodstock fed with the extract of *Anacyclus pyrethrum* compared to the control diet, which is similar to the present findings. It was observed that all the treated groups had higher values in relative fecundity, weight of male fish, size of eggs, and number of eggs compared to the control group. Also, the milt volume of the treated groups and sperm counts of the treated groups increased as the inclusion level increased, except for $SWLM_5$. A higher latency period was observed in $SWLM_2$ and $SWLM_4$ compared to the other treated groups and the control diet. It was observed in the results that all the treated groups had high relative fecundity compared to the control diet, with significant differences among the dietary groups. This report was in accord with the result of Sotoudeh *et al.* (2007) who reported a high performance in relative fecundity, gonadosomatic index, mean egg diameter, and female ovary weight in *C. gariepinus* of treated groups compared to the control.

This may be due to phytochemical constituents of the plants such as saponins, alkaloids, terpenoids, flavonoids, etc., which might have altered the biosynthetic processes underlying the growth and development of the fish and its ovaries in the treatment groups in comparison with the control. These constituents may have been responsible for the positive influence of *C. odorata* leaves meal on reproductive indices of *C. gariepinus* broodstock. The increase in milt volume and sperm counts observed for *C. gariepinus* fed with a diet supplemented with *C. odorata* leaves meal could be linked to the presence of steriods, highly effective antioxidants capable of increasing testosterone production, a key hormone involved in milt quality and production.

Based on the results of the intestinal morphology, it was observed that the area of absorption depended on the villi height and the villi width, while the ratio of the villi height to the cryptal depth was also considered. Generally, the treated groups had a better area of absorption and cryptal depth compared to the control, and this is also in agreement with the findings of Bello *et al.* (2012), who tested the effect of Walnut (*Tetracarpidium conophorun*) and Onion (*Allium cepa*) on the gut morphology of *C. gariepinus* juveniles. The results of the histopathological analysis on the transverse section of the testis of *C. gariepinus* male broodstocks fed with different inclusion levels of *C. odorata* leaves meal showed no indication of any observable lesion which indicated that *C. odorata* leaves had no negative impact on the milt of the fish. The transverse section of the egg of *Clarias gariepinus* female broodstocks fed with different inclusion levels of *C. odorata* leaf meal revealed that there was no indication of any observable lesion. However, moderate atrophy of follicles was observed in eggs of *C. gariepinus* fed with *C. odorata* leaves, which agrees with the report of Amulejoye *et al*. (2020) that dietary *M. indica* leaf extract had a positive impact on the eggs of *C. gariepinus*

Conclusion

It is therefore concluded that *C. odorata* leaves meal has a promising effect on the fertility of *Clarias gariepinus* and could be used as organic growth and pro-fertility agents instead of using synthetic drugs that

are expensive and have residual side effects on the host. The inclusion of *C. odorata* leaves meal could enhance the growth performance, hematological indices, and reproductive indices in *C. gariepinus* broodstock. Viable sperm and eggs are essential components of any successful animal production operation and the success of the reproduction process is dependent on a supply of high-quality gametes.

Conflicts of interest

The authors declare that no conflict of interest.

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