



GROWTH, REPRODUCTIVE PERFORMANCE, HEALTH STATUS AND OVARIAN HISTOLOGY OF FEMALE *Clarias gariepinus* BROODSTOCK FED HERBAL MIXTURE SUPPLEMENTED DIETS

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Abstract

This study was conducted to assess the efficacy of the Combination of Medicinal Herbs (CMH) {maca root, *Lepidium meyenii*; Snot apple, *Azanza garckeana*; tiger nut, *Cyperus esculentus*; date palm, *Phoenix dactylifera*; thorn apple, *Datura stramonium*} on growth, blood profiles, reproductive performance and ovary histology of female *Clarias gariepinus* broodstock. One hundred and forty (140) *C. gariepinus* with a mean weight of $650.00 \pm 0.01\text{g}$ were randomly allotted to seven treatments at different inclusion levels; control (0), CMH2 (0.5%), CMH3 (1.0%), CMH4 (1.5%), CMH5 (2.0%), CMH6 (2.5%) and CMH7 (3.0% inclusion). Each treatment was stocked with 10 *C. gariepinus* per treatment, replicated twice and fed at 5% body weight for 56 days. Growth parameters, blood profiles, reproductive indices and ovary histology were taken using standard methods. The results of growth performance revealed significantly higher ($p < 0.05$) values in the treated groups compared to the control. The blood profiles show no significant difference ($p > 0.05$) in packed cell volume, haemoglobin, total protein, alkaline phosphatase, and neutrophils while significant differences ($p < 0.05$) were observed in creatinine, lymphocytes, among the dietary groups. Relative fecundity, and hatchability were significantly higher ($P < 0.05$) in the treated groups compared to the control. The ovary histology revealed that no abnormal changes were observed in the treated groups and the control. It was concluded that a combination of these medicinal herbs could be used in the diet of *C. gariepinus* to enhance weight gain, and blood profiles, and act as a bio-fertility agent in aquaculture.

Keywords: *Clarias gariepinus*; Medicinal herbs; Growth; Reproductive performance; Blood profiles; Intestinal morphology; Ovarian histology.

Introduction

Fish and its numerous products play a vital role in food security and in meeting the nutritional needs of the human population both in developing and developed countries (Food and Agriculture Organisation FAO, 2014). It is a good source of high-value protein as well as an essential source of

micronutrients including vitamins, minerals and polyunsaturated omega-3 fatty acids (FAO, 2012). The annual average global per capita consumption of fish increased from 9.9 kg to 17 kg between the years 1960 and 2000 further expanded to 18.9 kg in 2010 (FAO, 2014). In the same year, fish was the major source of protein providing the global

population with 16.7 per cent of animal protein intake and 6.5 per cent of all the protein consumed (FAO, 2014). This shows the huge impact and contribution of Aquaculture to sustaining the human population. Therefore, aquaculture is widely acknowledged as a key means to meeting increased future food requirements for the human population (Ibrahim *et al.*, 2010).

High demand for fish has necessitated the development of fish seed production which has been identified as a rational way of augmenting the dwindling fish supply from the capture fisheries (Dada and Fagbenro, 2008). Several studies have revealed that medicinal plants can influence fertility in humans, animals and fish (Dada and Fagbenro, 2008). Also, it has been reported that medicinal plants possess anti-inflammatory, anti-microbial, anti-diabetic and antiviral properties (Adedeji *et al.*, 2006), as well as pro-fertility effects in fish which could help enhance sperm and egg characteristics in *Clarias gariepinus* (Amulejoye *et al.*, (2025); Dada and Ajilore, 2009). There has not been any documented report on the effect of the combination of medicinal plants on indigenous fish species such as *C. gariepinus*. This study was therefore carried out to assess the effect of the combination of medicinal plants on the growth performance, blood profiles, reproductive indices and intestinal morphology of female *C. gariepinus* broodstock.

Materials and Methods

Study Location

This study was conducted at the Department of Fisheries and Aquaculture Technology Teaching and Research Farm, Olusegun Agagu University of Science and Technology, Okitipupa.

Plant Collection and Identification

Maca root (*Lepidium meyenii*) and Snot apple (*Azanza garckeana*) were purchased

in Jigawa State, Nigeria while tiger nut (*Cyperus esculentus*) date palm (*Phoenix dactylifera*), thorn apple (*Datura stramonium*) were obtained from a known market in Ibadan, Nigeria and were authenticated at Herbarium unit of Olusegun Agagu University of Science and Technology, Okitipupa, where a voucher specie was deposited for future reference.

Preparation of plant materials

The combination of medicinal herbs {maca root, *Lepidium meyenii*; Snot apple, *Azanza garckeana*; tiger nut, *Cyperus esculentus*; date palm, *Phoenix dactylifera*; thorn apple, *Datura stramonium*} was air-dried for four weeks after which it was ground into a fine powder, the combination of medicinal herbs was thoroughly mixed in a ratio of 0.5: 1: 1:0.5:0.5 on a dry *w/w* basis, respectively, decided based on previous findings and was stored at room temperature until required.

Collection and Acclimatization of Experimental Fish

One hundred and forty female *C. gariepinus* (mean weight 650 ± 0.01 g) were procured from a known farm in Irele, Ondo State, Nigeria. The broodstock was acclimatized for two weeks in a fibre holding tank at the Research and Teaching Farm, Olusegun Agagu University of Science and Technology, Okitipupa. During this period, they were fed commercial diets (blue crown, 6 mm) of 40% crude protein twice daily at 5 % body weight.

Experimental Diets and Set up

The ground combination of medicinal herbs at a different inclusion level of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % were added to feed ingredients such as fishmeal, soybean, groundnut cake, yellow maize, wheat offal, Di-calcium phosphate, salt, vitamin premix, vegetable oil, lysine and methionine to formulate a 40% crude protein diet. Each diet mixture treated separately was extruded through a 6 mm mincer pelleting machine to form a noodle-like suitable size for the adult

C. gariepinus. The pelleted diets were sun-dried, packed in labelled polythene bags and stored until required (Table 1). Water was sourced from the Research and Teaching Farm borehole and 14 fibre holding tanks were filled with water to a depth of 1.2 m each. One hundred and forty *C. gariepinus* were stocked into the 14 fibre holding tanks containing ten (10) female *C. gariepinus* per treatment tank. The diets were assigned to

the tanks as designated and were fed at 5 % 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 fibre holding tank every two weeks 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 reproductive indices of female *C. gariepinus* were determined.

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

INGREDIENTS	CONTROL	CMH2 (0.5%)	CMH 3 (1.0%)	CMH 4 (1.5%)	CMH 5 (2.0%)	CMH 6 (2.5%)	CMH 7 (3.0%)
Fishmeal	13.43	13.43	13.43	13.43	13.43	13.43	13.43
Soya bean	26.86	26.86	26.86	26.86	26.86	26.86	26.86
Groundnut cake	26.86	26.86	26.86	26.86	26.86	26.86	26.86
Yellow maize	12.93	12.93	12.93	12.93	12.93	12.93	12.93
Wheat offal	12.93	12.93	12.93	12.93	12.93	12.93	12.93
Starch	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DCP	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vit. Premix	3.00	2.50	2.00	1.50	1.00	0.50	-
Vegetable oil	0.50	0.50	0.50	0.50	0.50	0.50	0.5
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.5
Lysine	0.50	0.50	0.50	0.50	0.50	0.50	0.5
Methionine	0.50	0.50	0.50	0.50	0.50	0.50	0.5
Combination of medicinal herbs	-	0.50	1.00	1.50	2.00	2.50	3.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

*vitamin - minerals premix. Each 2.0 kg of premix contain; 12.5 million international units (MIU); D₃, 2.5 MIU; E, 40g; K₃ 2g; B1,5.5g; BB6,5g; Niacin 55g; Calcium Pantothenate 11.5g; Chlorine chloride 500g; Folic acid, Biotin,0.08g; Manganese, 120g; Iron, 100g; Zinc, 80g, Copper,8.5g; Iodine, 1.5g; Cobalt,0.3g; Selenium, 0.12g; Anti-oxidant, 120g. CMH = Combination Medicinal Herbs

Biological Evaluation

The growth performance and nutrient utilization indices were calculated as described by Olaifa and Bello, (2011). Weight gain = Final body weight – initial body weight

Weight gain (%) =

$$\frac{(\text{Final body weight} - \text{initial body weight}) \times 100}{\text{Initial body weight}}$$

Increase in standard length (CM) = L₂ – L₁

Where L₂ = Final standard length; L₁ = Initial standard length

Specific growth rate (SGR) =

$$\frac{100 (\log \text{final body weight} - \log \text{initial body weight})}{\text{Time (days)}}$$

Feed conversion ratio (FCR) =

Dry weight of feed fed (g)

Fish weight gain (g)

Survival rate (%) =

$$\frac{\text{Initial Number of Fish Stocked} - \text{Mortality}}{\text{Initial Number of Fish Stocked}} \times 100$$

The initial Number of Fish stocked

Condition factor (k) = 100W/L³

Where: W= weight of fish (g); L=standard length (cm)

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

Where a = Initial mean weight of fish; b= final mean weight of fish; h= experimental periods in days.

Evaluation of Reproductive Indices

At the end of the feeding trials, 2 females were randomly selected per dietary treatment for

reproductive indices such as egg size estimation, relative fecundity, percentage fertilization, percentage hatchability, gonadal-somatic index (GSI), the weight of ovary and belly diameter were calculated as described by Olusola *et al.*, (2021), Francis *et al.*, (2013) and Dada, (2012).

Artificial Propagation

Two (2) female spawners from each treatment were taken and used for artificial propagation techniques- hormone administration, stripping, fertilization and incubation as described by Olusola *et al.*, (2022).

Statistical Analysis

One-way Analysis of variance (ANOVA) and descriptive statistics was performed using SPSS 22 for the window software package at ($p < 0.05$) significance level. Significant means were subjected to multiple comparisons at a 0.05 level.

Results

Growth performance and nutrient utilization of *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

The highest values of weight gain were recorded in CHM₄ and CHM₇ (530.00g) while the lowest was recorded in control (330.00g), and the highest percentage weight gain (81.54%) was recorded in CHM₄ and CHM₇ respectively. The Specific growth rate was highest in CHM₄ and CHM₇ (0.46%) and the lowest value was recorded in the control (0.32%). the highest nitrogen metabolism (28130.76) was recorded in CHM₄ and CHM₇ respectively and the lowest was recorded in the control (25056.36). no mortality was recorded among the dietary groups for 56 days of feeding trials and the value of the production performance index was highest in CHM₄ and CHM₇ (946.36) respectively (Table 2).

Table 2: Growth performance and nutrient utilization of female *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

Parameters	CONTROL	CMH2 (0.5%)	CMH 3 (1.0%)	CMH 4 (1.5%)	CMH 5 (2.0%)	CMH 6 (2.5%)	CMH 7 (3.0%)
IBW	650±0.01 ^a	650±0.02 ^a	650±0.02 ^a	650±0.01 ^a	650±0.02 ^a	650±0.01 ^a	650±0.01 ^a
FBW	980.00±1.05 ^a	1100.00±0.89 ^b	1160.00±0.80 ^{bc}	1180.00±2.50 ^{bc}	1016.67±0.91 ^a	1160.00±1.29 ^{bc}	1180.00±3.21 ^c
WG	330.00±2.83 ^a	450.00±2.83 ^c	510.00±2.83 ^d	530.00±2.83 ^e	366.00±2.83 ^b	510.00±2.83 ^d	530.00±2.83 ^e
PWG	50.77±2.83 ^a	69.23±2.83 ^b	78.46±2.83 ^{bc}	81.54±2.83 ^c	56.31±2.83 ^a	78.46±2.83 ^{bc}	81.54±2.83 ^c
SGR	0.32±0.03 ^a	0.41±0.03 ^{ab}	0.45±0.03 ^b	0.46±0.03 ^b	0.35±0.03 ^{ab}	0.45±0.03 ^b	0.46±0.03 ^b
SR	100.00±2.83 ^a	100.00±2.83 ^a	100.00±2.83 ^a	100.00±2.83 ^a	100.00±2.83 ^a	100.00±2.83 ^a	100.00±2.83 ^a
NM	25056.36±0.03 ^a	26901.00±2.83 ^c	27822.32±2.82 ^d	28130.76±2.83 ^e	25609.75±0.03 ^b	27823.32±2.83 ^d	28130.76±2.83 ^e
FCR	0.89±0.01 ^c	0.71±0.00 ^d	0.60±0.00 ^a	0.62±0.00 ^{ab}	0.88±0.01 ^e	0.65±0.01 ^c	0.64±0.01 ^c
PPI	589.36±21.72 ^a	803.50±17.68 ^b	910.65±20.71 ^c	946.36±21.72 ^c	653.50±13.44 ^a	910.65±20.72 ^c	946.36±21.72 ^c

CMH = Combination Medicinal Herbs, IBW= Initial body weight, FBW = Final body weight, WG = Weight gain, PWG = Percentage weight gain, SGR = Specific growth rate, FCR = Feed conversion rate, SR = Survival rate, NM = Nitrogen metabolism, PPI = Production performance index, mean with same superscript along the row are not significantly different at $p > 0.05$

Reproductive performance of female *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

Reproductive performance was better in the treated groups compared to the control. The value of belly diameter, latency period and fertilization show no significant difference ($p > 0.05$) among the dietary groups while significant differences were recorded in the

weight of ovary, size of eggs, relative fecundity, number of eggs, GSI, weight of eggs, and hatchability among the dietary groups. The treated groups performed better than the control in hatchability, fertilization, relative fecundity and belly diameter. Although, no significant difference ($p > 0.05$) was observed in the belly diameter and fertilization but significant difference ($p < 0.05$) was observed

in hatchability and relative fecundity among the dietary groups (Table 3).

Table 3: Reproductive performance of male *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

Parameters	CONTROL	CMH2 (0.5%)	CMH 3 (1.0%)	CMH 4 (1.5%)	CMH 5 (2.0%)	CMH 6 (2.5%)	CMH 7 (3.0%)
Belly diameter	12.50±2.83 ^a	12.25±2.83 ^a	13.00±2.83 ^a	13.00±2.83 ^a	13.00±0.00 ^a	13.10±2.83 ^a	12.50±2.83 ^a
Weight of ovary	123.85±2.83 ^d	118.74±2.83 ^{cd}	73.28±2.83 ^a	118.81±2.83 ^{cd}	145.11±2.83 ^c	99.68±2.83 ^b	110.12±2.83 ^{bc}
Size of eggs	1.12±2.83 ^a	1.13±0.18 ^a	1.00±0.00 ^a	1.00±0.00 ^a	1.45±0.01 ^a	1.38±0.18 ^a	1.00±0.00 ^a
R. fecundity	113.68±2.83 ^a	113.06±2.83 ^a	137.57±2.83 ^{bc}	149.71±2.83 ^d	185.80±2.83 ^c	140.54±2.83 ^{cd}	126.66±2.83 ^b
GSI	0.14±0.03 ^a	0.12±0.03 ^a	0.90±0.03 ^b	0.17±0.03 ^a	0.21±0.03 ^a	0.13±0.03 ^a	0.14±0.03 ^a
Number of eggs	102310.56±0.03 ^c	101756.16±0.03 ^b	110056.32±0.03 ^c	103300.56±0.03 ^d	130062.24±0.03 ^f	112432.32±0.00 ^g	101308.48±0.03 ^a
Latency period	12.15±2.83 ^a	11.55±2.83 ^a	11.55±2.83 ^a	12.15±2.83 ^a	11.35±0.03 ^a	12.15±2.83 ^a	11.35±0.03 ^a
Weight of eggs	129.18±2.83 ^a	128.48±2.83 ^a	138.96±2.83 ^{ab}	130.43±2.83 ^a	164.22±2.83 ^c	141.96±2.83 ^b	127.94±2.83 ^a
Hatching time	25.00±0.00 ^a	24.35±2.83 ^a	24.50±2.83 ^a	24.25±2.83 ^a	24.15±2.83 ^a	24.40±0.00 ^a	24.58±2.83 ^a
Hatchability	60.29±0.03 ^a	93.75±0.03 ^d	81.76±0.03 ^c	93.75±2.83 ^d	92.80±2.83 ^d	65.03±2.83 ^{ab}	68.50±0.03 ^b
Fertilization	82.55±6.82 ^a	93.28±7.01 ^a	94.17±7.01 ^a	94.44±7.33 ^a	93.71±7.78 ^a	94.44±7.33 ^a	92.55±7.33 ^a

CMH = Combination Medicinal Herbs, mean with the same superscript along the row is not significantly different at $p>0.05$

Blood profiles of female *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

The results of haematology show variation of the parameters assayed, the values obtained at the end of the study were relatively similar among the dietary groups

and the values obtained before the commencement of the study. There were no significant differences ($p > 0.05$) among the dietary groups of the parameter assayed except creatinine, lymphocytes, monocytes and eosinophils (Table 4)

Table 4: Mean blood profiles of female *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

Parameters	Before	CONTROL	CMH2 (0.5%)	CMH 3 (1.0%)	CMH 4 (1.5%)	CMH 5 (2.0%)	CMH 6 (2.5%)	CMH 7 (3.0%)
PCV	34.00±2.83 ^a	35.00±2.83 ^a	36.00±2.83 ^a	33.00±2.83 ^a	34.00±2.83 ^a	32.00±2.83 ^a	33.00±2.83 ^a	35.00±2.83 ^a
Hb	11.30±2.83 ^a	11.70±2.83 ^a	12.00±2.83 ^a	11.00±2.83 ^a	11.30±2.83 ^a	10.70±2.83 ^a	11.00±2.83 ^a	11.70±2.83 ^a
Glucose	5.10±2.83 ^a	4.20±2.83 ^a	4.40±2.83 ^a	4.70±2.83 ^a	4.90±2.83 ^a	5.00±2.83 ^a	4.30±2.83 ^a	4.80±2.83 ^a
Sodium	131.70±2.83 ^a	130.80±2.83 ^a	130.70±2.83 ^a	134.00±2.83 ^a	129.60±2.83 ^a	130.80±2.83 ^a	133.40±2.83 ^a	130.30±2.83 ^a
Potassium	3.80±2.83 ^a	3.40±2.83 ^a	3.70±2.83 ^a	4.00±2.83 ^a	3.90±2.83 ^a	3.90±2.83 ^a	3.70±2.83 ^a	3.60±2.83 ^a
Chloride	97.00±2.83 ^a	100.00±2.83 ^a	95.00±2.83 ^a	97.00±2.83 ^a	98.00±2.83 ^a	95.00±2.83 ^a	99.00±2.83 ^a	101.00±2.83 ^a
Albumin	39.00±2.83 ^a	41.00±2.83 ^a	39.00±2.83 ^a	38.00±2.83 ^a	41.00±2.83 ^a	37.00±2.83 ^a	38.00±2.83 ^a	40.00±2.83 ^a
Total Protein	79.00±2.83 ^a	80.00±2.83 ^a	74.00±2.83 ^a	79.00±2.83 ^a	78.00±2.83 ^a	77.00±2.83 ^a	75.00±2.83 ^a	78.00±2.83 ^a
Globulin	40.00±0.00 ^a	39.00±0.00 ^a	41.00±0.00 ^a	35.00±0.00 ^a	37.00±0.00 ^a	40.00±0.00 ^a	37.00±0.00 ^a	38.00±0.00 ^a
Urea	4.60±2.83 ^a	3.80±2.83 ^a	3.30±2.83 ^a	2.80±2.83 ^a	3.60±2.83 ^a	4.20±2.83 ^a	3.70±2.83 ^a	3.80±2.83 ^a
Creatinine	65.10±2.83 ^{ab}	66.10±2.83 ^{ab}	66.40±2.83 ^{ab}	65.30±2.83 ^{ab}	60.50±2.83 ^a	71.00±2.83 ^b	68.30±2.83 ^b	69.80±2.83 ^b
Calcium	2.30±0.28 ^a	2.10±0.28 ^a	2.10±0.28 ^a	2.35±0.35 ^a	2.00±0.00 ^a	2.20±0.28 ^a	2.30±0.28 ^a	2.20±0.28 ^a
Alkaline	18.60±2.83 ^a	19.70±2.83 ^a	17.40±2.83 ^a	18.00±2.83 ^a	19.30±2.83 ^a	18.90±2.83 ^a	22.00±1.41 ^a	15.90±2.83 ^a
Phosphates	3.60±2.83 ^a	3.80±2.83 ^a	4.30±2.83 ^a	3.90±2.83 ^a	3.70±2.83 ^a	4.50±2.83 ^a	4.60±2.83 ^a	3.90±2.83 ^a
Cholesterol	1.20±0.28 ^a	0.90±0.28 ^a	1.00±0.28 ^a	1.10±0.28 ^a	1.10±0.28 ^a	1.05±0.21 ^a	0.90±0.28 ^a	0.80±0.28 ^a
Triglycerides	30.00±2.83 ^{ab}	33.00±2.83 ^{ab}	31.00±1.41 ^{ab}	30.00±2.83 ^{ab}	35.00±2.83 ^b	32.00±2.83 ^{ab}	28.00±2.83 ^a	33.00±2.80 ^{ab}
Lymphocytes	70.00±2.83 ^a	63.00±2.83 ^a	70.00±2.83 ^a	68.00±2.83 ^a	65.00±2.83 ^a	66.00±2.83 ^a	70.00±2.83 ^a	67.00±0.00 ^a
Neutrophil	0.00±0.00 ^a	3.00±2.83 ^a	0.00±0.00 ^a	2.00±0.00 ^a	0.00±0.00 ^a	2.00±0.28 ^a	2.00±0.00 ^a	0.00±0.00 ^a
Mono	0.00±0.00 ^a	1.00±0.28 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.00±0.71 ^a	0.00±0.00 ^a	0.00±0.00 ^a
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CMH = Combination Medicinal Herbs, mean with the same superscript along the row is not significantly different at $p>0.05$

Intestinal morphology of female *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

The result of the intestinal morphology revealed that the villi width, cryptal depth, area of absorption, goblet cells and villi height

/ cryptal depth ratio were significantly among the dietary groups (Table 5)

higher ($p > 0.05$) in the treated groups

Egg histology of *Clarias gariepinus* fed a

Table 5: Mean intestinal morphology of female *Clarias gariepinus* fed a combination of medicinal herbs for 56 days

	Villi height	Villi width	Cryptal depth	Cryptal width	Muscle thickness	Goblet cell	Area of absorption	Villi height / cryptal depth ratio
Control	15443.82±23.29 ^c	155.87±21.14 ^a	371.89±20.25 ^d	142.29±12.83 ^a	190.70±7.00 ^a	4.33±2.67 ^a	241705.27±334.05 ^a	4.21±0.32 ^{at}
CMH2 (0.5%)	1163.06±21.40 ^a	205.58±2.31 ^b	312.26±6.57 ^b	210.78±0.34 ^c	211.02±0.99 ^b	6.67±0.66 ^a	239028.82±363.85 ^a	3.73±0.13 ^a
CMH3 (1.0%)	1247.93±5.92 ^b	202.63±0.96 ^b	253.22±1.30 ^a	204.31±0.58 ^c	247.28±10.73 ^{cd}	6.67±2.67 ^a	252873.83±206.33 ^a	4.96±0.28 ^c
CMH4 (1.5%)	1960.14±22.42 ^c	203.12±0.26 ^b	344.71±3.11 ^{bc}	206.0.5±1.01 ^c	238.25±3.36 ^c	14.00±1.00 ^{bc}	398160.53±503.65 ^c	5.69±0.01 ^d
CMH5 (2.0%)	1615.88±2.42 ^d	183.91±1.40 ^b	332.82±5.64 ^b	187.51±1.98 ^b	253.43±7.26 ^{cde}	19.00±2.00 ^d	297165.21±186.28 ^b	4.86±0.09 ^c
CMH6 (2.5%)	1975.20±6.90 ^e	187.29±0.81 ^b	389.25±1.64 ^d	185.62±4.53 ^b	267.28±5.04 ^e	17.33±0.67 ^{cd}	369938.65±285.66 ^c	5.07±0.01 ^c
CMH7 (3.0%)	1645.21±1.67 ^d	185.65±1.09 ^b	349.21±2.37 ^{bc}	187.57±1.01 ^b	262.98±2.48 ^{de}	11.33±0.67 ^b	305437.34±170.10 ^b	4.71±0.03 ^{bc}

CMH = Combination Medicinal Herbs, mean with the same superscript along the row is not significantly different at $p > 0.05$

combination of medicinal herbs for 56 days

There were abnormal changes in the eggs of the control group and no abnormal changes

were recorded in all the treated groups fed a combination of medicinal herbs. The results of histopathological analysis are shown in the photomicrograph (plate 1A – G).

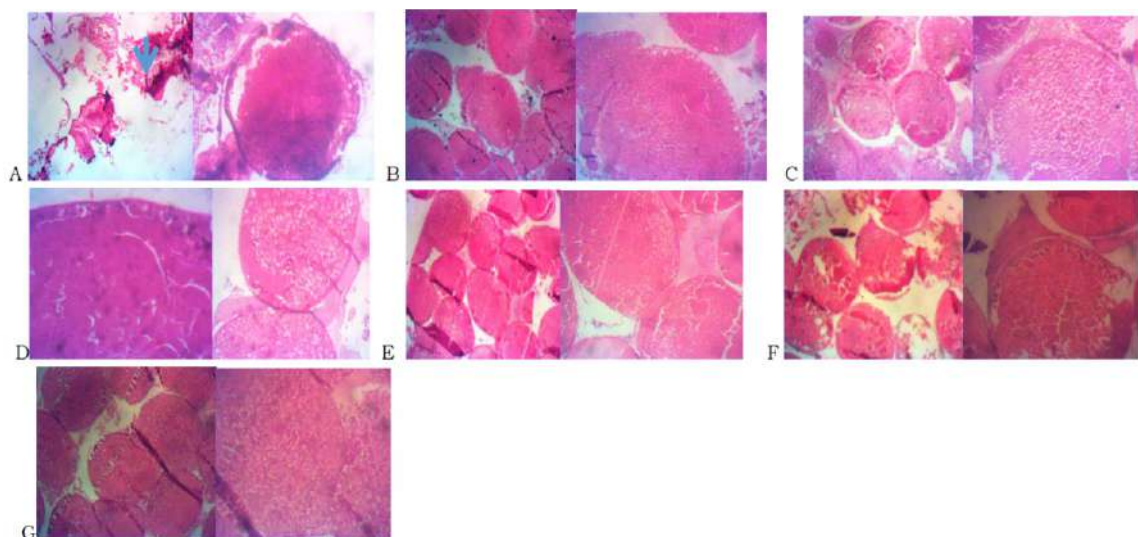


Plate 1 (A – G): Transverse section of the egg of *Clarias gariepinus* broodstock fed different graded levels of the combination of medicinal herbs. **(A)** Control showed that there were observable lesions and degeneration of follicles (arrows). **(B)** Egg of egg of *Clarias gariepinus* broodstock at 0.5% inclusion indicating that there were no observable lesions. **(C)** Egg of egg of *Clarias gariepinus* broodstock at 1.0 % indicating that there is no observable lesion. **(D)** Egg of egg of *Clarias gariepinus* broodstock at 1.5% showing that there is no observable lesion. **(E)** Egg of *Clarias gariepinus* broodstock at 2.0% indicating that there is no observable lesion. **(F)** Egg of egg of *Clarias gariepinus* broodstock at 2.5% indicating that there is no observable lesion. **(G)** Egg of egg of *Clarias gariepinus* broodstock at 3.0% inclusion indicating that there is no observable lesion [H & E x 100, 400].

Discussion

A general increase in weight gain was observed in *C. gariepinus* fed graded levels of the herbal mixture when compared to the value obtained at the commencement of the study. The weight gain, percentage weight gain, specific growth rate, feed conversion ratio, nitrogen metabolism and production performance index were significantly higher ($p < 0.05$) in the treated groups when compared with the control. No mortality was recorded across the dietary groups, there was no significant difference ($p > 0.05$) among the dietary groups. This study accords with the report of Fawole *et al.*, (2022) who observed better weight gain, specific growth rate and feed conversion ratio in the *C. gariepinus* fed combination of medicinal herbs compared to the control.

The reproductive indices such as weight of ovary, relative fecundity, gonadal somatic index, number of eggs, the weight of eggs and hatchability were significantly higher ($p < 0.05$) in the treated groups when compared to the control. Belly diameter, hatching time and fertilization were not significantly different ($p > 0.05$) among the dietary groups. Among the treated groups, HM₅ performed better than the other treated groups. The reason for this performance could be attributed to the presence of metabolites in the plants (saponins, flavonoids and alkaloids). This result is agreed with the work of Olusola *et al.*, (2023) who reported better weight of ovary, relative fecundity, gonadal somatic index, number of eggs, the weight of eggs and hatchability in *C. gariepinus* fed medicinal herbs supplemented diets. This result was in accord with the work of Francis *et al.*, (2013) who reported better performance in egg size, fertilization and hatchability in *Heterobranchus bidosalis* fed *Vernonia amygdalina* compared to the control. Also, Dada and Adeparusi (2012) reported better

values of weight of ovary, relative fecundity, gonadal somatic index, number of eggs, the weight of eggs and hatchability in *C. gariepinus* fed two medicinal plants supplemented diets. However, this report was in agreement with the result of Ekpo *et al.*, (2017) who reported a lower performance in gonad somatic index and weight of ovary in *C. gariepinus* of treated groups compared to the control.

The results of blood profiles and seminal fluid of *C. gariepinus* fed different levels of herbal mixture revealed that the treated groups had higher values of packed cell volume except CMH₂, CMH₃ and CMH₅ when compared to the control. The value of haemoglobin was higher in the treated groups although no significant difference ($p > 0.05$) was observed in the packed cell volume and haemoglobin among the dietary groups. This result was similar to the work of Zaid *et al.*, (2020) who reported higher values of haemoglobin in *C. gariepinus* fed herbal mixture supplemented diets compared to the control. The value of total protein, globulin and albumin were higher ($p > 0.05$) in the treated groups when compared to the control. This study recorded a lower value of globulin in the treated groups compared to the control. An increase in the globulin in control could be an indication of chronic infections, liver damage and kidney dysfunctions. The result of packed cell volume, haemoglobin, glucose, albumin, total protein, alkaline phosphatase, globulin, and cholesterol of *Clarias gariepinus* fed a combination of medicinal herbs supplemented diets were not significantly different ($p > 0.05$) and influenced by the treatment diets, this could be ascribed to the quality of the experimented diets. The result of the lymphocytes and monocytes were higher in the control when compared to the treated groups, an indication that the fish in the treated groups had better immune responses to the foreign bodies and the higher

value of neutrophils showed better non-specific immune response of *C. gariepinus* against the control, no significant difference ($p > 0.05$) was observed among the dietary groups. The increase in neutrophils and reduction in lymphocytes could be due to the presence of some bioactive phenolic compounds in the combination of medicinal herbs that showed antimicrobial and growth-promoting activities. This study agrees with the work of Fawole *et al.*, (2022) who reported better value of neutrophils and lymphocytes in *C. gariepinus* fed polyherbal mixture supplemented diets compared to the control.

The values of cholesterol, triglycerides, and seminal fluids such as sodium, potassium, chloride and calcium were relatively similar ($p > 0.05$) among the dietary groups. Blood glucose is a pointer to energy status and the changes in the glucose could be affected by physiological conditions. The values of glucose recorded in the study were higher in the treated groups when compared to the control. A higher value of glucose in the treated groups could be attributed to higher available energy present in the blood. This is similar to the report of Fawole *et al.*, (2022) who reported higher values of glucose in *C. gariepinus* fed polyherbal mixture supplemented diets. Also, the result of alkaline phosphatase shows that the treated groups recorded a lower value of alkaline phosphatase against the control, which was an indication of better liver functions and there was no significant difference ($p > 0.05$) among the dietary groups. The value of creatinine was higher in the treated groups when compared to the control except CMH₄ and there was no significant difference ($p > 0.05$) among the dietary groups. Urea recorded in this study shows no significant difference ($p > 0.05$) among the dietary groups, this shows that the protein quality

was good and this value was lower than the value obtained before the commencement of the study. The higher value recorded was an indication of poor-quality feed. The cholesterol values recorded indicate that treatment was not significantly different ($p > 0.05$) which revealed adequate metabolized and utilization of diets in the tissues.

The results of intestinal morphology revealed that the villi height and villi width were significantly higher ($p < 0.05$) in the treated groups compared to the control. Muscular thickness and area of absorption were significantly higher ($p < 0.05$) in the treated groups when compared to the control. The higher values of area of absorption recorded in the treated groups were an indication of better utilization of the fish diet that resulted in better absorption of feed, which was a supportive function of what was recorded in the growth performance, that the treated groups had better weight gain, specific growth rate, and production performance index. The reason for better area of absorption may be as a result of phytochemical constituents present in these plants (flavonoids and saponins) which stimulate epithelial cell proliferation and therefore contribute to the maturation and integrity of the intestinal (gut) structure. The value of goblet cells was higher in the treated groups when compared to the control and there was a significant difference ($p < 0.05$) among the dietary groups. This report agrees with the work of Bello *et al.* (2012) who reported higher values in cryptal depth, villi length and villi width of the treated groups than the control of *C. gariepinus*-fed onion bulb and walnut leaves-based diets. This study was similar to the report of Olusola *et al.*, (2022) who observed higher values of villi height, villi width, cryptal width, muscular thickness and goblets cells in the treated groups of *C. gariepinus* fed cinnamon bark supplemented diets. Also, this study was in accord with the report of Fawole *et al.*,

(2022) who reported higher values of area of absorption, muscular thickness and villi height/ cryptal depth ratio in *C. gariepinus* fed polyherbal mixture supplemented diets. The result of the histology revealed that no abnormal changes were observed in the treated groups and the control. The treated groups indicated that oocyte vitellogenesis was more developed and the follicle walls among the treatment groups were normal (plate A –G). This results correlates increased fecundity or hatchability of the treated groups compared to the control and there was a significant difference ($p < 0.05$) among the dietary groups. The reason for this might be as a result of these plants that contain inherent bioactive substances such as sterols, flavonoids, and saponins, coupled with vital nutrients that contribute to their benefits. This study accord with Sepehrfar *et al.*, (2023) who reported that most plants contain phytochemicals, which are natural bioactive compounds that can potentially enhance sexual development and reproductive performance in aquaculture species

Conclusion

The results of this study indicated that the supplementation of the herbal mixture had no negative influences on the growth performance, reproductive indices, blood profiles and ovary histology of *C. gariepinus* broodstock. It is therefore concluded that a combination of medicinal herbs could be used in the diet of *C. gariepinus* to enhance growth as well as bio-fertility agents to enhance productivity in fish farming to boost fish seed production.

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