The Effect of variegated grasshopper meal (*Zonocerous variegatus*) on Growth, Nutrient utilization and Gene expression study in juvenile *Clarias gariepinus*(Burchell, 1822)

ABSTRACT

Over the years there has been research on how to improve fish yield. This study investigated the effects of variegated grasshopper meal (*Zonocerous variegatus*) on growth performance, nutrient utilization, and gene expressions in juvenile *Clarias gariepinus* (African catfish). The variegated grasshopper meal diets (VGDMs) were incorporated with the other feed ingredients at 25% (VGDM1), 50% (VGDM2), 75% (VGDM2), and 100% (VGDM3) diets while the remaining group served as control. Growth performance, nutrient utilization, and gene expression study were evaluated. The mRNA expression of Growth hormone (GH), Heat Shock Protein (HSP70), Melatonin (MEL 1C), tumour necrosis factor-alpha (TNF-α), and interleukin 1 beta (IL-1β) were investigated in the liver and muscle of *C.gariepinus* fed with varying doses of grasshopper meals (VGDMs) using reverse transcriptase–polymerase chain reaction. There were significant ($p>0.05$) variations in all parameters in all the treatments. There was a significant upregulation ($p<0.05$) in the gene expression of GH, HSP70, MEL 1C, and TNF-α in VGDM1 compared to control in both tissue groups. In addition, there was significant downregulation ($p<0.05$) in interleukin 1 (IL-1β) gene expression in liver VGDM2. This study suggests that dietary variations involving VGMD have the potential to boost growthand improve the immune responses of this fish species.

Keywords: *Clarias gariepinus*; *Zonocerous variegatus*; Growth; Gene expression

1. INTRODUCTION

Aquaculture is a rapidly expanding global food production sector, marked by increasing intensification in nearly every region worldwide [1]. This growth has not kept pace with the rising demand for aquatic fish food driven by the continuous increase in the global population. This is partly due to challenges in production management, among other factors [2]. Many aquatic organisms are cultivated globally in both fresh and marine waters. However, the African catfish (*Clarias gariepinus*) stands out as a significant freshwater species due to its high production levels and strong disease resistance in several countries [3].

Fish feed accounts for roughly 40% to 60% of production costs infish culture [4-5]. Fishmeal (FM) is the primary component in fish feed, known for its palatable protein. For decades, it has been the main protein source in fish diets due to its high-quality protein content, essential amino acids, vitamins, minerals, and other unidentified growth factors [6-7]. Due to the rapid growth in fish farming, FM prices have risen over the past decade and are expected to continue increasing to support ongoing growth [8]. Therefore, insect protein sources are being explored as an alternative for protein in dietary formulations, either as a partial or complete substitute for fish meal [9] for several reasons, including their low cost and their status as a more sustainable resource [10]. Consequently, recent research primarily focuses on reducing and/or potentially eliminating the use of fish meal (FM) in fish diet formulations [10].

Insects have been deemed a suitable protein source for animal feed, comparable to conventional protein sources such as fishmeal and soybean meal, in terms of amino acid composition, lipids, vitamins, and minerals [11]. Insects naturally constitute a significant part of the diet for many fish species [12], research has shown that cultured fish can adapt well to insect protein, exhibiting performance comparable to those fed with conventional protein sources [13]. Insects have high protein and fat contents and have been considered promising high-quality feed

components [14-16]. Insects could replace 25% to 100% of fishmeal or soybean meal in feeds for livestock and aquaculture, depending on the insect, livestock, and fish species [17]. The nutritional composition of the different insect species varies and so do the requirements of livestock and fish species that may be fed with the insects. Thus, the use of insects as feed requires system-specific investigations, as is common practice in all animal nutrition scientific research [18]. An example of such an insect being incorporated into a fish meal is a grasshopper.

The variegated grasshopper *Zonocerus variegatus* (Linn.) is a giant grasshopper with a high dry season population in southwest Nigeria. It belongs to the suborder Caeliferaand it is herbivorous. It is easily recognized by the multicoloured markings on its body and the disagreeable odour. It is a polyphagous insect that feeds on and defoliates many farm crops [19-20]. It is an animal protein supplement of the Akokos, a tribe native to the Southwestern part of Nigeria [21]. They provide animal dietary protein supplements for rural populations in the Southwest of Nigeria, thus alleviating animal protein scarcity in this zone. They could be found variously processed (smoked and fried [21].

Gene expression studies in fish are pivotal for understanding the underlying molecular mechanisms that regulate various biological processes. With information on how genes are transcribed and translated into proteins, which in turn influence the physiological, developmental, and adaptive responses of fish to their environments. Fish, being a diverse and ecologically significant group of organisms, are affected by a wide range of environmental conditions such as changes in water temperature, salinity, pollutants, and oxygen levels. Gene expression studies in fish play a crucial role in aquaculture, helping to improve breeding programs for traits such as growth rate, disease resistance, and stress tolerance.

This study investigated the effects of variegated grasshopper meal (*Zonocerous variegatus*) on growth performance and gene expression study of juvenile *Clarias gariepinus*. Gene is the unit of hereditary material and has a lot of impact on the physiology and morphology of organisms [22]. The expression of genes associated with growth, immune response, and inflammations was also investigated in the liver and muscle of the variegated grasshopper-fed fishes. The genes studied include growth hormone (GH), heat shock protein (HSP70), tumor necrosis factor α (TNF α), interleukin 1β (IL-1β), and melatonin receptor (MEL 1C).

2. Materials and Methods

2.1 Experimental Site

The experiment was conducted at the Department of Fisheries and Aquaculture unit at Central Laboratory, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria, between August and October 2023.

2.2 Preparation of Experimental Diets

Forty percent (40%) crude protein basal diets were formulated for *C. gariepinus* using the Pearson Square Method [23]. The diet was prepared using the following locally available feed ingredients: 70% fish meal, soybean meal, maize, Methionine, Lysine, Vitamin-mineral, Premix, Bone meal, starch, and salt as described in Table 1.

The grasshopper was oven-dried at a temperature of 55-60^{0}C, the dried form was dewinged, and all the limbs were removed before blending into powdered form. The proximate analysis of the grasshopper meal was conducted with a crude protein of 59.87.Variegated Grasshopper Meal Diets (VGMD) was incorporated with the other feed ingredients at 0%, 25%, 50%, 75%, and 100% diets and coded as VGMD0 (control), VGMD 1(25%), VGMD 2(50%), VGMD 3(75%) and VGMD 4 (100%) respectively as shown in Table 1. All the ingredients were

thoroughly mixed into a homogenous mass and pelletized using a locally manufactured pelleting machine through a 4mm diet. Diets were air-dried and packed into well-labeled polythene bags until ready for use. The diets were crushed and used to feed *C. gariepinus*.

Table 1: Composition of experimental diets formulated with Grasshopper meal used for feeding trials for 84 days in plastic tanks

INGREDIENTS (g)	**VGMD0 Control**	**VGMD1 25%**	**VGMD2 50%**	**VGMD3 75%**	**VGMD4 100%**
Soybean meal(CP=42)	30	30	30	30	30
Grasshopper meal(CP=59.87)	0	10.9	21.7	32.6	43.4
Fish meal(CP=65)	40	30	20	10	0
Maize(CP=12)	12	12	12	12	12
Di-Calcium Phosphate (DCP)	0.5	0.5	0.5	0.5	0.5
Lysine	0.6	0.6	0.6	0.6	0.6
Methionine	0.4	0.4	0.4	0.4	0.4
Salt	0.1	0.1	0.1	0.1	0.1
Vitamin C	0.5	0.5	0.5	0.5	0.5
Toxin Binder	0.3	0.3	0.3	0.3	0.3
Starch	14.1	13.2	12.4	11.5	10.7
Fish Oil	0.5	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5	0.5
Chromium oxide	0.5	0.5	0.5	0.5	0.5
Total %	100	100	100	100	100

Note: $VGMD_0$=0% Grasshopper Meal inclusion; $VGMD_1$=25% Grasshopper Meal inclusion; $VGMD_2$=50% Grasshopper Meal inclusion; $VGMD_3$=75% Grasshopper Meal inclusion; $VGMD_4$=100% Grasshopper Meal inclusion. DCP= Di-Calcium Phosphate

2.3 Collection and acclimatization of experimental fish sample

Eight hundred *C. gariepinus* juveniles of size ranging 7.8–9.2 cm (total length) with an average weight of 10.0 g ± 0.2g were obtained from a local fish farm in Ondo State, Nigeria, and transported to the research laboratory of the Department of Fisheries and Aquaculture Laboratory Central Lab, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria where it was acclimatized to laboratory conditions in aerated $1m^3$ plastic tanks for 2 weeks.

2.4 Experimental design, set-up, and management

The experimental design was completely randomized with each treatment in triplicate using the least significant difference (LSD). The feeding trial of *C. gariepinus* lasted for 12 weeks (84 days) and was carried out in 15 plastic containers (60 cm x 45 cm x 50 cm) at the rate of 15 juveniles per container, of three replicates per treatment (n=45 juveniles per treatment) The containers were covered with nets to prevent fish from escape as described by [24]. After acclimatization, 15 pieces of *C. gariepinus* (initial average weight of 10.0 g ± 0.2g) were weighed before the commencement of the experiment and randomly distributed into the 15 experimental bowls. The fish were starved for 24hours before the experiment to empty their gut and increase their appetite for the reception of the new feed. The new diets were fed to triplicate groups of fishes to apparent satiation twice daily (8:00–9:00 hours and 15:00–16:00 hours). The amount of feed given at each feeding time was recorded and uneaten feed was siphoned daily. Water was changed at 50% level every day to prevent fouling resulting from food residues and

fish excreta. Experimental fish were weighed bi-weekly with the use of a sensitive digital scale (model EK5350).

2.5 Evaluation of growth parameters of experimental fish

Growth performance and nutrient utilization of *C. gariepinus* were determined in terms of Mean Weight Gain (MWG), Final weight (FW), Protein intake (PI), Specific Growth Rate (SGR),Feed Intake (FI), Feed Conversion Ratio (FCR), Gross Efficiency of Feed Conversion (GEFC), Protein Efficiency Ratio (PER), Feed Efficiency Ratio (FER), Gross Feed Conversion Ratio (GFCR), Relative Growth Rate (RGR), Survival Rate (SR), Nitrogen metabolism (NM) and Condition Factor (CF) using the following formulae according to [25]:

i. **Mean Weight gain (MWG)** = $(W_f - W_i)/N$ ---------- Equation

Where, W_1 = initial weight (g)

W_2 = final weight (g)

N = no of fish in the tank

ii **Final Weight (FW)** = Final weight – Initial weight---------- Equation 2

iii **Protein Intake (PI)** = Total feed consumed x percentage protein/100---------- Equation 3

iv **Specific Growth rate (SGR) (%)** = $\frac{100 \log (W_2 - W_1)}{(T_2 - T_1)}$ ----------Equation 4

Where,

log W_2 - W_1= logarithms of initial mean and final mean weights of fish respectively and

T_2-T_1 = Experimental period in days.

v**. Feed intake (FI)** = 5% body weight of fish × No. of fish/treatment /tank----------Equation 5

vi. **Feed Conversion Ratio (FCR)** = $\frac{\text{Dry weight of feed fed (g)}}{}$ ----------Equation 6

Fish weight gain (g)

vii. **Gross Efficiency of Feed Conversion (GEFC)** = Feed Consumed/Final weight-- Equation 7

viii. **Protein Efficiency Ratio (PER)** = Wet body weight gain (g) ----------Equation 8

Crude protein fed (percentage)

ix. **Feed Efficiency Ratio (FER)** = Weight gain/Dry feed fed---------- Equation 9

x. **Gross Feed Conversion Ratio (GFCR)** =1/FCR x 100 ---------- Equation 10

xi. **Relative Growth rate (RGR) (%)** = $\frac{W_2 - W_1}{W_1} \times 100$ ----------Equation 11

Where, W_1 = initial average weight (g) at the beginning of the experiment

W_2 = final average weight (g) at the end of the experiment

xii. **Survival Rate (SR) (%)** = $\frac{N_f}{N_i} \times 100$ ------Equation 12

Where N_f = final number of fish that survived at the end of the feeding trial

N_i = initial number of fish stocked

xiii. **Nitrogen metabolism(NM)** $= \frac{0.549 (b-a) h}{2}$---------- Equation 13

Where, b = final body weight of fish

a = initial body weight

h = number of experimental days

xvi. **Condition Factor (CF)** = $K = \frac{100 \times w}{L^3}$----------Equation 14

Where, k = condition factor

w = weight of fish

l = length of fish

2.6 Gene Expression Study

Isolation of Total RNA

Total RNA was isolated from tissue samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNAse I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity was confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

cDNA conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min[26].

PCR amplification and agarose gel electrophoresis

Polymerase chain reaction (PCR) for the amplification of the gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (InqabaBiotec, Hatfield, South Africa). PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse; Table 2), and Ready Mix Taq PCR master mix. Under the following conditions: Initial denaturation at 95 ◦C for 5 min, followed by 30 cycles of amplification (denaturation at 95 ◦C for 30 s, annealing for 30 s, and extension at 72 ◦C for 60 s) and ending with final extension at 72 ◦C for 10 min. The amplicons were resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using “image J” software [27].

Table 2 PRIMER SEQUENCES

Gene	Forward Primer	Reverse Primer
GH	5'-CTGGAGAGACACTTTGGAGAAC-3'	5'-CCAAACCCTCACCACTTCTT -3'
Mel 1C	5'-TGTCTGAATGCTGCCGTCTA-3'	5'-ACTGCGATGTTGTTGGTCAC-3'
HSP 70	5'-AGAGCCAAGAGAACCCTGTC-3'	5'-TCTCAGGGCTTTCTCCACTG-3'
TNF-α	5'-ACCACGCTCTTCTGTCTACTG-3'	5'-CTTGGTGGTTTGCTACGAC-3'
IL-1β	5'-CCGGATGGGTAGGATAAAGTT-3'	5'-ACCCACTGAGGTAGGAAAGA-3'
GADPH	5'GCAAGGATACTGAGAGCAAGAG-3'	5'-CATCTCCCTCACAATTCCATCC-3'

The genes investigated include growth hormone (GH) heat shock protein (HSP70), tumour necrosis factor α (TNF α), interleukin 1β (IL-1β), and melatonin receptor (MEL 1C).

2.7 Statistical analysis

Statistical analysis was done with PRISM 8 (GraphPad Software, Inc.) using a two-tailed t-test with $p<0.05$ being used as statistical significance. The data were presented as mean ± SD (standard deviation) and used to plot bar charts [22].

3. Results

3.1 Growth performance and nutrient utilization of *C. gariepinus* fed with Variegated Grasshopper meal diets.

The growth performance and nutrient utilization of *C. gariepinus* fed with Variegated Grasshopper meal diets showed that there were no significant differences ($p<0.05$) for the values of the initial weight(IW), final weight (FW), Feed conversion ratio (FCR), Gross efficiency of feed conversion (GEFC) and Condition factor (K) for all the treatments (Table 3). *C. gariepinus*fed diet VGMD2 had the highest value for final weight gain (454.17±35.88g), while

the lowest values for final weight gain were recorded at VGMD4 (231.64±56.99g). The Feed intake (FI), Protein intake (PI), Specific growth rate (SGR), Protein efficiency ratio (PER), Relative growth rate (RGR), Nitrogen metabolism (NM), and Survival rate (SR) were significantly different ($p<0.05$) in all the treatment. Variegated Grasshopper Meal Diets (VGMD) was incorporated with the other feed ingredients at 0%, 25%, 50%, 75%, and 100% diets and coded as VGMD0 (control), VGMD1 (25%), VGMD2 (50%), VGMD3 (75%) and VGMD4 (100%) respectively. PI had the highest value (22.16±2.66g) at VGDM0 and the lowest (13.99±0.75g) at VGDM4. The value for FCR and GEFC ranges from 1.45 ±0.12g (VGDM0) to 1.94±0.62g (VGDM4) and 55.43±2.88g (VDGM1) to 69.24±5.79g (VGDM0), respectively. PER and FER had their values ranging from 0.48±0.15g (VGDM4) to 0.93±0.12g (VGDM0) and 0.55±0.03g (VGDM1) to 0.69±0.06g (VGDM0) respectively. GFCR had the highest value (69.24±5.79g) at VGDM0 and the lowest (54.70±14.96g) at VGDM4. RGR, NM and K values ranged from 167.60±81.70g (VGDM4) to 437.76±62.92g (VGDM0), 208.86±102.04g (VGDM4) to 538.77±81.13g (VGDM0) and 0.70±0.18g (VGDM4) to 0.87±0.15g (VGDM3), respectively. Fish on VGDM1 had the highest value for survival rate (97.78±3.85g) and the lowest value in VGDM0 (77.78±7.69g). Also, there was a significant difference ($p<0.05$) among FW, PI, SGR, FI, PER, RGR, and NM within the treatment groups, and no significant differences among FCR, GEFC, FER, GFCR, SR, and K within the treatment groups.

Table 3. Growth performance and nutrient utilization of *C.gariepinus* fed with variegated grasshopper meal diets.

Parameters (g)	**VGDM0**	**VGDM1**	**VGDM2**	**VGDM3**	**VGDM4**
	Control (0%)	**25%**	**50%**	**75%**	**100%**
Initial weight	105.69±1.41[a]	107.51±1.11[a]	107.63±0.91[a]	108.05±1.26[a]	108.04±0.47[a]

Final weight	444.20±41.99^{b}	387.08±72.88^{b}	454.17±35.88^{b}	401.58±22.54^{b}	231.64±56.99^{a}
Weight gain	338.51±40.58^{b}	279.57±71.77^{b}	346.54±34.97^{b}	293.53±21.28^{b}	123.60±56.52^{a}
FMWG	33.27±4.74^{c}	26.46±5.29^{ab}	34.26±1.45^{bc}	29.29±2.59^{b}	19.28±5.91^{a}
SGR	2.79±0.19^{c}	2.15±0.36^{ab}	2.56±0.08^{bc}	2.30±0.16^{bc}	1.58±0.57^{a}
RGR	437.76±62.92^{c}	269.29±74.67^{ab}	363.59±21.55^{bc}	299.38±38.87^{b}	167.60±81.70^{a}
CF	0.82±0.05^{a}	0.76±0.02^{a}	0.71±0.10^{a}	0.87±0.15^{a}	0.70±0.18^{a}
Survival Rate	77.78±7.69^{a}	97.78±3.85^{b}	91.11±7.69^{ab}	93.33±11.55^{ab}	82.22±13.88^{ab}
Feed Intake	55.39±6.66^{c}	47.48±7.30^{bc}	52.52±1.01^{c}	42.91±2.14^{ab}	34.98±1.86^{a}
Protein Intake	22.16±2.66^{c}	18.99±2.92^{bc}	21.01±0.40^{c}	17.16±0.86^{ab}	13.99±0.75^{a}
PER	0.93±0.12^{c}	0.63±0.12^{ab}	0.81±0.05^{bc}	0.72±0.05^{b}	0.48±0.15^{a}
FER	0.69±0.06^{a}	0.55±0.03^{a}	0.63±0.03^{a}	0.68±0.08^{a}	0.55±0.15^{a}
FCR	1.45±0.12^{a}	1.81±0.09^{a}	1.58±0.08^{a}	1.42±0.15^{a}	1.94±0.62^{a}
GEFC	69.24±5.79^{a}	55.43±2.88^{a}	63.35±3.37^{a}	68.48±7.61^{a}	54.70±14.96^{a}
GFCR	69.24±5.79^{a}	55.43±2.89^{a}	63.35±3.37^{a}	68.41±7.61^{a}	54.70±14.96^{a}
NM	538.77±81.13^{c}	333.61±91.84^{ab}	451.10±25.32^{bc}	379.64±45.97^{b}	208.86±102.04^{a}

a,b,c,d= indicate that the mean on the same row but with different superscripts are statistically significant ($p<0.05$)

3.3 Gene expression of *C. gariepinus* fed variegated grasshopper meal diets

3.3.1 Growth hormone (GH) gene expression response in liver and Muscle of *C. gariepinus* fed variegated grasshopper meal diets

The mRNA expression of Growth hormone (GH) in liver of *C. gariepinus* fed variegated grasshopper meal diets (VGDMs) revealed that there was upregulation in the gene expressed in VGDM1 and VGDM4 when compared to control (VGDM0) in both the liver and muscle tissue (Fig. 1). There was significant ($p<0.05$) GH repression in the liver of VGDM2 and VGDM3 to VGDM1.

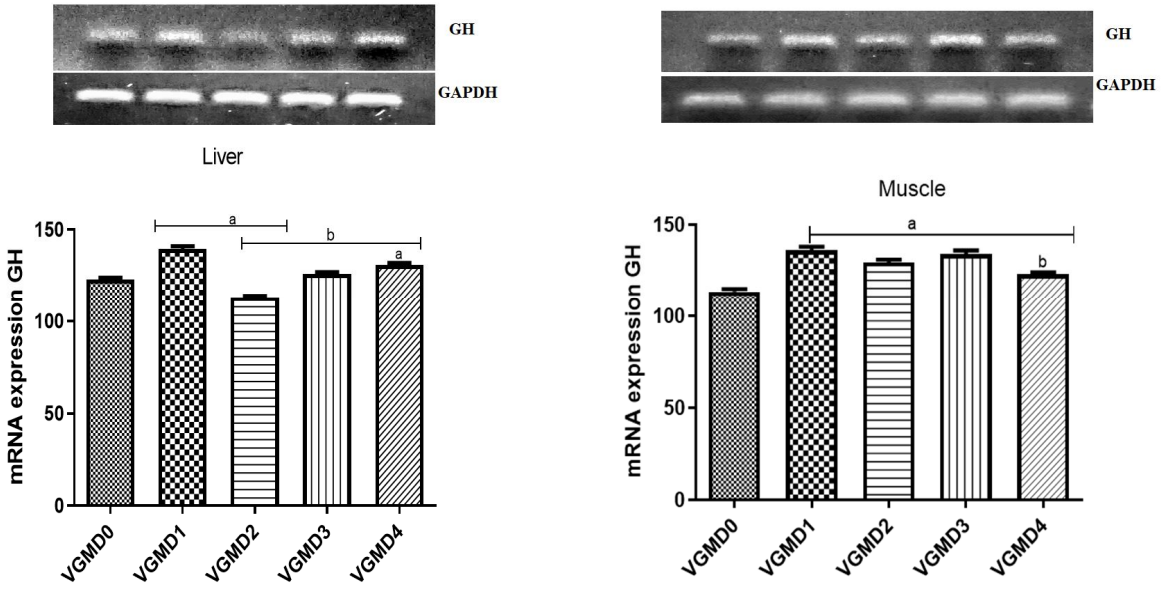

Fig. 1: A snapshot representation of growth hormone (GH) gene expression response in liver and muscle of *C. gariepinus* fed variegated grasshopper meal diets (VGMDs). “a” connotes significant regulation ($p <0.05$) to VGMD0. “b” connotes significant downregulation ($p <0.05$) to VGMD1.

3.3.2 Heat Shock Protein (HSP70) gene expression response in the liver of *C gariepinus* fed variegated grasshopper meal diets

The mRNA expression of Heat Shock Protein (HSP70)in the liver of *C. gariepinus* fed VGDMs showed that there was significant upregulation ($p<0.05$) between grasshopper fed-fish at 25% inclusion with respect to control (without grasshopper inclusion) in both the muscle and the liver tissue (Fig. 2). The mRNA expression of Heat Shock Protein (HSP70) gene expression in the liver of *C gariepinus* fed VGMDs was downregulated ($p<0.05$) in VGMD2 and VGMD3 to VGMD1 and control (VGMD0).

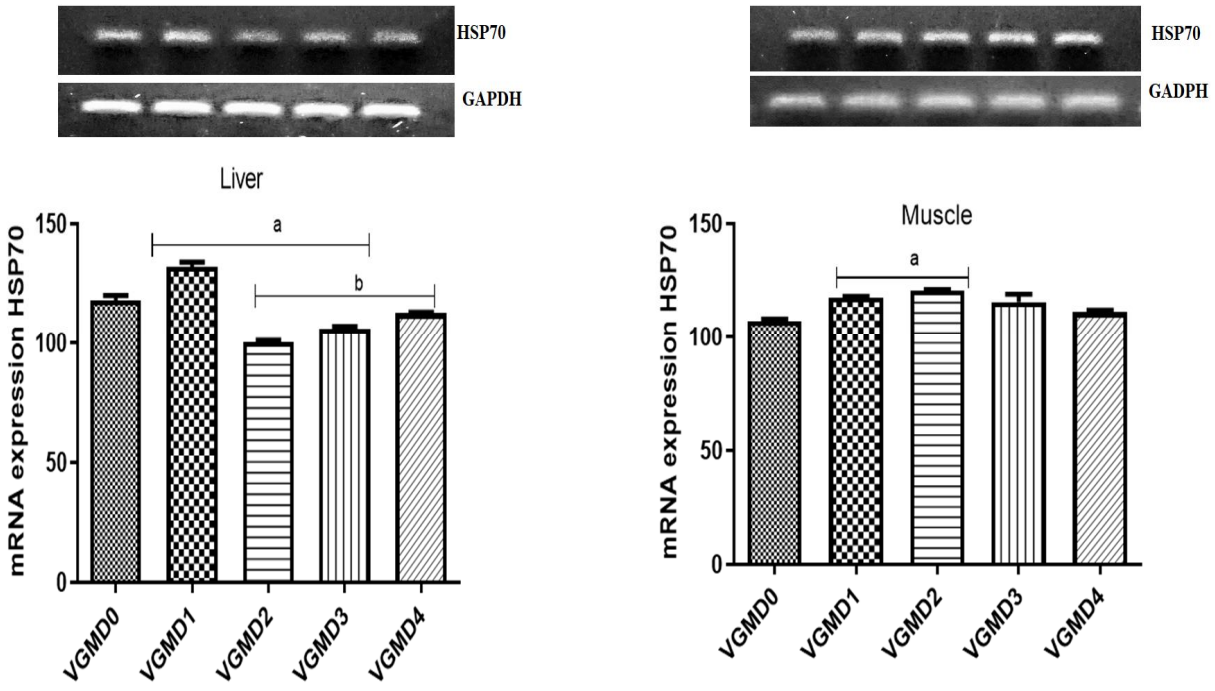

Fig. 2: A snapshot representation of heat shock protein 70 (HSP 70) gene expression response in liver and muscle of *C. gariepinus* fed variegated grasshopper meal diets (VGMDs). “a” connotes significant regulation ($p <0.05$) to VGMD0. “b” connotes significant downregulation ($p <0.05$) to VGMD1.

3.3.3 Tumor necrosis factor α (TNF α) gene expression response in the liver of *C gariepinus* fed variegated grasshopper meal diets

The mRNA expression of Tumor necrosis factor-alpha (TNFα)in the liver and muscle of *C. gariepinus* fed VGDMs was significantly ($p<0.05$) upregulated in VGDM1 when compared with control (Fig. 3). There were no significant differences ($p<0.05$) between VGDM1 and VGDM2 in the muscle's TNF-α gene expression. There was significant downregulation ($p<0.05$) in VGDM3 and VGDM4 when compared with VGDM1 in both the liver and muscle tissue (Fig. 3).

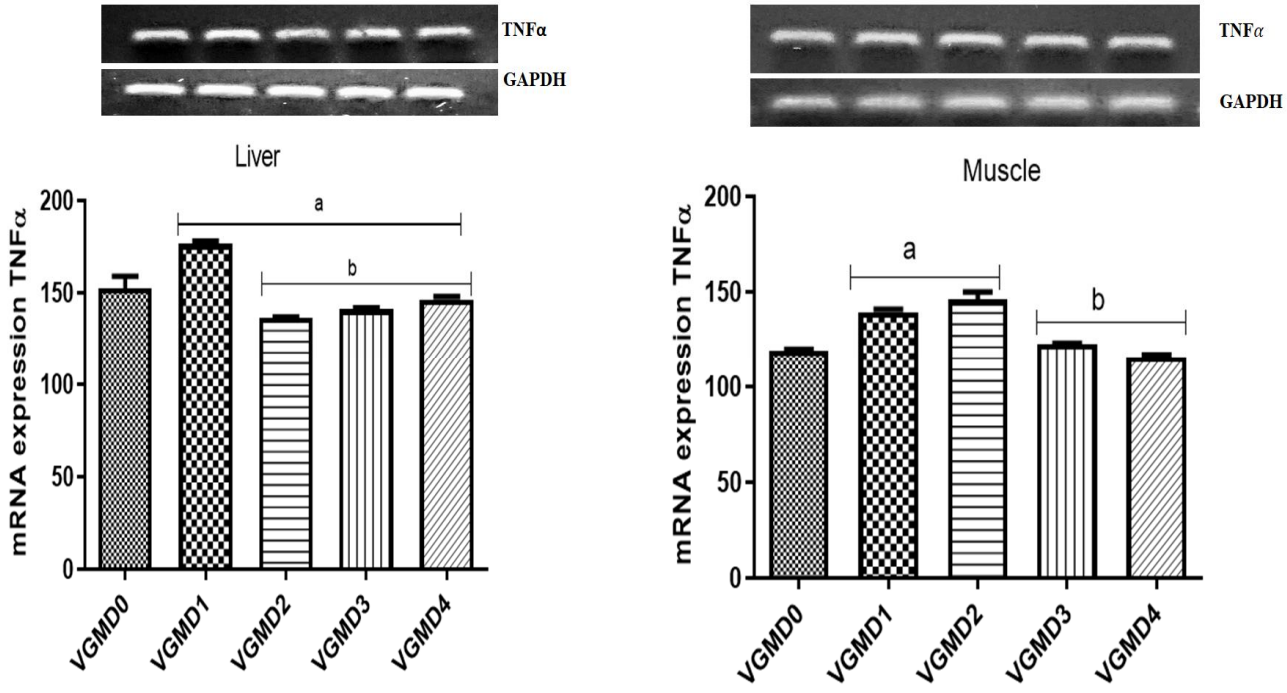

Fig 3: A snapshot representation of Tumor necrosis factorα (TNFα) gene expression response in liver and muscle of *C. gariepinus* fed variegated grasshopper meal diets (VGMDs). "a" connotes significant regulation ($p <0.05$) to VGMD0. "b" connotes significant downregulation ($p <0.05$) to VGMD1.

3.3.4 Interleukin 1β (IL-1β) gene expression response in the liver of *C gariepinus* fed Variegated grasshopper meal diets

The mRNA expression of Interleukin 1β (IL-1β)in the liver of *C. gariepinus*-fed VGDMs revealed that there was significant upregulation ($p<0.05$) in VGDM1 and VGDM2 compared to control in both tissue groups. There was significant downregulation in IL-1β expression in the liver's VGDM2-fed fish compared to VGDM1 (Fig. 4).

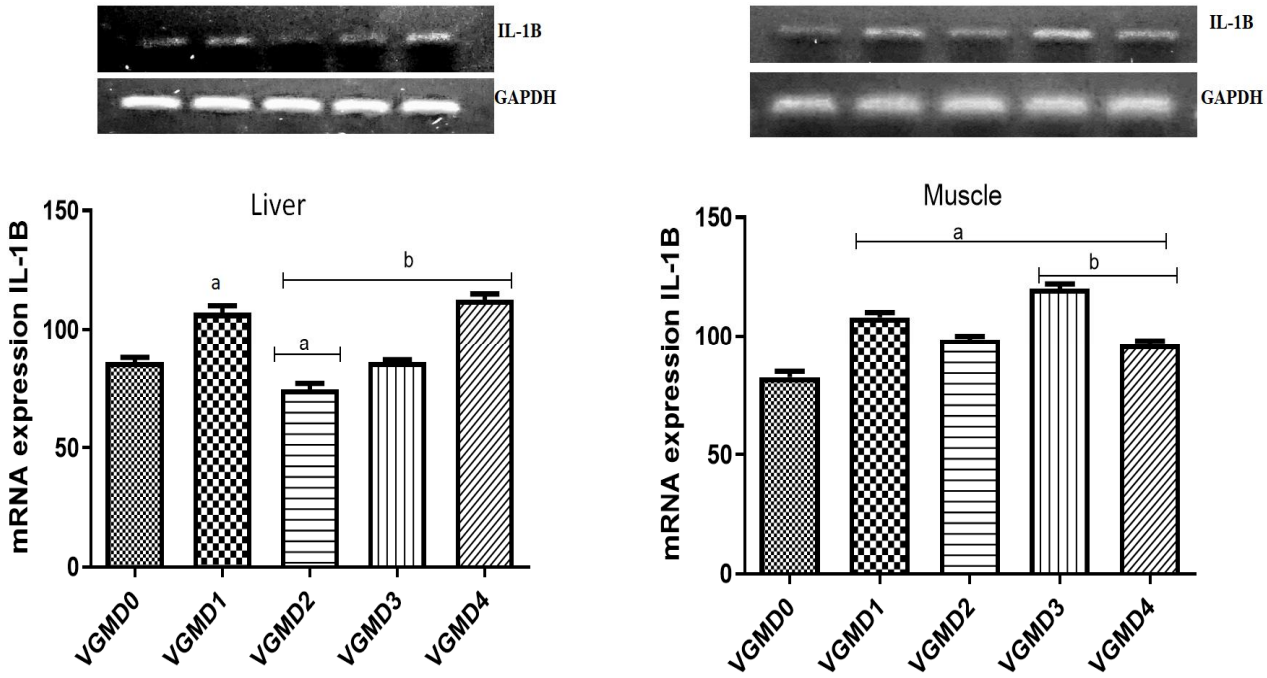

Fig 4: A snapshot representation of Interleukin 1β (IL-1β)gene expression response in liver and muscle of *C. gariepinus* fed variegated grasshopper meal diets (VGMDs). "a" connotes significant regulation ($p <0.05$) to VGMD0. "b" connotes significant regulation ($p <0.05$) to VGMD1.

3.3.5 Melatonin receptor (MEL 1C) gene expression response in the liver of *C gariepinus* fed Variegated grasshopper meal diets

The mRNA expression of the Melatonin receptor (MEL 1C)in the muscle of *C. gariepinus* fed VGDMs was significantly upregulated ($p < 0.05$) in the treatment groups compared to control (Fig. 5). There was significant upregulation ($p < 0.05$) in MEL 1C gene expressed in VGDM1 and VGDM2 compared to control (VGDM0) (Fig.5). MEL 1C gene expression was significantly downreregulated ($p < 0.05$) in VGDM2 relative to VGDM1 group.

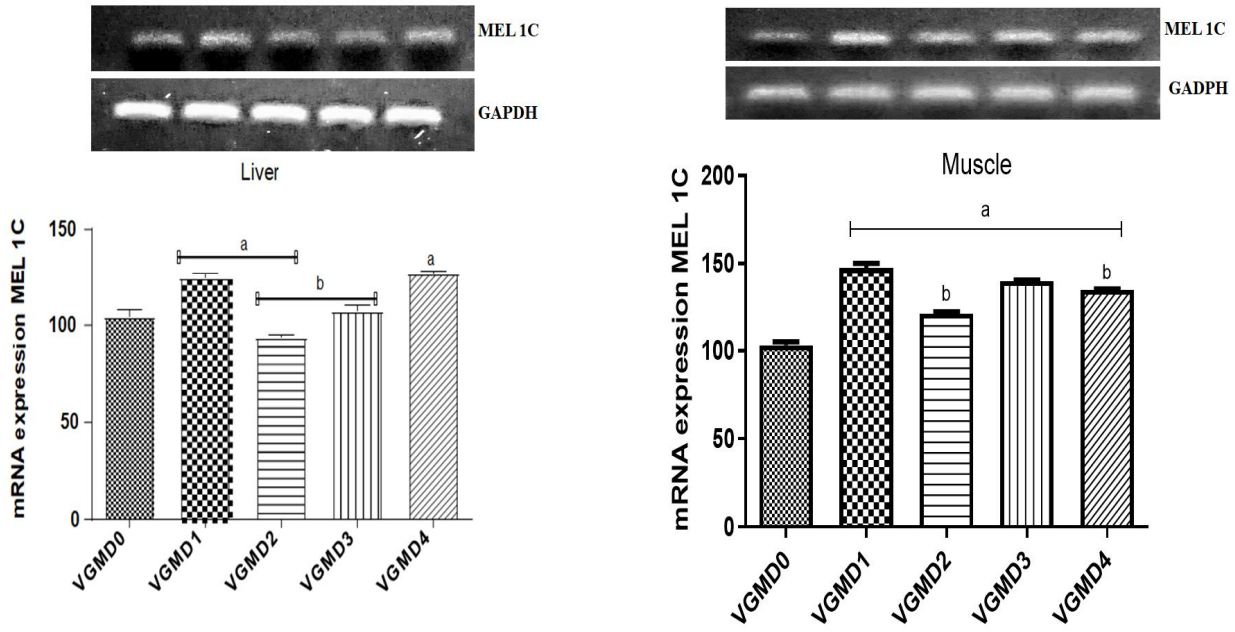

Fig 5: A snapshot representation of Melatonin receptor (MEL-1C)gene expression response in liver and muscle of *C. gariepinus* fed variegated grasshopper meal diets (VGMDs). "a" connotes significant regulation ($p < 0.05$) to VGMD0. "b" connotes significant downregulation ($p < 0.05$) to VGMD1.

4. Discussion

The baseline conditions and overall growth of the fish were similar across the dietary treatments. The lack of significant differences in initial weight, final mean weight gain, feed conversion ratio (FCR), gross efficiency of feed conversion (GFCR), and condition factor (CF) among the treatment groups is an interesting finding. This aligns with the concept that initial fish size and starting conditions can influence growth performance [28]. The substantial differences in final weight gains between the treatment group variegated grasshopper meal diets (VGMDs) and the control group VGMD0 are notable. VGMD2 resulted in significantly higher weight gain, indicating that this diet may be more effective in promoting growth. This finding is consistent with previous research suggesting that dietary composition can greatly impact the growth of fish [29]. The variations in protein intake, Protein efficiency ratio (PER), and Feed efficiency ratio (FER) across the treatments highlight the importance of dietary protein content. The highest protein intake in VGDM0 which was not significantly different from VGDM2 suggests that this diet was richer in protein, which could have contributed to improved growth. These results align with the well-established relationship between dietary protein levels and fish growth [30]. The varying FCR and GFCR values among treatments indicate differences in feed utilization efficiency. Lower FCR values, as observed across all treatment groups, are generally preferred as they indicate better feed conversion. The highest GFCR in VGDM0 suggests that this diet may not be the most efficient in converting feed into fish biomass. These findings are consistent with the literature on aquaculture feed efficiency [31]. The differences in Relative growth rate (RGR), Nitrogen metabolism, and Condition factor (CF) among treatments further highlight the impact of diet composition on fish growth and overall health. VGDM2 appears to provide the most favourable conditions for growth and nutrient utilization. These results align with the idea that diet plays a crucial role in shaping the physiological and metabolic responses of fish [32]. The

significant variation in survival rate among treatments is important for assessing the overall success of the feeding regimes. VGDM1 resulted in the highest survival rate which is not significantly different from VGDM2, suggesting that this diet may be better suited for maintaining fish health and viability. This finding is in line with studies emphasizing the importance of diet in fish survival and resistance to stress [33].

Morphological (physical) features of fishes depend on their genetic makeup and have a lot of say or determining power on their value [34]. Over the years, a lot of research has been going on to alter or modulate genes responsible for these physical features, and immune responses and to boost the survival rate of animals. Growth hormone (GH) is secreted by the anterior pituitary gland and is linked to many genes. Its function is to induce the growth and development of other genes [35]. There was significant upregulation of the GH gene expressed in the muscle of *C. gariepinus* fed with varying doses of grasshopper meal when compared to the control group (VGDM0) (Fig 1). There was also a significant upregulation of GH gene expression in the liver of VGDM1 and VGDM4 compared to control indicating that this diet exerted a measurable impact on GH expression in the liver of *C. gariepinus*. This is supported by the work of [36]. This upregulation suggested that the inclusion of VGDM in the diet has a stimulatory effect on GH gene expression, which is a critical regulator of fish growth. This finding aligned with previous research that has shown dietary composition can influence the expression of growth-related genes in fish as reported by [37] and as observed in the growth indices in Table 3. There is significant modulation of GH expression between VGDM1 and VGDM4 in both the liver and the muscle tissues. This suggests that the various VGDM formulations may have distinct effects on GH gene expression. These differences may be attributed to variations in nutrient composition, anti-nutritional factors, or other bioactive

compounds present in the different diets. Effects of these factors on growth patterns have also been reported [38].

Heat shock proteins 70 (HSP 70), is a highly conserved molecular chaperones that aid in protein folding and prevent aggregation under stress conditions, thus preserving cellular integrity [39]. The mRNA expression of the heat shock protein (HSP70) gene of *C. gariepinus*, when subjected to dietary variations involving Variegated Grasshopper Meal Diets (VGMDs) was investigated in both the liver and muscle (Fig. 2). There was a significant upregulation in VGMD1 in both tissues compared to the VGDM0 group. This suggests that the grasshopper-meal diet at moderate inclusion is capable of preventing stress which could be a result of temperature fluctuations, which pose a serious environmental challenge to aquatic organisms. In addition, it will also help both the liver and muscle tissues to function optimally for metabolism and growth respectively. This could be attributed to various factors present in the variegated grasshopper meal diets, such as differences in nutrient composition. [40-41] also reported the importance of HSP in aquatic organisms, in mitigating the adverse effects of temperature fluctuations, pollutants, and dietary challenges. Thus, the upregulation of HSP70 suggests an adaptive response of *C. gariepinus* to dietary changes, potentially contributing to its overall fish's defense, fitness, and survival.

Tumour necrosis factor-alpha (TNF-α) helps in response to inflammation [42]. There was a significant upregulation ($p < 0.05$) in the mRNA expression of the TNF-α gene in VGMD1 compared to VGMD0 in the liver and muscle of *C. gariepinus* as seen in Fig. 3. This suggests the inflammatory response of this fish species when subjected to dietary variations, particularly the incorporation of variegated grasshopper meal diets (VGDM) at low inclusion. This result is supported by prior work on the effect of dietary components on TNF-α expression in fish under

various inflammatory conditions [42]. There are no significant changes in the mRNA expression of both VGDM4 and the control. This finding suggests that, at the mRNA level, the dietary alteration with VGDM did not induce a noticeable inflammatory response mediated by TNF-α in the liver of *C. gariepinus*at high percentage inclusion. However, the study did reveal significant statistical differences ($p<0.05$) in TNF-α gene expression between specific treatment groups in the muscle tissue (Fig. 3). This could indicate a suppression of the inflammatory response in these treatment groups, suggesting an adaptive or regulatory mechanism. This is supported by previous works [43].

Interleukins are crucial pro-inflammatory cytokines that help in the immune responses of organisms [44]. There was a significant upregulation in the mRNA expression of Interleukin 1β (IL-1β) in VGDM1 compared to VGDM0 in both the liver and muscle of *C. gariepinus* (Fig. 4). This suggests that VGDM has the potential to boost immune responses of this fish species. The results of this study also revealed an interplay between diet and the immune response in fish. This result is supported by previous research indicating the effect of dietary components on immune-related gene expression in fish [45]. These differences may be attributed to variations in the composition of VGDM formulations, including nutrient levels, anti-nutritional factors, or bioactive compounds [46]. The downregulation observed in VGDM3 compared to VGDM1 in the liver and muscle might suggest a unique immunosuppressive response or a compensatory mechanism in the fish's immune system. This immunosuppressive ability of diet inclusion has also been reported [47].

Melatonin is a hormone that regulates blood pressure and sleep-wake cycles in vertebrates. It also plays a role in seasonal rhythmicity, or the circannual cycle, which includes fattening, moulting, hibernation, and reproduction [48]. There was significant upregulation in

melatonin receptor (MEL 1C)gene expression in VGDM1 and VGDM4 compared with VGDM0 in the muscle and the liver. This result aligned with previous findings indicating that dietary factors may have varying effects on the expression of melatonin receptors [49]. The observed statistical differences in MEL 1C expression between VGDM1 and VGDM2 in both tissues investigated are intriguing. This discrepancy may reflect nuanced responses to different compositions of VGDMs, highlighting the importance of diet formulation in eliciting gene expression changes. Similar findings have been reported in studies on VGMDs dietary effect on modulation of gene expression in fish species [50]. Understanding the mechanisms behind this downregulation may shed light on the complex interplay between dietary factors and melatonin receptor expression as reported by [51].

Conclusion

This study suggests that variegated grasshopper meal has some qualities that could effectively supplement fish meal in the diet of *C. gariepinus*. When variegated grasshopper meal was included at (50%) in the *C. gariepinus* diet, parameters relating to growth significantly improved, alleviating oxidative stress, and improving immune responses.

Declarations

Ethical Approval

Animal protocols were approved by the ethical committee of Adekunle Ajasin University, Akungba-Akoko.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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