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Performance Traits and Expression of Growth-Related Genes of *Clarias gariepinus* Fed Conventional and Non-Conventional Feed Ingredients

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ABSTRACT

This study investigated the impact of conventional (commercial) and nonconventional diets, specifically containing hydrolyzed feather meal (HFM), crustacean waste (CW), and chicken viscera (CV), on performance traits and *insulin growth factor 1 (igf-1)/ growth hormone gene* (*ghg*) expression in African catfish (*Clarias gariepinus*), with an average initial weight of 5.36 ± 0.03g. Eight diets were formulated, including a control diet and a commercial diet containing 37% crude protein, and administered in triplicate over a six-month feeding trial. Total RNA was extracted from liver samples via the Quick-RNA Miniprep Plus Kit, followed by real-time quantitative polymerase chain reaction (RT–qPCR). The results revealed that *gh* mRNA expression levels were significantly increased by the CV, CW, HFM, and commercial diets, whereas *igf-1* expression was notably increased by the HFM and HFM + CV diets compared to the control. A significant difference was detected in *gh/igf-1* cycle quantification (Cq) ($P< 0.05$), when compared to the control. Among the diets, the HFM + CW combination resulted in the highest *gh/igf-1* gene expression. The highest final weight gain (108.9 ± 0.007g), specific growth rate (2.32 ± 0.003%/day) and lowest feed conversion ratio (1.30±0.003) were recorded for the fish fed the HFM + CW diet. The fish fed diets supplemented with 8.43% CW and 8.43% HFM presented increased growth rates and increased expression of *gh/igf-1*. The incorporation of HFM + CW in aqua-feed formulations modulated growth performance and GH/IGF-1 expression in *Clarias gariepinus*, contributing to sustainable aquaculture practices.

INTRODUCTION

Nutrigenomics is a novel field focusing on how molecular mechanisms and nutrients influence aquaculture species **(Vera *et al.*, 2017; Hakim *et al.*, 2018)**. It emphasizes the importance of genes involved in growth, such as *growth hormone* (*gh*), which is critical for fish development **(Tian *et al.*, 2014)**. Increased *gh* expression benefits growth, and the *Gh*/insulin-like growth factor-I (*Igf-I*) axis regulates somatic growth in teleost fish through two receptors, *Gh* receptor (*GhR*)-I and *GhR*-II **(Jiao *et al.*, 2006; Fuentes *et al.*, 2013)**. *Igf-1*

plays a key role in muscle growth by balancing protein degradation and synthesis **(Rossi & Messina, 2014)** and serves as a biomarker for growth and nutritional status in aquaculture **(Moon *et al.*, 2022)**. Nutrition influences the expression of *gh* and *igf* genes through transcription factors in metabolically active organs like the liver, intestine, and adipose tissue **(Haro *et al.*, 2019)**. The TOR pathway, part of the Pi3K/Akt signaling cascade, is activated by Igf-1 and promotes protein synthesis in fish **(Xie *et al.*, 2019)**. *GhR*s are mainly expressed in the liver and other tissues, mediating *Gh* effects on growth and metabolism **(Canosa *et al.*, 2007)**. The liver's biological functions make it a crucial organ for assessing the nutritional and physiological status of fish **(Escaffre & Bergot, 1986; Fontagné *et al.*, 1998; Wang *et al.*, 2014; Hu *et al.*, 2016; Sun *et al.*, 2024)**. *Clarias gariepinus* is a resilient aquaculture species capable of thriving in diverse conditions, utilizing atmospheric oxygen, and converting various feedstuffs to flesh efficiently **(Okomoda, 2018; Langi *et al.*, 2024)**. Fish growth is influenced by feed utilization, which depends on the nutrient composition and digestibility of the feed **(Moshood *et al.*, 2014)**. However, the aquaculture industry utilizes only a small fraction of available feed, with studies showing that a significant portion of feed remains underutilized **(Miller & Atanda, 2011; Udo & Dickson, 2017)**.

Research has shown that diets and feed ingredients significantly impacted fish's expression of growth-related genes. It was reported that *Clostridium autoethanogenum* supplementation increased hepatic *igf-1* expression in tilapia **(Maulu *et al.*, 2021)**. Protein-rich diets decreased hepatic *igf-1* expression in genetically improved farmed tilapia, while poultry byproduct meal suppressed *gh/igf* axis gene expression in the gilthead seabream **(Karapanagiotidis *et al.*, 2019; Singha *et al.*, 2020)**. Feather meal also downregulated the *gh/igf* axis in the same species **(Psofakis *et al.*, 2020)**. Tryptophan supplementation enhanced *gh-igf* axis gene expression in the hybrid catfish **(Zhao *et al.*, 2019)**, while fish oil increased *gh-1* and *igf-1* expression in the yellow drum **(Wabike *et al.*, 2020)**. However, comparisons among commercial (vital feeds), chicken viscera, crustacean waste, and feathermeal ingredients in *Clarias gariepinus* are lacking. Hence, this study aimed to investigate the effects of a conventional diet and diets containing nonconventional ingredients on the mRNA expression of *gh* and *igf-1* and performance traits in *Clarias gariepinus.*

MATERIALS AND METHODS

The research was conducted at the Teaching and Research Farm of the Faculty of Agriculture, University of Port Harcourt, Choba, Rivers State, for six months. The site is located at latitude 4º.77ˈ.00"N and longitude 6º.45ˈ.00"E in the Obio-Akpor Local Government Area of Rivers State, Niger Delta, Nigeria, as seen in Fig. (1).

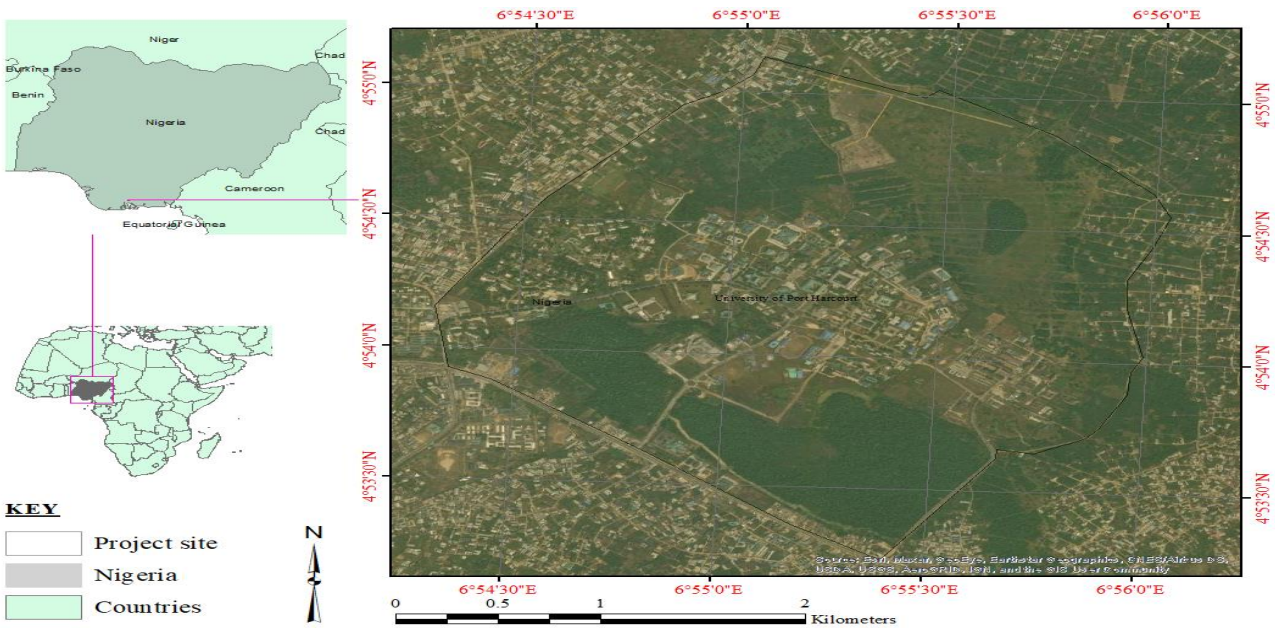

Fig. 1. A map of Africa showing Nigeria and Rivers State, the study area

1. Diet formulation

Nonconventional ingredients such as chicken viscera, and crustacean waste were processed for use in fish feed. Fresh chicken viscera were collected, cleaned, parboiled at 100°C for 30min, dried at 60°C for 9h, and ground into chicken viscera meal. Commercially processed hydrolyzed feather meal was sourced from Modern Agro Enterprises. The dried crustacean waste was purchased, sieved, and ground.

All ingredients in Table (1) were ground, thoroughly mixed, and sieved through a 60-mesh sieve. A total of 85ml of water per 100g of feed was blended into the mixture (Philips HR7628, Finland) to form dough for fish food **(Lovell, 1989)**. The dough was then extruded into 2– 3mm pellets via a fish feed extruder (ZNGP200, China), dried in an oven (BD100, Nigeria) at 35°C for 48h, sealed in plastic bags, and stored at –20°C until use.

Table 1. Composition (%) of isonitrogenous experimental diets (37% CP) for African catfish (*Clarias gariepinus*) containing nonconventional protein ingredients

	[1]Commercial Diet	Control	Crustacean Waste (cw)	Chicken Viscera (cv)	Hydrolyzed Feather Meal (hfm)	CW + CV	HFM + CW	HFM + CV
Maize bran	-	260.70	142.70	231.20	325.90	176.80	244.10	260.70
Fish meal	-	224.80	56.20	56.20	56.20	56.20	56.20	56.20
Soya bean meal	-	449.50	567.50	479.00	384.30	533.40	466.10	449.50
Crustacean waste	-	0.00	168.60	0.00	0.00	84.30	84.30	0.00
Feather meal	-	0.00	0.00	0.00	168.60	0.00	84.30	84.30
Chicken viscera	-	0.00	0.00	168.60	0.00	84.30	0.00	84.30
Fish oil	-	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Calcium diphosphate	-	20.00	20.00	20.00	20.00	20.00	20.00	20.00
D-L Methionine	-	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Cassava flour	-	10.00	10.00	10.00	10.00	10.00	10.00	10.00
[2]Vitamin/[3]Mineral PMX	-	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chromium(iv)oxide	-	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Total	-	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
Nutrient Composition (%Dry weight)								
Crude protein	373.60	394.30	391.30	345.30	374.00	397.30	390.70	393.70

Crude lipid	32.00	54.30	54.30	60.30	32.00	56.70	55.00	36.70
Ash	92.40	95.40	87.20	98.50	92.40	94.50	95.60	87.80
Moisture	74.00	74.00	80.30	76.30	74.00	77.30	64.00	73.30
Crude fiber	36.70	27.60	33.30	34.00	36.70	30.30	31.00	37.30

[1]Commercial diet (Vital) composition - crude protein- 37.36%, crude lipid- 3.2%, fiber- 3.67%, ash- 9.24%, moisture-7.40%. [2]Each 0.25 kg of the vitamin premix (El Bardeny Company) provides 10,000,000 IU of vitamin A, 2,200,000 IU of vitamin D3, 10,000 mg of vitamin E, 1,000 mg of vitamin K3, 1,000 mg of vitamin B1, 5,000 mg of vitamin B2, 1,500 mg of vitamin B6, 10,000 mg of vitamin B12, 10,000 mg of pantothenic acid, 30,000 mg of niacin, 1,000 mg of folic acid, 50,000 mg of biotin, and 600,000 mg of choline chloride. [3] The mineral premix (El Bardeny Company) contains 0.25 kg: 30,000 mg of iron, 60,000 mg of manganese, 50,000 mg of zinc, 4,000 mg of copper, 100 mg of cobalt, 300 mg of iodine, and 100 mg of selenium.

2. Experimental setup and fish

A total of 720 healthy catfish juveniles (initial average weight of 5.36g) were obtained from the Faculty of Agriculture, University of Port Harcourt, and acclimatized for two weeks. During acclimatization, the fish were fed commercial diets (Vital) thrice daily for apparent satiation. After this period, the fish were distributed across 24 concrete tanks (1.5 m × 0.9 m × 0.45 m) with a stocking density of 30 fish/m^3. Eight dietary treatments were conducted in triplicate, including a control diet, commercial feed, crustacean waste, chicken viscera, hydrolyzed feather meal, and combinations of these ingredients. The experiment followed a completely randomized design and lasted for 190 days (approximately 6 months and 10 days), with the fish being fed twice daily at 5% of their body weight. The concrete tanks were covered with nets to prevent the fish from jumping out and removing unwanted organisms. Dissolved oxygen (DO) was measured using a portable DO meter (HANNA HI 9146, Hanna Instruments, USA) whilst pH was measured with a pH meter (HANNA HI 9125, Hanna Instruments, USA) and temperature was measured electrochemically with a portable multimeter (HANNA HI 99300, Hanna Instruments, USA). Well-filtered water (pH 7.35) was added one week prior to stocking the fish. Throughout the experiment, the water temperature was maintained at 26.7 ± 0.05°C, the dissolved oxygen concentration was 6.80 ± 0.05 mg/L, and the pH was 7.78 ± 0.05. Weight in gram (g) was measured with a digital scale (Mettler Toledo PG 5002-SDR, Ohio, USA).

3. Proximate composition analysis

The proximate compositions of the experimental diets (Table 1) and unconventional protein sources (Table 2) were analyzed following the methods outlined by **AOAC (2002)**. The moisture content (%) was determined by drying the samples in an oven at 105°C until a constant weight was achieved. The dried samples were then finely ground using a mortar and pestle for further analysis. The crude protein content (N × 6.25) was measured via the Kjeldahl method; crude lipids were extracted with ether via a Soxhlet apparatus, and the ash content was determined via combustion at 550°C in a muffle furnace for 5h. Gross energy was measured via a bomb calorimeter (PARR 1281, USA).

Table 2. Proximate analysis of protein sources (nonconventional ingredients) used in the experimental diets (Mean± standard deviation)

Parameter	HFM	CV	CW
Crude protein (%)	80.92±0.01	48.09±0.02	44.65±0.00
Crude fat (%)	5.70±0.01	0.52±0.02	2.39±0.02
Crude fiber (%)	3.57±0.01	2.83±0.00	5.68±0.03
Moisture (%)	14.53±1.64	9.36±0.02	18.72±0.12
Ash (%)	4.59±0.01	1.05±0.00	2.96 ± 0.03
Gross energy (kJ/cal)	3460.85±1.78	1820.99±0.97	1847.53±2.10

4. Extraction of RNA and real-time qualitative PCR (qPCR) of *gh/igf-1* expression

RNA was extracted from the liver of each fish sample fed commercial (COMM), control (CTRL), crustacean waste (CW), chicken viscera (CV), hydrolyzed feather meal (HFM), CW + CV, HFM +CV and HFM +CW diets according to the manufacturer's protocol via a Quick-RNA Miniprep Plus Kit. The cDNA was synthesized via TOPscript™ RT DryMIX (Enzynomics, Daejeon, Korea). To examine the expression of the *growth hormone* (*gh*) and *insulin-like growth factor 1* (*igf-1*) genes in the African catfish (*Clarias gariepinus*), real-time PCR was performed via the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA) with the Luna Universal One-Step RT–qPCR Kit (E3005L, New England Biolabs, USA). The PCR protocol began with initial denaturation at 95°C for 15mins, followed by 40 cycles of amplification at 72°C for 30 secs, 65°C for 15 secs, and 72°C for 30 secs, for a total reaction volume of 20µL. The β-actin gene, recognized for its reliability and stability **(Habte-Tsion *et al.*, 2015; Liang *et al.*, 2016; Maulu *et al.*, 2021)**, was used as the reference gene. All the assays were performed in triplicate, and a negative control without cDNA was included in each run. Each assay included a negative control without cDNA. Primers for the *gh*, *igf-1*, and *β-actin* genes were designed on the basis of sequences sourced from the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.gov), as shown in Table (3). The primers for *gh*, *igf-1*, and *β-actin* were species-specific because their sequences are available for African catfish.

Table 3. Sequences of primers used for real-time qPCR assays

Gene Name	Primer		Amplicon Size (bp)
	Name	**Sequence**	
gh	Forward	GACTGTTCTCCATCGCTGTC	83
	Reverse	TCAAACCATACACCCTCAGC	
igf-1	Forward	ATGTAGGGAAGGTGCGAATG	123
	Reverse	CCTTTGTCAGCATCCTCTTTG	
β – actin	Forward	ATCACACCTTCTACAACGAGC	122
	Reverse	GAAGGTCTCGAACATGATCTG	

Gene bank accession numbers for GH-KR269816.1, IGF-1-AY776159.1 and *β-actin*-AY510710.2. qPCR- quantitative polymerase chain reaction, *gh- growth hormone*, *igf-1- insulin-like growth factor 1*

Relative *gh* mRNA expression levels in the fish liver were calculated via the following formula:

$\Delta Ct_{(sample)} = Ct_{(target\ gene\ control)} - Ct_{(\beta\ actin\ control)}$

$\Delta\Delta Ct = \Delta Ct_{(target\ gene\ of\ exposure)} - \Delta Ct_{(\beta\ actin)}$

Fold change = $2^{-\Delta\Delta Ct}$ **Arocho *et al.* (2006)**

Where, Ct= cycle threshold/crossing point/take-off point.

5. Statistical analysis and growth parameters

Data from all measured parameters were tested for homogeneity prior to analysis. Statistical evaluation was carried out via IBM's SPSS software for Windows, version 25 **(SPSS, 2017)**. One-way ANOVA was used to assess significant differences between treatment group means, followed by Duncan's *post- hoc* test to pinpoint specific differences between groups. The results are reported as the mean values with their corresponding standard error of the mean (SEM)/ standard deviation (SD). Statistical significance was set at $P< 0.05$.

Specific growth rate was calculated using the following equation:

SGR (%/day) $= \frac{LnWf - LnWi}{T} \times 100$ **(Edward *et al.*, 2010)**

Where, Wf = Final Weight

Wi = Initial Weight

T = Time in days

Ln = Natural Log

Feed conversion ratio was calculated using the following equation-

$$FCR = \frac{Feed\ intake\ (g)}{Weight\ gain\ (g)}$$

Ethical approval: This study was approved by the ethical panel and examiners of Lilongwe University of Agricultural and Natural Resources, Malawi.

RESULTS

1. Log fold change in the diets of *Clarias gariepinus*

The log-fold change in *growth hormone* (*gh*) gene expression in the CTRL diet was greater than that in the other diets; CW, HFM and HFM+CW were also upregulated, indicating increased expression while COMM, CV, CV+CW and HFM+CV were downregulated (Fig. 2). The log-fold change in *insulin growth factor* (*igf-1*) gene expression in the CTRL diet was greater than other diets; CW, HFM and HFM+CW were also upregulated, indicating increased expression while COMM, CV, CV+CW and HFM+CV were downregulated (Fig. 3).

2. Cycle quantification of the diets fed on *Clarias gariepinus*

The study further revealed that *gh* expression was significantly greater in the diet ($P<0.05$) with a combination of HFM+CW than in the CTRL. The study also revealed that growth hormone gene expression was upregulated by the HFM+CW diet because it had the highest mean cycle quantification (Cq) compared with the CTRL diet (Table 4). There was no significant difference ($P>0.05$) among the diets for *insulin growth factor-1* (*igf-1*), but there were slight variations among the diets. Interestingly, a significant interaction between *gh* and *igf-1* was also observed for all the diets with varying superscripts, except for the combination of HFM+CW having the highest expression values and same superscript on both *gh/igf-1*, indicating a good interaction level of the genes.

3. Relationship between *gh/igf-1* and weight gain

There was a significant difference ($P<0.05$) in the relationship between the *gh/igf-1* ratio and weight gain (g) of the fish, as shown in Fig. (4), with the highest in the HFM+CW diet and the lowest in the HFM diet.

4. Final weight of *Clarias gariepinus* fed the different diets

The final weights ranged from 17.73±0.01 to 108.9±0.007g, with the highest weight in the HFM+CW diet (108.9±0.007 g) and the lowest weight in the HFM diet (17.73±0.01g). There was a significant difference ($P< 0.05$) in the final weights of the fish, as shown in Fig. (5).

5. Specific growth rate of *Clarias gariepinus* fed the different diets

The specific growth rate (SGR) ranged from 0.93±0.009%/day to 2.32±0.003%/day, with the highest value in the combination of HFM+CW (2.32±0.003%/day) and the lowest value in the HFM (0.93±0.009%/day). There was a significant difference ($P<0.05$) in the SGR of the fish, as shown in Fig. (6).

6. Feed conversion ratio of *Clarias gariepinus* fed the different diets

The feed conversion ratio (FCR) ranged from 1.30 to 2.59 with the highest in HFM and the lowest in the combination of HFM+CW. The FCR of the fishes on the COMM diet was lower when compared with the HFM and HFM+CV diets but higher than CTRL, CW, CV, CV+CW and HFM+CW diets. There was a significant difference in FCR at $P<0.05$ across the diets (Fig. 7).

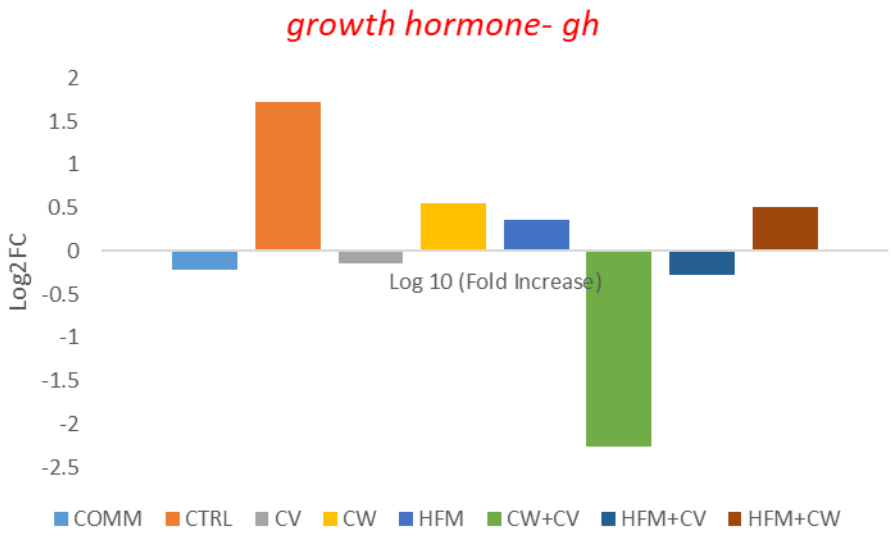

Fig. 2. The growth hormone (*gh*) gene expression in *Clarias gariepinus* fed a commercial diet or diets containing nonconventional ingredients

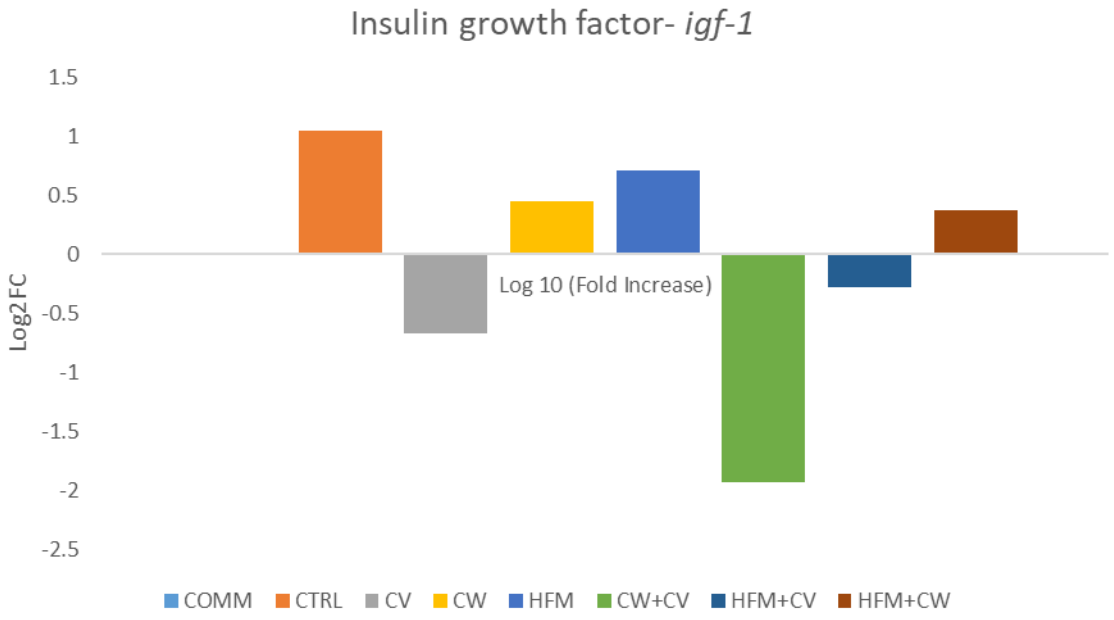

Fig. 3. The insulin growth factor (*igf-1*) in *Clarias gariepinus* fed a commercial diet or diets containing nonconventional ingredients

Table 4. Cycle quantification (Cq) of Clarias gariepinus fed a commercial diet and diets containing nonconventional ingredients (Mean±standard error)

Cq-Target Gene	**COMM**	**CTRL**	**CW**	**CV**	**HFM**	**CV+CW**	**HFM+CW**	**HFM+CV**	**F value**	**p value**
Growth Hormone (gh)	34.3±0.338^{ab}	31.0±0.971^{b}	32.2±0.134^{ab}	31.6±0.364^{b}	32.8±1.179^{ab}	33.8±0.182^{ab}	35.9±1.878^{a}	33.1±0.176^{ab}	3.27	0.024
Insulin growth factor-1 (igf-1)	35.2±0.556^{a}	36.1±0.612^{a}	35.3±0.566^{a}	36.9±1.658^{a}	34.6±1.455^{a}	35.9±0.668^{a}	37.3±1.602^{a}	36.2±0.000^{a}	0.76	0.628

Values are the means ± SEs of three fish diet replicates.

Mean values with different alphabetical superscripts in the same row are significantly different ($P< 0.05$).

Mean values with the same alphabetical superscripts in the same row are not significantly different ($P > 0.05$).

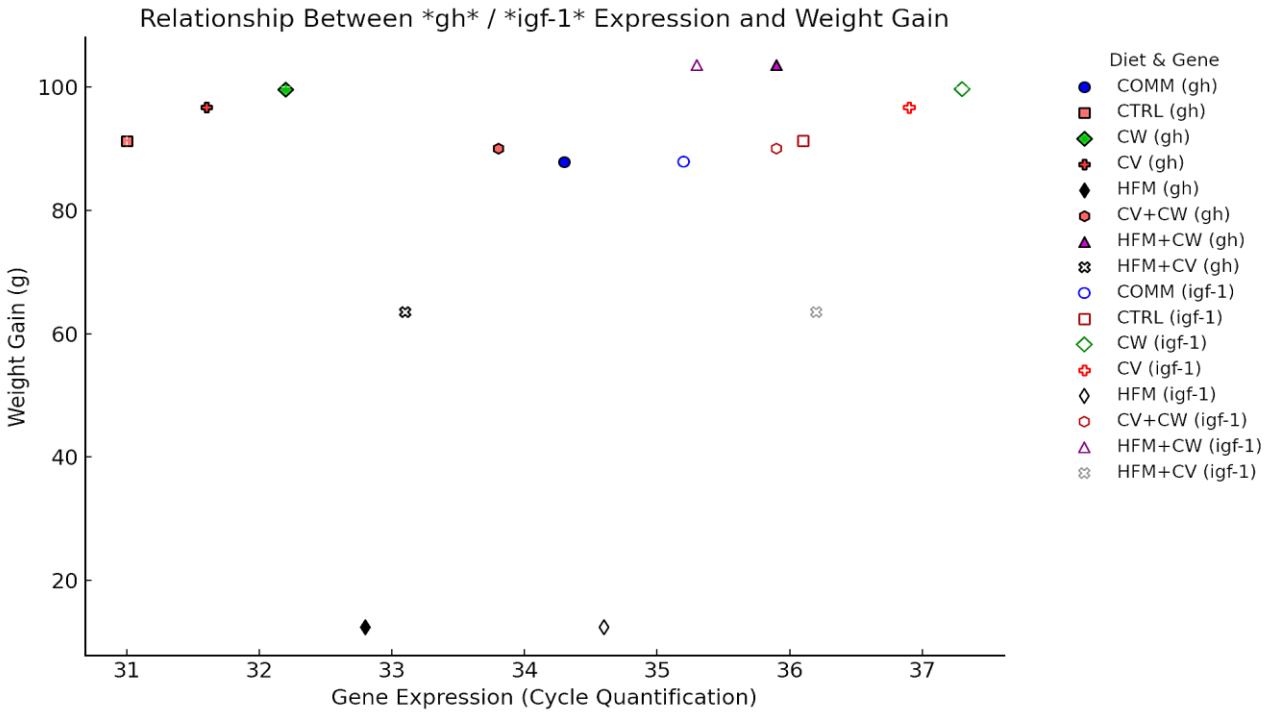

Fig. 4. Relationships between weight gain and gene expression in *Clarias gariepinus* fed a commercial diet or diets supplemented with nonconventional ingredients

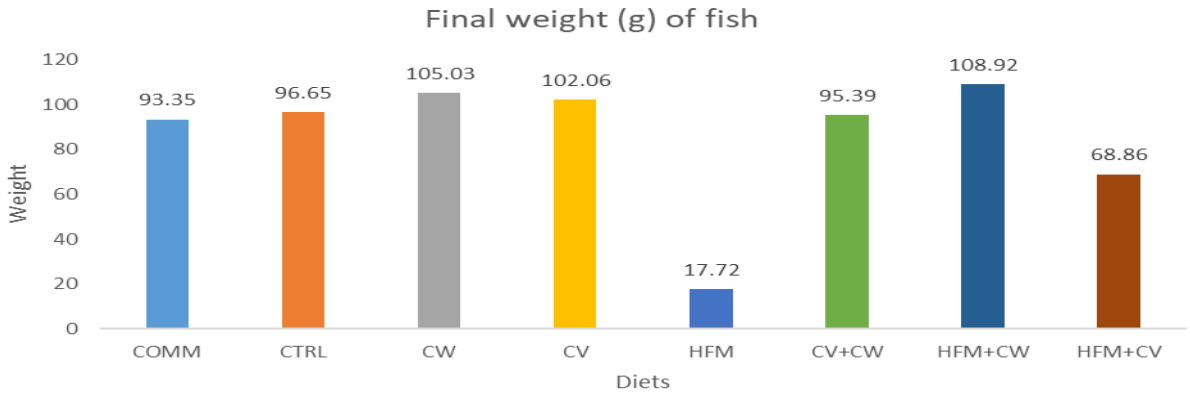

Fig. 5. Final weight (g) of *Clarias gariepinus* fed a commercial diet and diets containing nonconventional ingredients

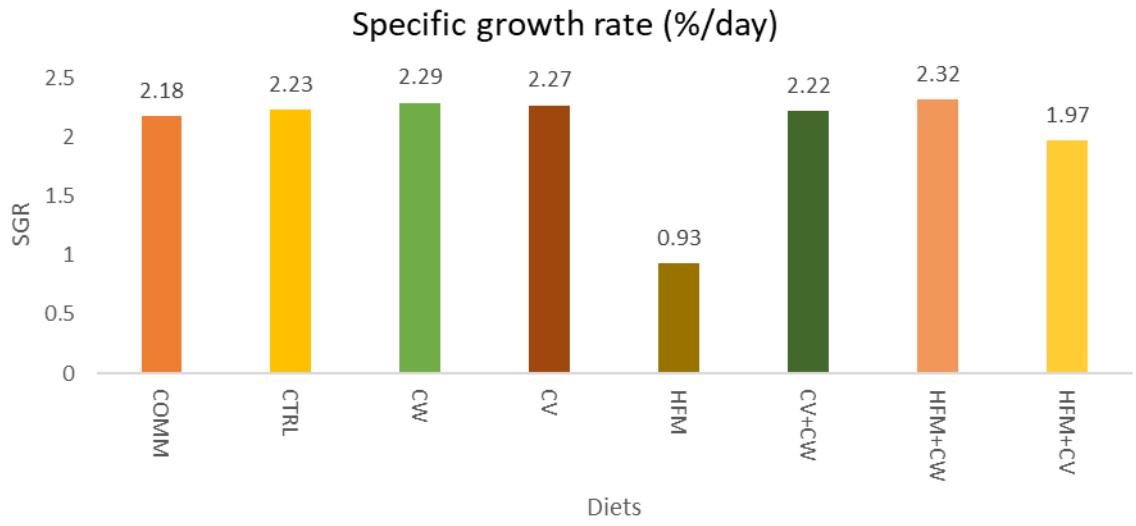

Fig. 6. Specific growth rate (%/day) of *Clarias gariepinus* fed a commercial diet and diets containing nonconventional ingredients

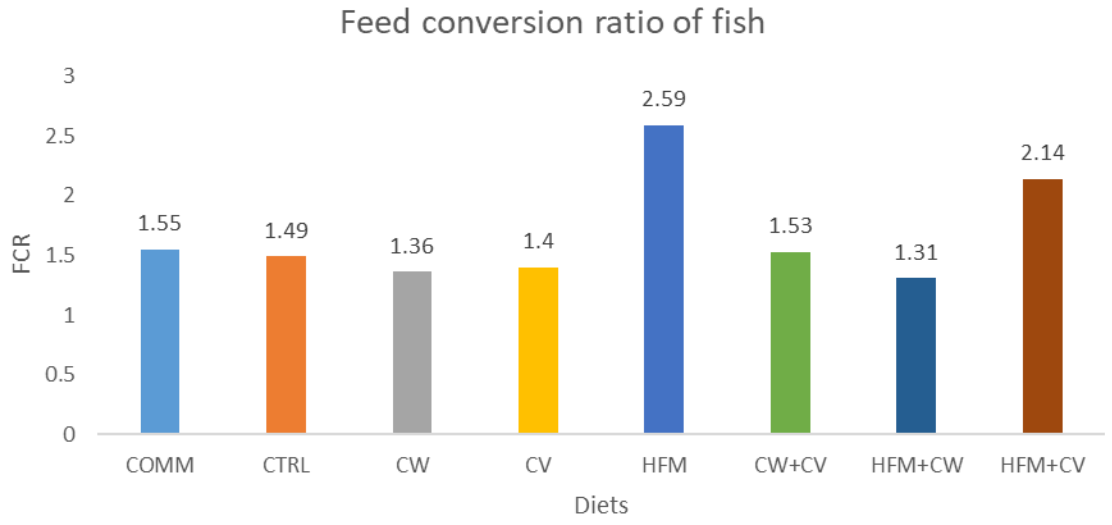

Fig. 7. Feed conversion ratio of *Clarias gariepinus* fed a commercial diet and diets containing nonconventional ingredients

DISCUSSION

Growth regulation in vertebrates, including fish, is primarily controlled by growth hormone (*gh*) and insulin-like growth factor (*Igf*) systems **(Sheridan, 2011; Kaneko *et al.*, 2019; Blanco, 2020)**. This study revealed that fish fed with a diet of HFM+CW

exhibited higher GH/IGF-1 gene expression in the liver compared to those fed alternative diets. This finding aligns with the trends reported by **Zhao *et al.* (2019)** and **Singha *et al.* (2020)** regarding dietary protein and tryptophan levels. Additionally, the upregulation of *gh* and *igf-1* in chicken gut meal was reported by **Peng *et al.* (2022)** in common carp. A review by **Ngasotter *et al.* (2023)** indicated that the application of chitin has led to the expression of specific growth-related genes (involved in plant growth- upregulation of growth-related genes in tomato roots treated with chitin nanofibers (ChNF) **(Egusa *et al.*, 2020)**, thereby promoting plant growth and nutrient utilization. Furthermore, crustacean meal has been associated with the upregulation of growth-related genes in the roots of lettuce and tomatoes **(Kandel *et al.*, 2022)**, which is consistent with the result of the study involving CW. This effect is attributed to chitin being a polymer of N-acetylglucosamine-glucose which serves as a major component that induces gene expression by signaling the liver and adipose tissue to regulate metabolism **(Vaulont *et al.*, 2000)**. Conversely, **Kumar *et al.* (2017)** and **Karapanagiotidis *et al.* (2019)** reported that high doses of poultry byproduct meal (PBM) in diets suppressed *gh/Igf-I* expression in the gilthead seabream, which is consistent with the observations made in the HFM+CV diet. The results of this study, in which *gh* was upregulated and *igf-1* was downregulated when CV was used to feed *Clarias gariepinus* align with the research conducted by **Peng *et al.* (2022)**. **Yuan *et al.* (2020)** observed an increased liver expression of the *gh/igf-1* axis in the juvenile blunt snout bream, with a partial replacement of fishmeal with cottonseed meal protein hydrolysate, which corresponds with the findings related to HFM in this study.

Growth performance is a valuable indicator of the nutritional status of fish **(Roques *et al.*, 2018)**. The dietary protein requirements of fish vary based on species, body size, feeding frequency, protein source, and other feeding conditions **(Alam *et al.*, 2008; Abdel-Tawwab *et al.*, 2010; Teles *et al.*, 2019)**. Poor growth rates in the catfish fed exclusively on feather meal may be attributed to the meal's high NFE content, which adversely affects feed utilization indices **(Familusi, 2020)**. Furthermore, the odor of chicken feather silage reduces fish appetite, and diets with a high inclusion of feather meal may suffer from experiencing a reduced palatability **(Somsueb & Boonyaratpalin, 2001; Rachmawati & Samidjan, 2019)**. In this study, final weight, mean weight gain, weight gain, and specific growth rate were at their highest values in correspondence with diet HFM+CW. When feather meal was blended with other protein sources, it did not negatively impact feed intake (%BW/day), thereby promoting growth **(Wang *et al.*, 2010; Xue *et al.*, 2012; Hu *et al.*, 2013)**. The superior results observed in the HFM+CW diet can be attributed to the low inclusion level of feather meal. This study aligns with that of **Bureau (2006)**, who recommended that feather meal inclusion should not exceed 5–10% due to its imbalanced amino acid profile and poor palatability. Crustacean meal serves as a flavor enhancer **(Shahidi & Wana, 1998)**, while *Clarias gariepinus* harbors

gut bacteria (*Bacillus cereus*) that secrete chitinase, facilitating chitin digestion and promoting increased feed consumption and growth **(Ajayi *et al.*, 2015; George & Onibokun, 2016)**. The low growth rates observed with HFM are consistent with the findings of **Wei-Kang *et al.* (2013)**, who reported that higher inclusion levels of feather meal negatively impacted growth performance in *Clarias gariepinus*. The mean length gain in the commercial diet was comparable to the results reported by **Torsabo *et al.* (2019)**, indicating that commercial diets (Vital feeds) effectively support growth. The feed conversion ratio (FCR) measures dietary efficiency, with lower values indicating better performance **(Oishi *et al.*, 2010)**. In this study, the combination of feather meal and crustacean waste (HFM+CW) produced the best FCR, consistent with the findings of **Özogul (2000)**, who associated the improved FCR with diets containing crustacean waste and low inclusion of feather meal. Crustacean waste enhances pigmentation by converting beta-carotene to astaxanthin **(Boonyaratpalin & Unprasert, 1989)**, which in turn improves feed efficiency. Similarly, **Poolsawat *et al.* (2021)** found no negative effects on nutrient utilization in tilapia when feather meal was included in their diet. The inclusion of feather meal and chicken viscera (HFM+CV) also supported an efficient FCR, attributed to the high ash and carbohydrate content in chicken viscera, which accelerates gut transit and feed intake **(Goda *et al.*, 2007)**. In contrast to the FCR results observed in this study, **Keremah (2013)** reported a decreased FCR in *Heterobranchus longifilis* fed crab meal, potentially due to increased protein metabolism. In comparison, the commercial diets (COMM) used in this study exhibited an FCR consistent with the findings of **Raimi *et al.* (2018)**.

CONCLUSION

The mRNA expression levels of the growth hormone gene (*gh/igf-1*) were found to be at their highest in fish fed a combination of feather meal and crustacean waste (HFM+CW), surpassing the performance observed with the commercial diet when compared to the control diet. The increase in growth hormone levels and overall growth performance were closely linked to the dietary combinations tested, confirming the role of nutrient composition in regulating the *gh/igf* axis in *Clarias gariepinus*. This finding aligns with the growth outcomes observed in both diets, where the commercial (COMM) diet demonstrated superior performance in terms of fish length, whereas HFM+CW exhibited better results in terms of fish weight, specific growth rate, and feed conversion ratio compared to the CTRL diet, thereby enhancing sustainable aquaculture.

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