Genetics of Aquatic Organisms 10(1), GA954
https://doi.org/10.4194/GA954

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RESEARCH PAPER

GENAQUA
GENETICS OF AQUATIC ORGANISMS

Growth Performance and Transcriptome Profiles of the Hybrid Between Hungarian and Vietnamese Common Carp (*Cyprinus carpio*)

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How to Cite

Luu, T.H.G., Nguyen, P.T., Vu, S.V., Gheyes, A., Kim, O.T.P., Vu, V.I. (2026). Growth Performance and Transcriptome Profiles of the Hybrid Between Hungarian and Vietnamese Common Carp (*Cyprinus carpio*). *Genetics of Aquatic Organisms, 10(1), GA954*. https://doi.org/10.4194/GA954

Article History
Received 21 September 2025
Accepted 04 January 2026
First Online 16 January 2026

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Keywords
Traits
Hybrid
RNA-Seq
Crossbred

Abstract

The Vietnam Hungarian hybrid carp has been used as broodstock in several selective breeding programs; however, its underlying transcriptomic mechanisms remain poorly understood. In this study, pure Vietnamese carp (V) were crossed with pure Hungarian carp (H) and growth performance was evaluated after one year of culture. The greatest heterosis was observed in the V♀×H♂ cross (VH) compared with the reciprocal cross (H♀ × V♂, HV) and pure Vietnamese carp (VV). To elucidate the molecular basis of this hybrid vigor, high-throughput RNA-Seq was performed on liver and muscle tissues from fast- and slow-growing individuals within VH hybrids. A total of 28 differentially expressed genes (DEGs) were identified, including 11 upregulated and 17 downregulated in fast-growing fish. Gene Ontology enrichment analysis showed that the upregulated DEGs were mainly associated with growth related processes, whereas the downregulated DEGs were primarily involved in cell polarity and immune responses. Notably, fast-growing VH hybrids exhibited a stronger genetic influence from Hungarian carp, as reflected in both morphological traits and gene expression patterns. Overall, these findings provide novel insights into the molecular regulation of growth in hybrid common carp and highlight the potential of selective breeding strategies to improve growth performance in common carp.

Introduction

Crossbreeding is a widely used and cost-effective strategy in fish breeding to enhance economically important traits, particularly growth performance, through heterosis effects that can rival gains achieved over multiple generations of selection (Rah et al*.,* 2013; Liu et al., 2017). In several aquaculture species, including tilapia, largemouth bass, and coral grouper, hybrids frequently exhibit superior growth and physiological performance compared with their parental lines (Dennis et al., 2020; Rasal et al., 2024; Hua et al., 2025). Recent transcriptomic studies, including mRNA and miRNA profiling, have further revealed the molecular basis of heterosis in hybrid tilapia (Xiao et al*.,* 2022). In common carp (*Cyprinus carpio* L.), systematic crossbreeding has long been practiced worldwide, and breeding programs in Vietnam using native and imported strains have achieved notable improvements in growth performance; however, inconsistent implementation has resulted in the loss of pedigree information in recent decades. To revitalize carp production, current efforts emphasize renewed crossbreeding between local common carp with Hungarian and Indonesian strains to enhance growth. Despite these practical successes, the molecular mechanisms underlying growth heterosis in

Vietnamese hybrid carp remain poorly understood, highlighting the need to integrate transcriptomic approaches, such as RNA sequencing, to identify growth-related genes and regulatory pathways and to improve selection efficiency and sustainable genetic improvement.

In this study, reciprocal crosses between Vietnamese and Hungarian common carp were established. Growth traits and survival rates were systematically compared among the two parental lines and their reciprocal hybrid lines. The objectives of this study were to (i) evaluate the presence and magnitude of heterosis between Vietnamese and Hungarian common carp; (ii) assess the relationships between body weight and standard length (body length) to support future selection decisions. (iii) identify candidate genes and biological pathways associated with by analyzing differential gene expression between fast- and slow-growing hybrids using transcriptomic approaches. The finding of this study provides a scientific foundation for improving growth performance and productivity in common carp aquaculture.

Material and Methods

Fish Material and Mating Design

Vietnamese (V) and Hungarian (H) purebred scaled common carp (*Cyprinus carpio)* were cultured for broodstock maturation at Research Institute for Aquaculture No. 1 (RIA1) hatchery located in Bac Ninh province, Vietnam. Sexually mature and healthy male and female fish were selected for breeding. A (2×2) diallel crossing design was applied between the two purebred lines, generating two purebred groups: 1) Pure Vietnamese common carp (V♀×V♂, VV) and 2) Pure Hungarian common carp (H♀×H♂, HH) as well as their reciprocal hybrids (V♀×H♂, VH; H♀×V♂, HV). In total, 24 parent fish from the two pure original lines (six males and six females each line) were used for inducing spawning by injecting with Luteinizing hormone-releasing Hormone Agonist (LRH-A) (10 µg/kg for sires and 30 µg/kg for dams) in combination with Domperidone (DOM) (3 mg/kg for sires and 10 mg/kg for dams) purchased from Dopa Aquatic Environment & Agricultural Materials Center, China. Following the hormone administration, males and females were maintained in separate tanks for 10 -12 hours. Eggs collected from the six females of each line were pooled and split into two equal portions: one portion was fertilized with a pooled sperm from the other line to produce hybrid crosses (VH and HV), whereas the remaining portion was fertilized with pooled sperm from the same line to produce purebred lines (VV and HH). Fertilized eggs from each cross were incubated separately in replicated 5L incubator under a continuous flow-through water system. Water temperature was maintained at 18–20°C throughout the incubation period.

Fry Rearing

Fish larvae started exogenous feeding on Day 4 after hatching. Subsequently, 2000 fish fry from each line (HH, VV, VH and HV) were transferred to and reared in separate net cages (5 m^2 each) for 60 days. Each hybrid line was replicated twice in the net cages. The pond was equipped with paddle wheel aerators to ensure adequate oxygen supply to each cage. All fry were fed the same diet, consisting of live food (zooplankton), soybean milk, egg yolk with feeding regimes adjusted according to the developmental stage and growth rate.

After 8 weeks of rearing, fry developed into fingerlings. Subsequently, 400 fish (100 fish for each line) were tagged with passive integrated transponder (PIT) tags and co-stocked into a 2000 m^2 pond with three replicate groups. Fish were reared until one year of age and fed at 3–5% of body weight per day, twice a day at 9:00 and 4:00 pm.

Measurement of Performance and Heterosis

Experimental fish were sampled every four weeks (30 individuals sampled per line from each pond). Fish were anaesthetized with 100 mg/L MS-222 (Tricaine Methanesulfonate, Western Chemical Inc., USA) to prevent injury and facilitate handling, measurement, and counting. Morphological traits, including body weight and standard length were measured using a digital balance (Pioneer PR Series 2200g, 0.01g, OHAUS PR2202JP/E) and a caliper (Terrinox TR150E 300mm with an accuracy 0.01 mm). The number of fish remaining alive after 365 days of rearing was recorded to calculate the survival rate. The absolute growth rate (AGR; g $fish^{-1}$ day^{-1}) was calculated using the following formula:

$$AGR\ (g/day) = \frac{W2 - W1}{t}$$

Where, W1 = initial weight of the fish (g); W2 = Weight of the fish at time t (g)

Heterosis, also known as hybrid vigour, is typically quantified using mid-parent heterosis (MPH) (Falconer & Mackay, 1996), which evaluates the extent to which the average performance of F1 hybrids exceeds the mean performance of their parental lines. Additionally, single-parent heterosis (SPH) quantifies the proportional improvement in the phenotypic value of a hybrid relative to that of one parent involved in the cross (Zheng et al., 2006; Le et al., 2023). Heterosis values were calculated using the following formulae:

$$\mathrm{MHP}(\%) = \frac{\text{Mean of F1} - \text{Mean of both parents}}{\text{Mean of both parents}}$$

$$\mathrm{SPH}\ (\%) = \frac{\text{Mean of F1} - \text{Mean of a single parent}}{\text{Mean of the single parent}}$$

Statistics

The phenotypic differences among four lines for each of the four performance parameters (survival, standard length, body weight, and growth rate) at different developmental stages were analyzed using one-way analysis of variance (ANOVA). Data were examined for outliers and compliance with assumptions of normality and homogeneity of variances. As these assumptions were satisfied ($P>0.05$), no data transformation was required. The correlations between body weight, body length, were calculated according to the description of Du and Chen (2010). Cochran's test was used to assess the homogeneity of the variances. In cases where a significant variance difference was detected, the means were evaluated using Tukey's Honest Significant Difference (HSD) test ($P<0.05$). Strain analysis was conducted using Spotfire Statistica version 14.3 (TIBCO Software Inc., 2025).

Transcriptome and Differential Expression Analysis

In this study, fish samples selected for RNA-Seq were chosen based on weight gain (WG, mg) after 365 days of culture from the VH and HV hybrid groups. Based on final body weight, individuals in this cohort of hybrid common carp were categorized into two phenotypic groups: fast-growing (FG; 608.5±45.4g), and slow-growing (SG, 424.6±33.5g). Muscle and liver tissues were collected from each fish, placed in labelled 1.5 mL tubes containing RNAlater solution, and stored at -80°C until RNA extraction. Only male individuals were selected in order to eliminate sex-related variation in transcriptomic analysis.

To minimize technical variation arising from library preparation and sequencing, RNA samples were first normalized to a uniform concentration and pooled prior to sequencing, with pooling performed separately for each phenotypic group. For each pool, equal amounts of RNA extracted from six individual samples were combined, ensuring that each individual contributed equally to the pooled sample, thereby generating three independent biological replicates. In total, 12 pooled RNA samples were prepared, representing two phenotypic groups (fast- and slow-growing), and tissue type (muscle and liver), with three biological replicates for each condition. The slow-growing (SG) group comprised muscle pools T1-T3 and liver pools G1-G3, whereas the fast-growing (FG) group included muscle pools T4-T6 and liver pools G4-G6.

Individual tissues were homogenized in TRIzol® reagent (Invitrogen) for total RNA extraction following the manufacturer's instructions. RNA quality was evaluated prior to library preparation, and only samples with an RNA Integrity Number (RIN≥8) were used. Library construction was performed using TruSeq RNA Sample Preparation v2 kit (Illumina, San Diego, CA, USA). Briefly, 3 µg of total RNA was purified to retain only mRNA by using poly-T oligo-attached magnetic beads. The purified mRNA was subsequently fragmented and reverse-transcribed into cDNA. The created cDNA underwent end-repaired and 3' adenylation, followed by adapter ligation. The pooled libraries were sequenced on the illumina NovaSeq 6000 plateform using a 150 bp paired-end configuration in accordance with Illumina specifications. Sequencing was designed to achieve a target depth of approximately 40-60 million paired-end reads per sample, corresponidng to an estimated average effective transcriptome coverage of 25x. Raw sequencing quality was assessed using FastQC v0.11.2 (Andrews, 2023). Low-quality reads and residual adapter sequences were removed using Trimmomatic v0.39 (Bolger et al., 2023). Clean reads were aligned to the *Cyprinus carpio* reference genome (GCA_905221575.1) using HISAT2 v2.2.1 (Kim et al., 2019). Gene-level expression qualification was performed by counting mapped fragments with the featureCount v2.1.1 (Liao et al., 2025). Differential Gene Expressions analysis was conducted using the DESeq2 v1.50.2 package (Love et al., 2024), which applies a negative binomial model while accounting for tissue type, growth phenotype, and the first surrogate variable identified by the *sva* v3.52.0 package (Leek et al., 2024). Comparison between fast-growing and slow-growing groups yielded lists of differentially expressed genes (DEGs). Functional annotation and enrichment analysis of DEGs were carried out and visualised using ShinyGO 0.81 (Ge et al., 2024) and the g:Profiler web tools (Kolberg et al., 2023).

Gene-set Enrichment Analysis

Genes identified from the differential expression analysis were ranked using the DESeq2 "*stat*" result, with positive and negative values indicating up- and down-regulation, respectively. Using the ortholog conversion tool from the g:Profiler package (Kolberg et al., 2023), common carp gene IDs were mapped to their corresponding zebrafish (*Danio rerio*) orthologs. Duplicated entries were subsequently removed and only the most recent gene ID versions were retained.

To further understand the biological implication of genome-wide expression differences between fast- and slow-growing fish, gene-set enrichment analysis (GSEA) was performed to identify significant enriched pathways. GSEA was performed using WebGestalt 2024 (Elizarraras et al*.,* 2024) with default parameters, drawing on multiple zebrafish-annotated functional databases.

Results

Growth and Survival Performances Among Four Lines

Growth performance of two purebreds (VV and HH) and two crossbreds (VH, HV) from Day 90 to Day 365 is summarized in Table 1. The mean body weight of both crossbred lines and the HH purebred line was

significantly greater than that of the VV purebred line (P<0.05). Throughout the early grow-out period, the HH strain consistently exhibited the highest average body weight. However, at Day 280 and 365, the VH line demonstrated superior growth performance, recording as the highest mean body weight (P<0.05).

There was no significant difference in body weight throughout the entire grow-out period between the two reciprocal crosses (VH and HV). However, VH displayed higher yields than HV at Day 365. The mean standard length of the VV was significantly lower than that of the other lines over the whole study period (P<0.05). The highest mean standard length was consistently observed in the HH line during the study. Mean standard length differed significantly between the two crossbred lines (VH, HV) on Day 250, 280 and 365 (P<0.05). The lowest absolute growth rates (AGR) among the four groups were observed between Day 250 and Day 280. In contrast, the highest AGR values in the VH line (3.18 and 3.06 g d−1, respectively) were recorded during two-time intervals: (1) Day 90-124 and (2) Day 281-365. The average survival rate of communally cultured common carp at Day 365 also varied among groups, with an overall mean of 51.25%. The highest survival rate was observed in the VV line (71.77%), followed by HV (70.05%) and VH (68.98%) (Figure 1).

Evaluation of Heterosis

Figure 2 presents the values of mid-parent heterosis ((H_M)) and single-parent heterosis relative to Vietnamese carp parent (H_V)) and Hungarian fish parent (H_H)) for hybrid offspring from two reciprocal crosses: HV (H♀×V♂) and VH (V♀×H♂), evaluated at different

Table 1. Growth characteristics of purebred and crossbred fish at different time points

	Standard length (cm)	Body weight (gram)	Growth rate (g/day)
90 days			
HV	12.42±0.78^{b}	31.16±7.99^{ab}	
VH	12.75±1.28^{bc}	34.15±7.60^{b}	
HH	13.33±0.91^{c}	40.59±9.63^{c}	
VV	11.75±1.38^{a}	28.28±11.75^{a}	
250 days			
HV	31.06±1.32^{a}	452.02±47.13^{b}	2.55±0.45^{b}
VH	32.65±1.77^{b}	458.53±65.60^{b}	2.58±0.44^{b}
HH	33.62±1.55^{c}	461.68±57.64^{b}	2.58±0.43^{b}
VV	30.41±1.67^{a}	368.01±56.11^{a}	2.00±0.39^{a}
280 days			
HV	31.75±1.32^{b}	513.03±48.64^{b}	2.02±0.29^{a}
VH	33.34±1.77^{c}	525.03±64.51^{b}	2.21±0.25^{c}
HH	34.07±1.54^{c}	524.72±57.85^{b}	2.10±0.26^{bc}
VV	30.87±1.66^{a}	423.86±56.93^{a}	1.86±0.20^{a}
365 days			
HV	32.96±1.32^{b}	741.55±56.83^{b}	2.69±0.34^{a}
VH	35.40±1.77^{c}	785.17±65.60^{c}	3.06±0.23^{c}
HH	35.37±1.55^{c}	754.90±61.78^{c}	2.71±0.19b^{c}
VV	31.85±1.67^{a}	609.06±58.19^{a}	2.19±0.18^{a}

* Growth traits (standard length, SL body weight, BW; absolute growth rate, AGR) of four lines at different rearing periods. Data are presented as mean±SD, and the different letters within the same column indicate statistically significant differences (P<0.05). V and H denote for Vietnamese and Hungarian common carp lines, respectively.

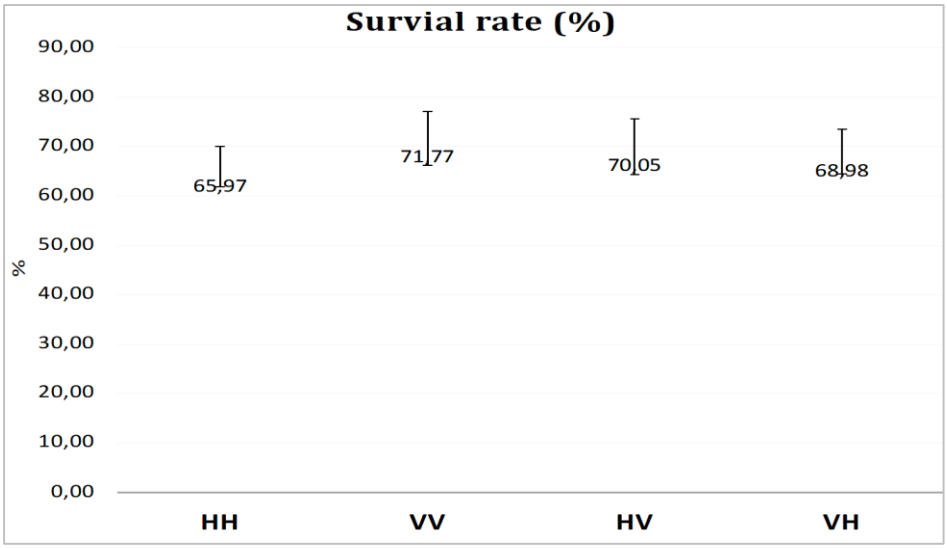

Figure 1. Survival rate of purebreds and crossbred fish reared in ponds.

ages. H_M for body weight ranged from 8.96% to 48.19%. Both H_M and H_V heterosis values for body weight were consistently positive but notable negative H_H heterosis was observed for body weight in the HV hybrid during the grow-out stage. For the VH hybrid, heterosis values for body weight were generally positive across sampling periods, except for a negative H_V heterosis value at Day 250. After Day 365, heterosis values for body weight in VH exceeded those observed in HV, with H_M, H_H, and H_V reaching 15.13%, 28.91% and 4.01%, respectively. However, no significant differences were detected among heterosis estimates (P>0.05).

Correlation Analysis Among Growth-related Traits.

The individual correlations between standard length and body weight was presented in Table 2. The correlations between body weight and standard length were significantly high, reaching to ~0.999 for HV, VH and HH at Day 250. When the data of all groups were pooled, standard length also showed a high correlation with body weight (P<0.05).

Transcriptome Characterization and Differential Expression Analysis

A total 12 cDNA libraries were constructed from two tissue types: liver (G1–G6) and muscle (T1–T6), derived from fast- and slow-growing groups of VH and HV crossbreds. The sequencing data are deposited in the NCBI Sequence Read Archive under accession number PRJNA1225025. These libraries were subjected to high-throughput RNA sequencing, and the resulting data were mapped to the common carp reference genome and assigned to genomic features for gene expression quantification. Supplementary Figure 1 and Supplementary Table 1 summaries the quality control metrics for these 12 sequencing samples, processed using Samtools, HISAT2, and featureCounts. Alignment rates were high (92.34%-96.50%), with muscle samples (T1-T6) mapping slightly better than liver samples (G1-G6). Total read counts ranged from 47.08M to 64.91M, with over 92% of reads mapping, indicating good sequencing quality (Supplementary Figure 1A). HISAT2 paired-end alignment showed most reads mapped uniquely (18.1M–24.6M per sample), with 82.00%–86.49% uniquely mapped reads and low level of multimapping (3.79%–6.12%). The proportion of unaligned reads were higher in liver samples, reaching up to 7.66% in G1 (Supplementary Figure 1B). FeatureCounts analysis revealed 68.76%-75.82% of reads assigned to genomic features, while unassigned reads due to low mapping quality, chimeric alignments, or annotation gaps. Although the proportion of unmapped reads was low (~1.3%–3.1%), suggesting effective read assignment overall, elevated multi-mapping was elevated in G2 (20.15%) and T3 (17.93%), and G5 exhibited the highest proportion of unassigned reads (13.62%) (Supplementary Figure 1C).

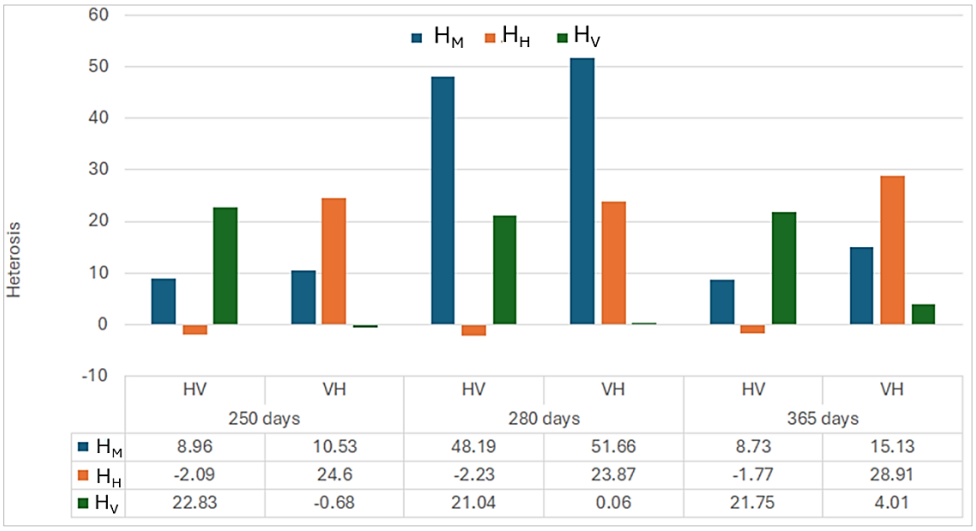

Figure 2. Mean mid-parent and single-parent heterosis for body weight for VH and HV crossbreds at different culture times. H_M: mid-parent heterosis, H_V: Vietnamese common carp heterosis, H_H: Hungarian common carp heterosis.

Table 2. Correlation coefficient (Rw) between standard length (SL) and body weight (BW) in VH, HV crossbreds and VV, HH purebreds

Lines	Day 90	Day 250	Day 280	Day 365
HV	0.918**	0.999**	0.802**	0.729**
VH	0.820**	0.998**	0.975**	0.927**
HH	0.920**	0.999**	0.955**	0.912**
VV	0.687**	0.982**	0.834**	0.809**

** Statistically significant (P<0.05).

The expression of individual gene copies is measured using the featureCounts tool in conjunction with the *Cyprinus carpio* annotation file (Cypcar_WagV4.0.113.gtf), which includes 162,529 gene transcripts and 44,807 coding genes. The statistical data on quantified gene expression are shown in Table 3. Overall, gene expression differences between slow-growing and fast-growing fish were minor. Fast-growing fish exhibited a higher total number of expressed genes (~34,522) compared to slow-growing fish (~33,665), while the mean expression count per expressed gene was lower (721.5 vs 801.2). Additionally, our data indicate that the liver contains approximately 10% more expressed genes than muscle tissue (Supplementary Data 1), reflecting abundant biological activity in the liver, which may require the coordinated expression of a greater number of genes.

RNA sequencing analysis of two fish groups revealed distinct gene expression profiles, providing insights into the molecular mechanisms underlying their phenotypic differences. Using a differential expression FDR cutoff of 0.05 and a fold change >2 (i.e., $\log_2$FC>1), we identified 28 differentially expressed genes, with 11 upregulated and 17 downregulated in fast-growing fish compared to slow-growing fish (Table 4). Gene symbols and biotypes were annotated using the g:Convert tool with Ensembl version 112 from the g:Profiler web server. The volcano plot in Figure 3 illustrates the selection of significant differentially expressed genes (DEGs) for the upregulated and down-regulated gene lists. Detailed information on all DEGs is provided in Supplementary Data 2.

Gene Ontology (GO) enrichment analysis of DEGs revealed significant enrichment across biological process (BP), cellular component (CC), and molecular function (MF) categories. Based on enrichment results obtained using the ShinyGO tool, upregulated genes were significantly enriched in growth-related functions (GO:0019825, GO:0005520) and oxygen transport activity (GO:0015671, GO:0019825, GO:0005833) (Figure 4A), indicating enhanced growth-related signaling and increased respiratory capacity in fast-growing fish, potentially supporting swimming activity and muscle development. Notably, transcripts of *igfbp3* and *igfbp1a*, which encode insulin-like growth factor-binding proteins, were upregulated in the fast-growing group, further supporting their role in growth regulation. Consistent with the observed phenotypic differences, the fast-growing group (608.5±45.4g) exhibited a significantly higher body weight than the slow-growing group (424.6±33.5g). In contrast, the down-regulated gene set showed limited functional enrichment, with only a small number of GO terms identified, primarily associated with cell polarity. Each of these GO terms was supported by a single gene (Figure 4). Among the down-regulated genes (Table 4), the *cd9a* gene (ENSCCRG00000057857), which is known to play an important role in immune responses, was identified, suggesting a relatively higher immune activity in the slow-growing fish.

From gene-set enrichment analysis (GSEA), all available common carp orthologs (n=25,558) were converted to their corresponding zebrafish (*Danio rerio*) orthologs, a closely related species, and ranked based on differential expression analysis, enabling GSEA across multiple well- established functional databases. KEGG-based analysis revealed clear pathway-level differences between fast- and slow-growing fish (Figure 5A).

Table 3. Gene expression statistics

Sample	Slow-growing sample (mean±sd)	Fast-growing sample (mean±sd)
Total expressed genes	33,664.8±2,034.4	34,521.8±2,054.1
Total expression counts (per sample)	26,908,272.2±2,446,478.13	24,863,868.3±2,080,358.2
Mean expression count per expressed gene	801.2	721.5

Table 4. List of significanty upregulated and downregulated genes in the comparison between fast-growing and slow-growing fish

Upregulated genes			Downregulated		
Ensembl ID	Symbol	Type	Ensembl ID	Symbol	Type
ENSCCRG00000072964		protein_coding	ENSCCRG00000068140		lncRNA
ENSCCRG00000063287		lncRNA	ENSCCRG00000019851		protein_coding
ENSCCRG00000055189	adamts8a	protein_coding	ENSCCRG00000079234		protein_coding
ENSCCRG00000078979	igfbp1a	protein_coding	ENSCCRG00000035048		protein_coding
ENSCCRG00000082123	igfbp3	protein_coding	ENSCCRG00000063980	fyba	protein_coding
ENSCCRG00000074530		protein_coding	ENSCCRG00000057118		protein_coding
ENSCCRG00000075400		protein_coding	ENSCCRG00000015940		protein_coding
ENSCCRG00000035718	creb5a	protein_coding	ENSCCRG00000009781	si:dkey-156m2.3	protein_coding
ENSCCRG00000080178	gpr1	protein_coding	ENSCCRG00000057857	cd9a	protein_coding
ENSCCRG00000065610	si:ch1073-475a24.1	protein_coding	ENSCCRG00000075775		protein_coding
ENSCCRG00000042808	per1a	protein_coding	ENSCCRG00000067865		IG_C_gene
			ENSCCRG00000033025	pnck	protein_coding
			ENSCCRG00000008021	pdzd11	protein_coding
			ENSCCRG00000068657		lncRNA
			ENSCCRG00000030515	mrps36	protein_coding
			ENSCCRG00000048219	sepsecs	protein_coding
			ENSCCRG00000041062	rps26	protein_coding

Pathways enriched in fast-growing individuals, as indicated by positive normalized enrichment scores (positive-NES), were primarily associated with cell-environment interactions and signal transduction, including ECM-receptor interaction, focal adhesion, cell adhesion molecules, and the MAPK and Notch signaling pathways. Muscle-related pathways, such as cardiac and vascular smooth muscle contraction, were also positively enriched. Among these pathways, ECM-receptor interaction showed the strongest and statistically significant enrichment (FDR≤0.05), whereas the remaining pathways exhibited moderate but consistent enrichment trends. Conversely, pathways enriched in slow-growing fish (negative NES) were predominantly related to core cellular and molecular processes, including proteasome, ribosome and ribosome biogenesis, DNA replication, RNA polymerase activity, mismatch repair, and aminoacyl-tRNA

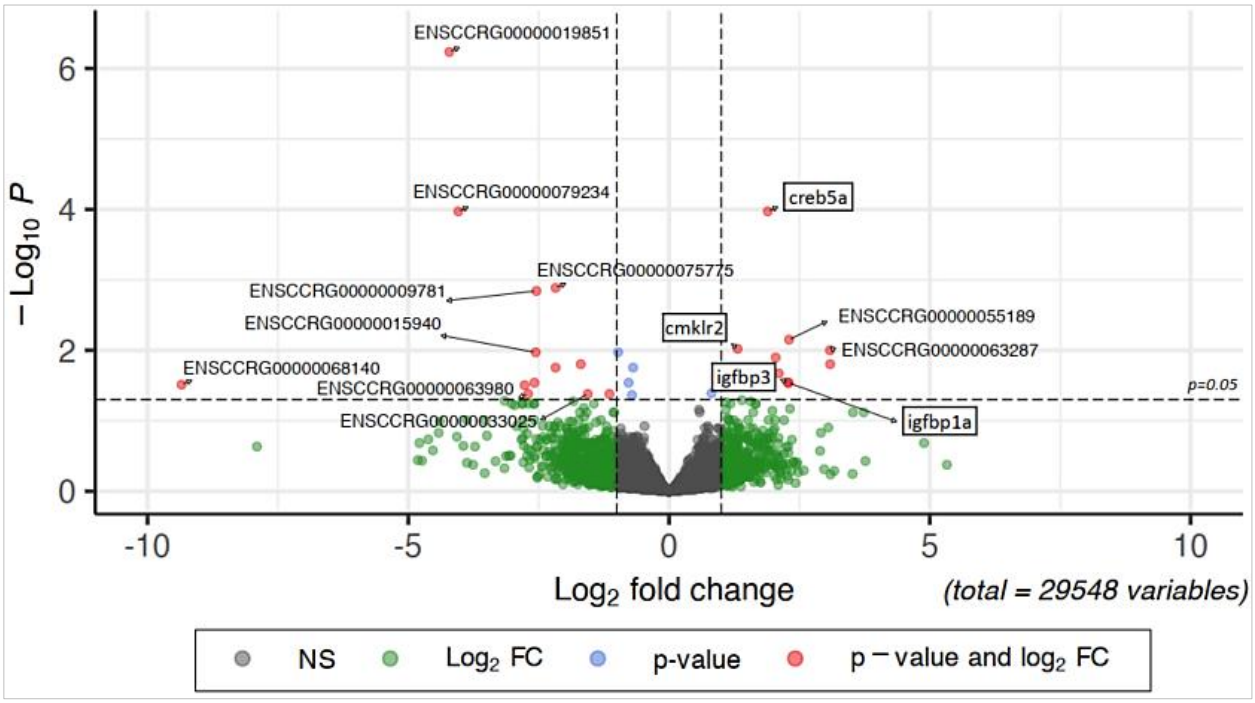

Figure 3. Distribution of genes based on differential expression fold change (Log_2FC) and statistical significance ($-Log_{10}$ adjusted p-value). Green and blue dots represent genes meeting the thresholds of $Log_2FC>1$ and adjusted $P<0.05$, respectively, while red dots indicate genes that satisfy both criteria.

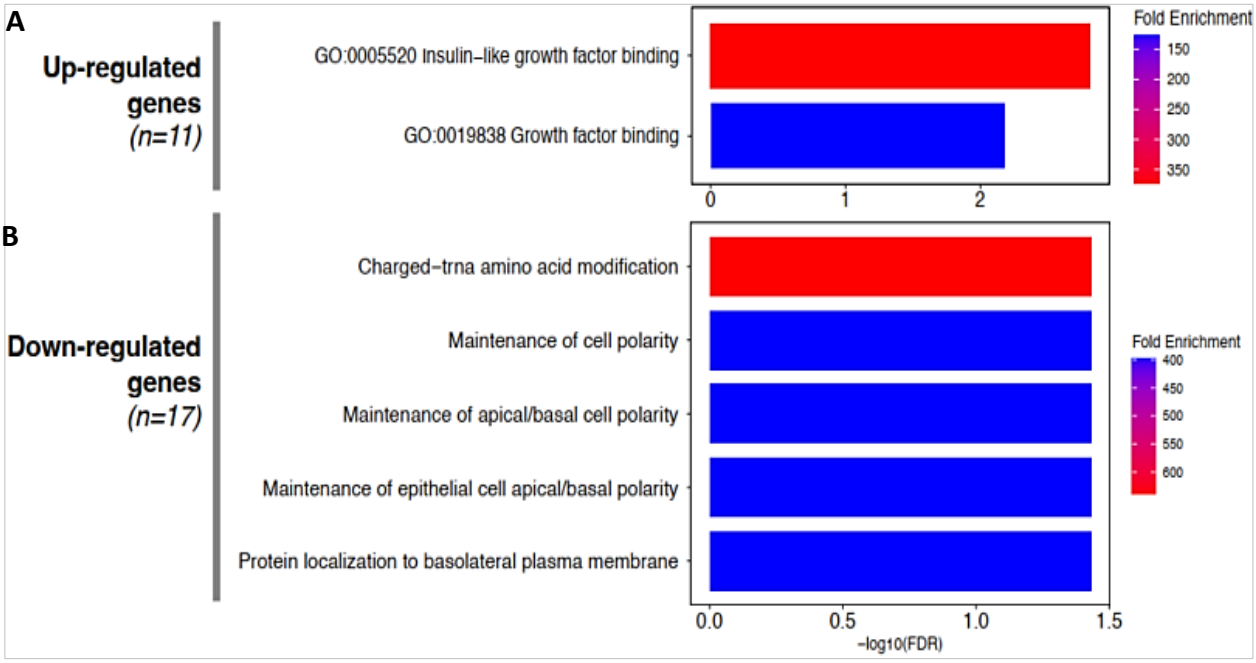

Figure 4. (A) Significant GO terms enriched in upregulated DEGs and on (B) downregulated DEGs of fast-growing fish compared with slow-growing fishes.

biosynthesis. Most of these pathways met the FDR≤0.05 threshold, indicating robust enrichment.

Similarly, GSEA based on the Reactome database revealed distinct functional enrichment patterns between fast- and slow-growing fish (Figure 5B). Pathways with significant positive normalized enrichment scores (NES; FDR≤0.05) in fast-growing fish were primarily related to erythrocyte function and gas exchange, including oxygen uptake and carbon dioxide release by erythrocytes, as well as O_2/CO_2 exchange processes. In contrast, pathways with significant negative NES, representing slow-growing fish, were predominantly associated with cell cycle regulation and mitotic progression, including cell cycle checkpoints, mitotic phases, separation of sister chromatids, and antigen processing–related pathways (Figure 5B).

Discussion

The growth potential of fish is primarily determined by genetic inheritance from both parental lines. This pattern was also evident in this study, which evaluated the growth performance of two purebred common carp strains (Hungarian and Vietnamese) and their reciprocal hybrids. Our results indicate that parental strains influenced both weight and length growth of the offspring. This influence is herein referred to as the hybrid combination effect. Collectively, these findings demonstrate that the genetic composition of the parental strains directly impacts the growth performance of their hybrid progeny. Consistent with our results, previous studies have shown that hybridization can provides valuable insights into parental genetic architecture, as crosses between genetically divergent strains may generate hybrids exhibiting non-additive genetic effects resulting from complex gene interactions (In et al., 2017; Audet et al*.,* 2025). These effects could be expressed as heterosis, where hybrid performance exceeds that of both parents, or as outbreeding depression, depending on the specific strain combination used for crossing (Chaivichoo et al*.,* 2020).

The highest growth performance was observed in the pure Hungarian carp strain, whereas the lowest values were recorded in the pure Vietnamese carp strain. These findings are consistent with previous studies, as Hungarian carp strains have undergone selective breeding and are adapted to the environmental conditions of aquaculture in Vietnam (Son et al., 2024). Among the crossbred groups, growth patterns varied across different stages. No clear size differences between hybrid lines were detected at the juvenile stage (90 days of age). However, after one year of growth, significant differences in growth performance were observed among the crossbred strains ($P<0.05$).

The VH crossbreds emerged as a promising candidate for further investigation of growth traits and genetic improvement in this study. VH crossbred fish exhibited superior performance in standard length, body weight, and growth rate (35.40±1.77cm; 785.17±65.60g; and 3.06±0.23 g/day, respectively) compared to other crossbred combinations. These results indicate that VH crossbreds demonstrate better growth performance in the rearing system. Given their strong growth traits and survival rates, VH crossbreds represent a promising variety for commercial production and further selective breeding.

The HV crossbred fish displayed positive mid-parent heterosis across all growth traits evaluated in this study. Similar patterns have been reported in previous studies on Rohu carp and other carp species (Gjerde et al., 2002; Nielsen et al., 2010). Crosses between genetically differentiated subpopulations are expected to increase heterozygosity, reduce the expression of recessive lethal genes and enhance overall fitness, resulting in heterosis (Sang et al., 2021; Whitlock et al., 2000). The results from this study suggested that heterosis in growth traits may be attributed to the genetic divergence between the Hungarian and the Vietnamese strains. Similar findings have been reported in other aquaculture species. A positive correlation between heterosis in growth and genetic distance was observed in both intraspecific hybrids of *Cyprinus carpio* and interspecific hybrids of *Oreochromis* spp (Wang & Xia, 2002). Similarly, a significant correlation was reported between genetic distance and heterosis in catfish (Koolboon et al., 2014).

In contrast to HV, the VH crossbreds exhibited lower heterosis in body weight and absolute growth rate (AGR). Moreover, crosses in which Hungarian common carp served as dams (VH and HH) achieved the highest performance across all growth traits throughout the entire grow-out stage. These findings highlight the substantial maternal influence on body weight. Consequently, the variations in growth traits between VH and HV, especially in comparison to VV purebreds, may be partly due to the maternal effect of the Hungarian carp. The maternal effect on growth has also been widely reported in other species, such as *Neogobius melanostomus* (Adrian-Kalchhause et al., 2018) and *Neolamprologus pulcher* (Reyes-Contreras et al., 2023). These findings suggest that both heterosis and maternal effects contribute to the outstanding growth performance of VH crossbreds.

To date, different statistical approaches have been utilized to assess the relationships between significant traits, including correlation assessment, path analysis, and regression methods. For instance, a strong significant relationship between standard length, body weight, and body fat content in cultured red sea bream was confirmed by correlation analyses (Kora et al. (2000). Additionally, key factors influencing body weight have been identified in other fish species, including sea trout (*Salmo trutta*) (Debowski et al., 1999) and salmon (*Salmo salar*) (Liu et al., 2014). In our present study, standard length significant direct effects on body weight ($P<0.05$). The correlation coefficients between these

traits were also high (0.81–0.92, Table 2). Hence, standard length could be considered the primary factors influencing the body weight in VH, VV, and HH.

This study employed VH and HV hybrids as study materials for transcriptome analysis to elucidate the molecular and regulatory mechanisms underlying growth traits. When comparing the transcriptomes of fast-growing and slow-growing groups, the fast-growing fish exhibited a higher average number of expressed genes and higher overall expression levels than the slow-growing group. This discrepancy may indicate variations in biological activity between the two groups, reflecting differences in developmental stages. Specifically, these patterns suggest that the sample groups may have undergone distinct developmental processes, such as the growth of specific cells or organs. The reduction in the total number of expressed genes and expression levels in one group compared with the other suggests gene regulation in response to differing developmental demands.

When analysing gene expression differences between the fast-growing and slow-growing fish groups, a total of 28 DEGs were identified, with 11 genes upregulated and 17 genes down-regulated. The results of GO enrichment revealed that "insulin-like growth factor binding", "gas transport", and "oxygen transport"

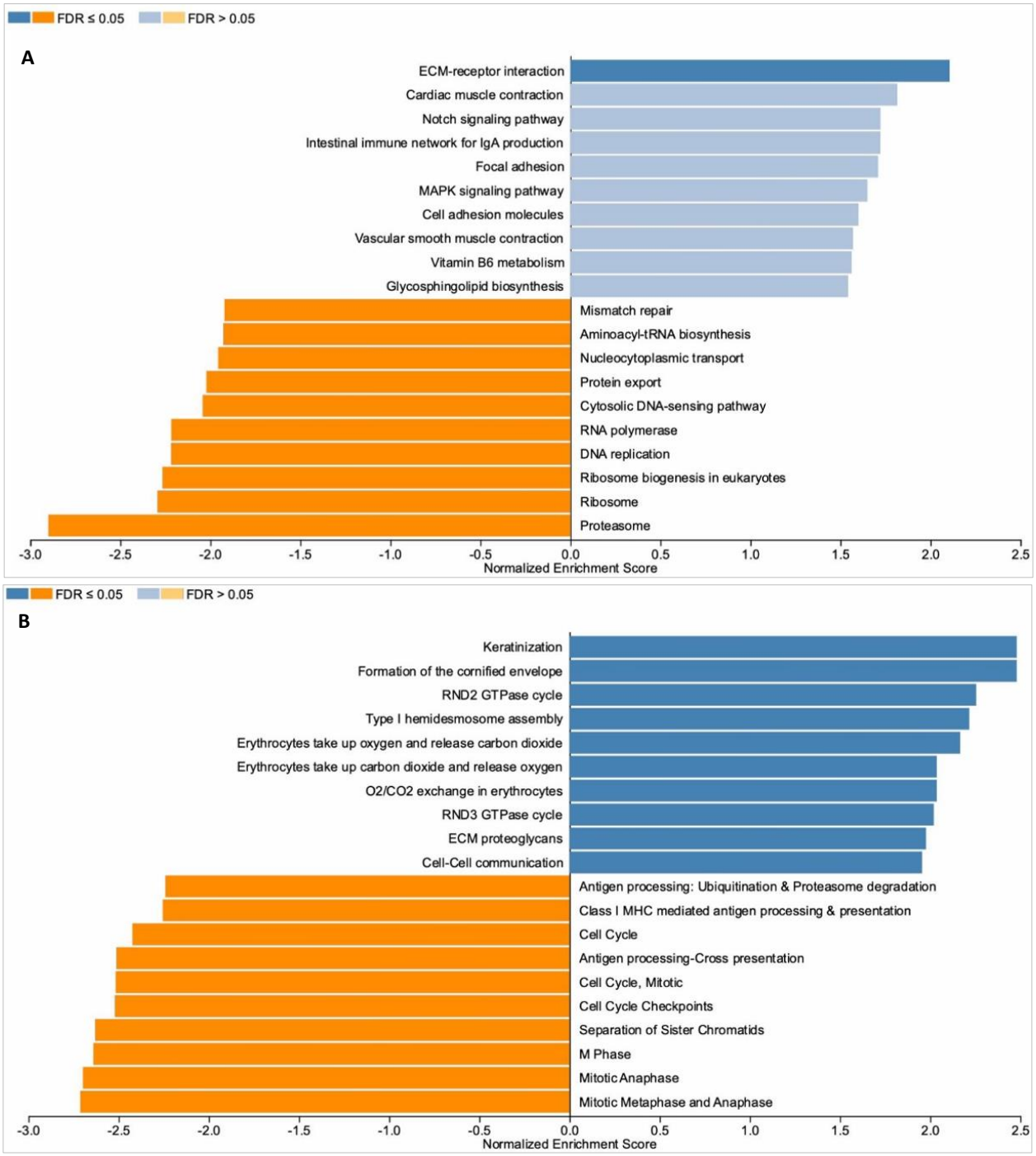

Figure 5. Gene set enrichment analysis results for (A) KEGG pathways and (B) Reactome pathways, using a ranked gene list (n=25,558) derived from differential expression analysis of fast-growing fish compared with slow-growing fish.

annotated genes, which are all important regulators of growth and metabolism, were significantly upregulated in the fast-growing group. Insulin-like Growth Factor (IGF) system, which consists of IGF ligands (IGF1, IGF2), IGF receptors (IGFRs), and IGF-binding protein (IGFBPs), plays an essential role in the neuroendocrine regulation of growth in all vertebrates (Allard & Duan, 2018). Generally, IGFs are upregulated in tissues of rapidly growing fish, such as Nile tilapia (*Oreochromis Niloticus*) (Herkenhoff et al., 2020) and channel catfish (*Ictalurus punctatus*) (Peterson et al., 2004). The up-regulation of IGF1, IGFBP-5 and IGFBP-4 was reported to be involved in the switching to fast growth of Atlantic salmon skeletal muscle (Bower et al., 2008). Moreover, Single Nucleotide Polymorphisms (SNPs) in genes coding for proteins belonging to the IGF system have been significantly associated with the growth traits (Tran et al., 2021). In addition, our results showed the increased expression of genes involved in gas and oxygen transport. Enhanced oxygen transport efficiency in fast-growing fish may provide the energy required to support higher metabolic activity. Consistent with this interpretation, a previous study on rainbow trout (*Oncorhynchus mykiss*) demonstrated that fast-growing fish were more tolerant to hypoxic condition than slow-growing fish (Roze et al*.,* 2013).

Our results showed that the cd9a gene, which is related to immune responses, was significantly downregulated in the fast-growing group. The reduced expression of this gene in the fast-growing group is consistent with the concept that some individuals within populations may prioritize somatic growth over immune responsiveness and vice versa (Visse et al., 2015). This is a functional compromise between growth and immunity, which is also observed in some fish species such as Atlantic salmon (*Salmo salar*) (Visse et al., 2015) and coho salmon (*Oncorhynchus kisutch*) (Alzaid et al., 2018). Previous studies have shown that selection for fast growth leads to a significant reduction in immune function, but selection for immune function did not consistently affect growth rate (Van et al., 2011). The fast- and slow-growing common carp groups in this study are hybrids of purebred Vietnamese and Hungarian common carp strains. Vietnamese purebred carp strains are characterized by slower growth but exhibit stronger disease resistance and higher environmental adaptability, whereas Hungarian common carp exhibit faster growth but lower adaptability to Vietnamese farming conditions, weaker immunity responses, and reduced survival rates (Son et al., 2024; Nedoluzhko et al*.,* 2021; Thai et al*.,* 2007).

Therefore, the fast-growing hybrid group in this study may have inherited these growth-related characteristics from the parental Hungarian carp strain. This finding shows the important role of genetic background in regulating gene expression related to growth potential and adaptability to aquaculture conditions in common carp. These results also highlight the importance of metabolic pathways and immune-related mechanisms in interpreting the genetic basis of morphological variation in fish populations, thereby providing valuable insights for selective breeding and aquaculture management programs aimed at improving fish health and productivity.

Further research on the functional roles of differentially expressed genes is essential to clarify their specific contributions to the observed morphological variations between the two fish groups. Nevertheless, these genes present promising candidate genes associated with growth traits and could be actively targeted in genome-informed selective breeding approaches, such as genomic selection, to achieve faster genetic improvements.

Conclusion

This study reveals distinct performance differences among two purebreds (HH, VV) and two crossbreds (VH, HV) of common carp during the grow-out period. VH crossbreds showed the highest body weights, while HH purebreds had the greatest standard lengths. Positive mid-parent heterosis observed in VH crossbreds indicates strong hybrid vigour. Significant correlations between body weight and morphological traits suggest that selection for a single trait may improve other growth-related traits in breeding programs.

Transcriptomic analysis of fast- and slow-growing fish identified a total of 28 differentially expressed genes potentially associated with growth performance. The fast-growing hybrid common carp (between Vietnamese and Hungarian lines) demonstrated a pronounced genetic contribution from the Hungarian carp, as evidenced by both morphological traits and gene expression profiles. This gene expression analysis not only enhances our understanding of the molecular mechanisms underlying growth variations in common carp but also highlights the considerable genetic potential for improving growth rates through selective breeding strategies.

Ethical Statement

All experimental procedures involving fish were conducted in accordance with the animal care and use guidelines approved by the Scientific Committee of the Research Institute for Aquaculture No. 1 (RIA1), Vietnam. All protocols related to fish handling, sampling, and tissue collection were reviewed and approved by the RIA1 Scientific Committee prior to the commencement of the study.

Funding Information

This work was financially supported by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) through the Grant number 07/2020/TN.

Author Contribution

LTHG: Conceptualization, methodology, investigation, data curation, formal analysis and writing-original draft; PTN: Transcriptomic analysis and writing; SVV and AG: growth analysis and writing- review & editing; OTPN and VVI: Conceptualization, methodology, supervision, writing-review & editing

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper**.**

Acknowledgements

Staff at the Center of Technology Transfer and Service (RIA1) for facilitating fish hatchery experiment.

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