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Genetic improvement of feed efficiency in large Yellow Croaker

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Introduction

Aquaculture contributes significantly to global food security, and improving feed efficiency (FE) is essential for both economic and environmental sustainability (Naylor *et al*., 2021). Large yellow croaker (*Larimichthys crocea*), the most cultured marine fish species in China, is particularly affected by feed efficiency limitations. However, selective breeding for FE remains challenging in fish because accurately tracking individual feed intake in group-rearing conditions is difficult (de Verdal *et al*., 2018). Existing methods for measuring individual feed intake in fish face significant constraints. Social isolation methods remove natural schooling behavior, which can affect feeding behavior and growth performance (Besson *et al*., 2019; Rodde *et al*., 2021). X-ray methods using radio-opaque markers can only estimate intake for single meals and require repeated anesthetization that disrupts normal behavior (Difford *et al*., 2023). Video observation methods are labor-intensive and can be compromised by fish shading during feeding, which affects accuracy (de Verdal *et al*., 2017). To address these challenges, this study aimed to develop an intelligent phenotyping platform for quantifying individual feed intake under group-rearing conditions and to use this platform to characterize feed efficiency and evaluate its genetic basis and the feasibility of genomic selection for its improvement in large yellow croaker.

Materials & Methods

Deep learning-based phenotypic measurement platform. We developed an integrated platform comprising two modules: Module 1 combines computer vision with RFID technology for individual feed intake quantification. Specifically, a feeding station equipped with RFID readers provides feed, and a restricted passage allows only one fish to enter at a time. The RFID readers detect PIT tags implanted in fish for individual identification, while cameras capture video data that is analyzed using a YOLOv5s model for feed pellet detection. Module 2 enables automated measurement of body size traits by placing individual fish on a measurement platform, where cameras capture images that are analyzed using YOLOv8 and HigherHRNet models for keypoint detection and morphometric measurement (Fig 1).

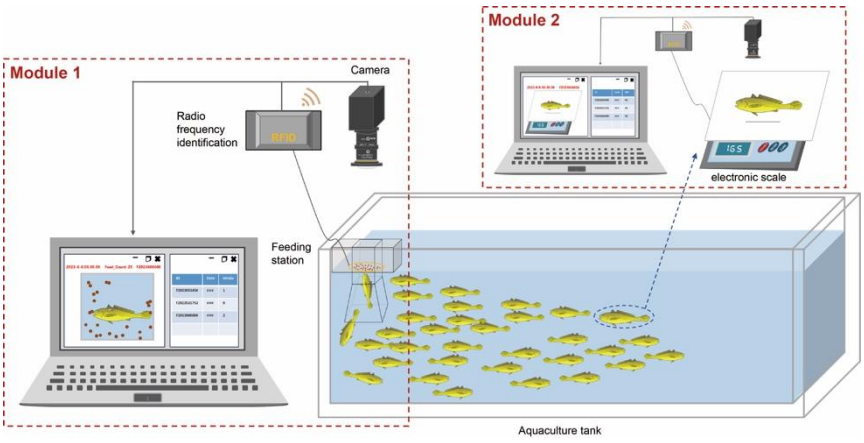

Figure 1. Deep learning-based phenotypic measurement platform

Animals and phenotyping. For phenotypic evaluation, the same group of 1,207 12-month-old large yellow croaker (mean body weight, 47.56 ± 22.8 g) was sequentially phenotyped at 15, 19, and 24°C. These temperatures were chosen to represent three feeding states relevant to large yellow croaker production: cold-induced fasting or reduced feeding (15°C), recovery of feeding activity (19°C), and normal satiation feeding within the optimal growth range (24°C). Each cycle included 14-day measurement periods for individual feed intake and body size traits. Within each temperature condition, FCR was calculated as feed intake divided by body weight gain, and RFI was defined as the difference between observed and predicted feed intake, i.e., $RFI = FI - (b_0 + b_1MBW^{0.8} + b_2BWG)$, where FI is observed feed intake, b_0 is the intercept, b_1 and b_2 are the partial regression coefficients for $MBW^{0.8}$ and BWG, respectively, MBW is the mean of initial and final body weight, and BWG is body weight gain. At 24°C (optimal growth temperature), feeding behavior traits including average daily feed intake, total feeding frequency, and feeding behavior variability were also quantified.

Genotyping and genetic analysis. Genotyping was performed using the 55 K liquid SNP array for large yellow croaker on the DNBSEQ-T7 platform (Wang *et al*., 2023). After quality control excluding individuals and SNPs with >10% missing rate and SNPs with minor allele frequency <0.05, 46,821 SNPs were retained. Heritability was estimated using GBLUP, whereas genetic correlations were obtained using a genomic multi-trait model, both implemented in the HIBLUP software. GWAS was performed using linear mixed models with initial body weight, feed intake, and the first two principal components derived from principal component analysis of genome-wide SNP data (PC1 and PC2) as covariates to control for population structure. Significant SNPs were identified using Bonferroni correction, and candidate genes were screened within 100 kb of significant loci.

Genomic selection and breeding. GBLUP was used to estimate breeding values for RFI based on the 24°C reference population. From 811 candidate broodstock of the same population, the 50 females and 20 males with the lowest RFI GEBVs were selected to establish the high-feed-efficiency line, which was subsequently evaluated under common-garden conditions.

Results & Discussion

Our deep learning platform delivered reliable performance across two complementary modules, with Module 1 quantifying individual feed intake at 94.5% accuracy and 100 FPS, and Module 2 measuring body size traits with correlations above 0.99 compared with manual measurements and achieving 97% accuracy for keypoint detection. While the platform demonstrates promising performance in controlled settings, practical deployment faces constraints as it is currently applicable only to specific controlled environments and fish of particular size ranges. Future scaling efforts will need to address adaptation to diverse environmental conditions and broader size ranges to enhance applicability across commercial aquaculture operations.

Using this platform, feed efficiency was characterized across three temperature conditions. Average daily feed intake and average daily gain both increased with temperature, whereas FCR decreased from 1.37 ± 0.75 at 15°C to 1.32 ± 0.57 at 19°C and 1.20 ± 0.38 at 24°C, with coefficients of variation of 55.0%, 43.18%, and 31.67%, respectively. Consistent with these phenotypic changes, RFI heritability remained low at 15°C and 19°C (0.01 ± 0.11 and 0.08 ± 0.08, respectively) but increased to 0.24 ± 0.07 at 24°C (Table 1), suggesting that reduced feed intake and growth under colder conditions limited the phenotypic expression of RFI. Across thermal environments, GWAS identified 24 SNPs and 65 candidate genes associated with FCR and RFI, implicating pathways that govern nutrient absorption and transport, lipid and amino acid metabolism, appetite and energy-balance regulation, and insulin/IGF signaling, with

representative candidate genes including *dgat1*, *pappa2*, *che*, *pqlc2*, and *wars* (Volkoff, 2016; Tang *et al*., 2016; Chen *et al*., 2017; Cabrera-Salcedo *et al*., 2017; Davoudi *et al*., 2022).

Table 1. Temperature-dependent heritability estimates for feed efficiency traits.

Temperature	h^2 (FCR)	h^2 (RFI)	Sample size
15°C	0.19 ± 0.16	0.01 ± 0.11	306
19°C	0.10 ± 0.10	0.08 ± 0.08	482
24°C	0.10 ± 0.06	0.24 ± 0.07	799

Building on these findings, genomic selection was implemented to develop a high-feed-efficiency line. Common-garden validation showed that the selected line outperformed the control line with 10.05% lower FCR, 0.07 g/day lower RFI, 5.74% higher average daily gain, and 4.60% higher survival. These results are consistent with findings in rainbow trout, where low-RFI families showed higher growth performance and nitrogen utilization efficiency (Silverstein *et al*., 2005), and in pigs, where L-RFI lines achieved 16% improvement in feed efficiency (Montagne *et al*., 2014). The selected line also demonstrated enhanced stress resilience, with lower plasma cortisol levels and reduced fecal nutrient content (16.29% lower crude protein and 31.18% lower crude fat), consistent with studies showing that RFI is negatively correlated with stress response in Nile tilapia and pigs (Martins *et al*., 2011; Colpoys *et al*., 2019). Feeding behavior traits displayed moderate heritability (h^2 = 0.23-0.26) but strong genetic correlations with RFI (rg = 0.53-0.79), indicating their potential utility as auxiliary selection indicators.

Conclusions

This study demonstrates that intelligent phenotyping combined with genomic selection can achieve substantial genetic improvement in feed efficiency in large yellow croaker. In addition, the results obtained with this platform indicate that temperature affects both the phenotypic performance and genetic basis of feed efficiency, with 24°C providing the most informative environment for selection on RFI in large yellow croaker. Overall, these findings provide a practical framework for improving feed efficiency in marine fish. However, because the feed-efficiency trial lasted only two weeks, future studies should extend the trial duration over a larger proportion of the grow-out period to confirm the robustness of the observed phenotypic and genetic patterns.

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