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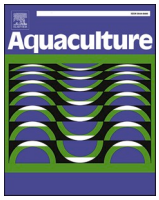

Investigating genomic prediction accuracies for muscle fatty acids composition in Malabar red snapper (*Lutjanus malabaricus*)

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ABSTRACT

This study evaluated the genomic prediction accuracy of fatty acid composition in fillets of Malabar red snapper (*Lutjanus malabaricus*) reared in a sea-based farm and sampled at 18 months of age, with an average body weight of 454 ± 102 g. In addition to body weight, phenotypic (534 individuals) and genotypic (39,780 SNPs) data were available for 16 fatty acid traits, grouped into four categories: polyunsaturated Omega-3 fatty acids, Omega-6 fatty acids, saturated and monounsaturated fatty acids, and various fatty acid ratios and indices. Genomic predictive abilities varied across traits, yielding low to moderate accuracies (0.29–0.48). These accuracies were comparable to, or slightly higher than, theoretical expectations based on heritability and training population size. Notably, moderate to high prediction accuracies were observed for DHA (0.47 ± 0.16), EPA (0.43 ± 0.15), and total Omega-3 (0.47 ± 0.19), highlighting the potential of genomic selection to enhance the nutritional quality of farmed red snapper. The absence of significant SNPs supports the polygenic nature of these traits and underscores the appropriateness of genomic selection. These findings demonstrate the feasibility of incorporating genomic selection into breeding programs to improve fatty acid composition in Malabar red snapper.

1. Introduction

In recent decades, the global growth in aquaculture has been the primary driver of increased aquatic food production (FAO, 2020, 2022, 2024). Malabar red snapper (*Lutjanus malabaricus*) is a commercially important fish species, highly valued for its nutritional content, especially long-chain Omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Purushothaman et al., 2024). Omega-3 fatty acids, well-known for their cardiovascular, anti-inflammatory, and cognitive benefits, are essential for human health and are a key driver of consumer demand for seafood products (Nigam et al., 2018; Saidaiah et al., 2024). However, the ongoing shift towards more sustainable aquaculture, particularly the substitution of fishmeal and fish oil with plant- or microbial-based alternatives has raised concerns about maintaining high levels of these valuable nutrients in farmed species (Hooft et al., 2024; Kiron et al., 2022; Napier and Betancor, 2023; Tocher et al., 2019; Mensah et al., 2025; Rocha et al., 2023). This shift necessitates strategies to maintain or enhance the deposition of nutritionally valuable unsaturated fatty acids, particularly long-chain Omega-3 s such as EPA and DHA, which are essential for human cardiovascular and cognitive health (Nigam et al., 2018; Purushothaman et al., 2024; Tocher et al., 2019).

Selective breeding has been pivotal in the aquaculture industry, historically enhancing traits such as growth rate, disease resistance, and feed efficiency (Houston et al., 2020; Nguyen, 2024; Zenger et al., 2019). While these traits remain of economic importance, there is a growing interest in improving the nutritional profile of fish, particularly through increasing health-promoting fatty acids like EPA and DHA in

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muscle tissue (Blay et al., 2021; Horn et al., 2018; Yáñez et al., 2015). Omega-3 fatty acids are desirable for both market value and human health, but achieving improvements through conventional breeding is challenging, due to the complexity of these traits and their relatively low heritability compared to other traits like growth (Blay et al., 2021; Horn et al., 2018; Purushothaman et al., 2025). Additionally, it is increasingly recognized that improving the nutritional quality of farmed fish could contribute significantly to meeting the rising global demand for dietary Omega-3 fatty acids.

The biosynthesis of Omega-3 fatty acids in fish involves key enzymatic processes regulated by genes such as fatty acid desaturase (*Fads*) and the elongation of very long-chain fatty acids (*Elovl*). These enzymes convert precursor molecules such as alpha-linolenic acid (ALA) into long-chain fatty acids like EPA and DHA, which are then stored in the muscle tissue (Hastings et al., 2001). Genetic variation in the *Fads* and *Elovl* genes can lead to differences in enzyme activity and efficiency, affecting the overall capacity of fish to produce long-chain Omega-3 fatty acids. By identifying single nucleotide polymorphisms (SNPs) associated with these traits, it is possible to select and breed fish with improved fatty acid profiles, even when they are fed diets rich in plant-based ingredients (Yáñez et al., 2015). Understanding these pathways is thus important for developing genomic tools to improve fatty acid composition, especially in the context of sustainable aquaculture practices.

Genomic tools, such as genome-wide association studies (GWAS) and genomic selection (GS), offer powerful tools for identifying genetic markers associated with complex traits like fatty acid composition. GWAS identifies single nucleotide polymorphisms (SNPs) associated with specific traits, providing insights into the genetic architecture underlying traits of interest (Gonzalez-Pena et al., 2016; Oikonomou et al., 2022; Omeka et al., 2022; Yanez et al., 2023; Yoshida and Yáñez, 2021). Alternatively, GS uses genome-wide markers to predict the genetic merit of individuals, offering greater accuracy in selecting for difficult-to-measure traits such as muscle fatty acid content (Bangera et al., 2017; D'Agaro et al., 2021; López et al., 2015; Yanez et al., 2023). In aquaculture species, the integration of genomic technologies has demonstrated their potential to accelerate genetic gains for fatty acid content.

In recent years, GWAS and GS have been successfully investigated in species like Atlantic salmon (*Salmo salar*) (Horn et al., 2020; Horn et al., 2018), and mapping quantitative trait loci for Omega-3 fatty acids in Asian seabass (*Lates calcarifer*), with the intent to enhance traits related to Omega-3 fatty acid content through selection (Xia et al., 2014). These studies have revealed that fatty acid composition is influenced by both genetic and environmental factors, and that marker-assisted selection could significantly accelerate genetic gains. However, while growth and disease resistance traits are typically feasible to select, the genetic improvement of fatty acid profiles is challenging, involving complex genetic architecture. For example, fatty acid compositions are assumed to be influenced by many genes with small effects, making it harder to identify major Quantitative Trait Loci (QTL) or candidate genes. Moreover, there is high genotypeby-environment interaction between genes with diet, water temperature and salinity, resulting in the masking of genetic effects. In addition, obtaining adequate quantitative data for fatty acid compositions is challenging since it is labor-intensive and expensive, in comparison to easy-to-measure traits like growth. This limits the ability to scale up data, which is essential for achieving the statistical power required for GWAS. These complexities pose a challenge for breeding programs targeting improved fatty acid profiles, which is also reflected in the limited literature available for genomic selection of fatty acid profiles in comparison to other aquaculture traits (Houston et al., 2020).

GWAS and GS are complementary tools for understanding and utilizing the genetic basis of complex traits such as fatty acid composition. While GWAS identifies significant loci or QTL linked to traits, GS captures the combined effects of genome-wide markers to predict breeding values (Houston et al., 2020; Yanez et al., 2023). In fish, key genes such as *fatty acid desaturases* and *elongases of very* long-chain fatty acids are central to the biosynthesis of long-chain Omega-3 fatty acids, converting precursors like ALA into EPA and DHA (Hastings et al., 2001; Horn et al., 2018). Integrating GWAS and GS thus enables both the discovery of genetic markers associated with Omega-3 biosynthesis, as well as the selection of fish with superior fatty acid profiles.

With this background, the objective of this study was to explore the genetic basis of muscle fatty acid composition in Malabar red snapper. We assessed the genomic predictive ability for 16 fatty acid profile traits in Malabar red snapper raised commercially in Singapore. This research offers a foundation for developing genomic tools that can enhance the nutritional value of Malabar red snappers in Singapore and the region.

2. Materials and methods

2.1. Ethics statement

This study was approved by James Cook University Singapore, following the approval from the Institute Animal Care and Use Committee (IACUC) under the reference number 2021-A010. All experimental procedures adhered to the guidelines set forth by the National Advisory Committee on Laboratory Animal Research (NACLAR).

2.2. Sampling for proximate and SNPs analysis

To support robust GWAS, GS and the evaluation of nutritional traits such as muscle fatty acid composition, juvenile Malabar red snapper were sourced from three geographically-distinct commercial hatcheries; Johor and Kedah in Malaysia, and Singapore, to ensure sufficient genetic diversity and population structure as described by Liang et al. (2025). The brooders originated from both wild-caught and domesticated sources, however no physical tagging was available for these broodstock, thus limiting the traceability of their true origin. Given that no family information was available, principal component analysis was performed to understand the population structure. These two-month-old fingerlings were pooled into a single nursery tank and later transferred to a grow out facility. In line with prevailing industry practices, the hatcheries relied on mass spawning protocols, where multiple broodstock were allowed to spawn naturally in sea cages. Fertilized eggs were collected from the cage surface and transferred to earthen ponds maintained under green-water culture systems. These systems provided natural feeds via phytoplankton and zooplankton blooms, after which the larvae were weaned onto dry formulated feed during early development.

After approximately four weeks, the fingerlings were collected, packaged in oxygenated plastic bags, and transported to a commercial floating barge farm located in the Eastern Johor Strait, Singapore. Upon arrival, the juveniles were stocked in a 50 m^3 circular fiberglass tank operating on a continuous flow-through seawater system with mechanical sand filtration and supplemental oxygenation. Key water quality parameters during rearing included temperature (29–31 °C), salinity (27–30 ppt), pH (7.9–8.2), and dissolved oxygen (>5 mg/L). Throughout the culture period, fish were fed a consistent commercial pellet diet (Uni-President, Dĩ An, Vietnam) containing 44 % crude protein and 7 % crude fat. Feeding was carried out to satiation four times daily. No formulated or experimental diets were applied, ensuring uniform nutritional conditions across the entire population. At harvest (approximately 18 months of age), 604 fish were euthanized using 200 ppm Aqui-S. Muscle tissue samples (~15 g) were collected from the dorsal–caudal region and stored at 80 °C for fatty acid profiling. For genotyping, caudal fins were sampled and genotyped using a custom Axiom Red Snapper 70 k array. At the time of sampling, the average fish body weight and total length were 452.9 ± 102.2 g and 289.0 ± 25.7 cm, respectively (Liang et al., 2025; Purushothaman et al., 2025).

2.3. Fillet fatty acid extraction and analysis

The fatty acid composition of Malabar red snapper fillets were analyzed at the Sustainable Technology & Analytical Research (STAR) Laboratory, Republic Polytechnic, following the protocol by O'Fallon et al. (2007). Each 15 g sample was first freeze-dried for 1-week, followed by grinding to determine moisture content. 0.5 g of ground tissue was mixed with a C13:0 internal standard, 10 N potassium hydroxide (KOH), and methanol, followed by incubation at 55 °C. After incubation, 24 % sulfuric acid (H_2SO_4) was added to catalyze the reaction. This transesterification process produced fatty acid methyl esters (FAME), which were subsequently extracted using hexane (C_6H_{14}). The extracts were stored at −20 °C until analysis. Gas chromatography (GC) was performed using a SP2560 capillary column equipped with a flame ionization detector. Fatty acids were identified and quantified by comparing the retention times to those of known standards, with results expressed as area percentages of total fatty acids.

2.4. Genotyping

SNP genotyping procedures were previously described by (Liang et al., 2025) and (Purushothaman et al., 2025). Briefly, fin tissue samples were genotyped using a custom Axiom Red Snapper70k SNP array, which included 70,904 biallelic SNPs. This array was designed based on genomic data from both wild and farmed red snapper populations across Southeast Asia and Australia. Genotyping output for each fish was obtained in the form of CEL binary files, which were subsequently processed for SNP-calling using default thresholds suggested by the Axiom Analysis Suite v5.4. SNPs were retained if they met the following criteria: call rate $\geq$ 0.97 and a dish QC call rate $\geq$ 0.82. Only SNPs categorized as "PolyHighResolution", which indicate high-confidence biallelic calls, were kept for downstream analyses. This was followed by further filtering to exclude SNPs with minor allele frequency (MAF) $\leq$ 0.05, Fisher's Linear Discriminant (FLD) $<$ 7 and genotype class count $<$10 for any of the three genotypes (AA, AB, BB). After applying these stringent filters, a total of 39,780 high-quality SNPs across 534 fish samples were retained. The final dataset was exported to numeric format using PLINK2 (Purcell et al., 2007) for subsequent analyses.

2.5. Genome-wide association study

We performed a GWAS using the multi-locus mixed linear analysis with leave-one-chromosome-out (MLMA-LOCO) approach implemented in Genome-wide Complex Trait Analysis (GCTA) (Yang et al., 2011; Yang et al., 2014). This model accounts for genetic relatedness of individuals with a genomic relationship matrix (GRM) derived from 39,780 markers. The leave-one-chromosome-out (LOCO) strategy was employed to mitigate biases due to confounding effects from markers on the same chromosome as the tested variant. The statistical GWAS model can be written as:

$$y = X\beta + Wu + \varepsilon \quad (1)$$

where $\boldsymbol{y}$ is the vector of phenotypic values for trait of interest (e.g., 16 fatty acid traits), $\boldsymbol{X}$ is the design matrix of a fixed effect for fish body ranking at sampling (fish ranked as normal or abnormal looking); fish with external body lesion(s), fin rot or pale in skin pigmentation were classified as abnormal-looking. The $\boldsymbol{\beta}$ is the vector of fixed-effect coefficient, $\boldsymbol{W}$ is the design matrix for random effect, $\boldsymbol{u}$ is the random polygenic effect excluding the contributions from the chromosome containing the SNP under test, is normal distributed; $\boldsymbol{u} \sim \left(\boldsymbol{0}, \boldsymbol{I}\sigma_{\boldsymbol{u}}^{\boldsymbol{2}}\right)$. The $\boldsymbol{\varepsilon}$ is the residual error, following a normal distribution; $\varepsilon \sim \left(\boldsymbol{0}, \boldsymbol{I}\sigma_{\varepsilon}^{\boldsymbol{2}}\right)$, with $\boldsymbol{I}$ a n × n identity matrix. The $\sigma_{\boldsymbol{gu}}^{\boldsymbol{2}}$ and σ_{ε}^{2} represent the genetic and residual variances, respectively. This LOCO approach prevents inflation of SNP effect testing due to overfitting or linkage disequilibrium between the tested SNP and other SNPs on the same chromosome, ensuring unbiased estimation of SNP effects. For each SNP, its effect size and *p*-value were estimated by testing the null hypothesis. Multiple testing correction methods, such as the Bonferroni correction (0.05 divide by number of SNPs used, i.e., a significant SNP is defined if surpassing a threshold of -log(0.05/39780) ~ 5.9) and false discovery rate were applied to identify significant associations (Yang et al., 2011; Yang et al., 2014).

2.6. Empirical genomic prediction

For assessing genomic predictive ability of each fatty acid trait, we employed the genomic best linear unbiased prediction (GBLUP) algorithm as VanRaden (2008). The Average Information Restricted Maximum Likelihood (AIREML) subroutine from the BLUPF90+ module of the BLUPF90 software family (Misztal et al., 2002) was used to analyze the continuous trait characteristics of all fatty acid traits. It provides precise estimates of variance components by maximizing the restricted likelihood, which adjusts for fixed effects and avoids biases in variance estimation that can arise in standard maximum likelihood methods (Strandén et al., 2024). This approach handled missing data by combining phenotypic and genotypic information to estimate breeding values. The GBLUP model notation can be expressed as:

$$y = Xb + Za + e \quad (2)$$

where $\boldsymbol{y}$ is the vector of phenotypic values (i.e., 16 fatty acid traits), $\boldsymbol{b}$ is the vector of fixed effect as Eq. 1, $\boldsymbol{a}$ is vector of genomic breeding values (GBVs), assumed to follow a multivariate normal distribution; $\boldsymbol{a} \sim \left(\boldsymbol{0}, \boldsymbol{G}\sigma_{\boldsymbol{g}}^{\boldsymbol{2}}\right)$. $\boldsymbol{X}$ and $\boldsymbol{Z}$ are the design matrices related to the fixed and random effects. The letter $\boldsymbol{e}$ refers to residual variance corresponded to each phenotypic value; $\boldsymbol{e} \sim \left(\boldsymbol{0}, \boldsymbol{I}\sigma_{\boldsymbol{e}}^{\boldsymbol{2}}\right)$. Here, $\sigma_{\boldsymbol{g}}^{\boldsymbol{2}}$ and $\sigma_{\boldsymbol{e}}^{\boldsymbol{2}}$ were described as above. The $\boldsymbol{G}$ and $\boldsymbol{I}$ matrices represent, respectively, the genomic relationship matrix (GRM) between individuals and the identity matrix. The $\boldsymbol{G}$ matrix was constructed using the genotype frequencies by VanRaden (2008) as following:

$$\boldsymbol{G} = \frac{\boldsymbol{MM}'}{\sum \boldsymbol{2p}(\boldsymbol{1}-\boldsymbol{p})} \quad (3)$$

where $\boldsymbol{M}$ is the matrix of genotypes (m_{ij} = 0, 1 or 2 for homozygous reference, heterozygous, and homozygous alternate genotypes, respectively) and $\boldsymbol{p}$ is the allele frequency for each SNP. The $\boldsymbol{M}'$ is the transpose of matrix $\boldsymbol{M}$.

Furthermore, we fitted bivariate models between harvest body weight and each fatty acid to estimate their genomic estimated breeding values (GEBVs) and benchmarked them against the Eq. 2 method (i.e., univariate models). These bivariate models used the bivariate function of Eq. 2, employing the same fixed factors and GRM. For both univariate and bivariate models, heritability, phenotypic and genetic correlation were computed for full data and each training sets in the 5-fold cross-validation procedure.

2.7. Prediction accuracy and reliability

To evaluate the performance of the predictive model, a 5-fold cross-validation procedure with 5 replicates was performed (Fushiki, 2011). The accuracy of genomic predictions for a validation set was defined as the Pearson correlation between predicted GEBVs and observed phenotypic values, divided by the square root of heritability of the full dataset (Purushothaman et al., 2025).

For all testing sets, we visualized the GEBV against real phenotypes and residual as scatter plots to assess model fitness. In each testing test, for reliability of GEBV estimates, we used root mean square error (RMSE) and the coefficient of determination (R^2). These two complementary metrics provide a comprehensive outlook of predictive performance in which RMSE measures the magnitude of prediction errors, indicating how far predicted values deviate from actual values. This

means that a lower RMSE suggests a more accurate model, with 0 indicating a perfect prediction while R^2 evaluates the proportion of variance in the observed data explained by the model, representing its predictive ability. A higher R^2 (nearly 1) suggests the best goodness-of-fit, while 0 indicates the model performed no better than random chance. These metrics are widely used because RMSE accounts for large errors due to its squared term, making it sensitive to outliers and ideal for assessing prediction accuracy. R^2 provides an intuitive measure of how well the model explains variation, making it useful for comparing predictive performance across traits. By combining RMSE and R^2, we quantified both the accuracy and explanatory power of our predictions over multiple fatty acid trait characteristics, ensuring a robust evaluation of between model traits performance.

2.8. Theoretical genomic prediction accuracy

In this study, we aimed to estimate the theoretical (i.e., deterministic) genomic prediction accuracy of Malabar red snapper fatty acid profiles using the population parameters defined in Eq. 4. This theoretical value served as a benchmark against the empirical prediction accuracy. Comparing theoretical and empirical accuracies allowed us to identify potential sources of bias in the prediction model, particularly those arising from overfitting, limited sample size, or inaccuracies in trait heritability estimates.

$$\textit{Theoretical genomic prediction accuracy} = \sqrt{\frac{Nh^2}{Nh^2 + \frac{M_e}{h^2}}} \quad (4)$$

where N is number of individuals in the training set having both phenotypes and genotypes (i.e., 534*0.8 = 427); h^2 is the trait heritability; M_e is effective number of independent chromosome segments (a proxy for genetic architecture) with $M_e \approx 2N_eL$. Here, N_e is the effective population size and L is the genome length in Morgan (we assumed 1 cM ~ 1 Mb). The genome length (L) of Malabar red snapper was approximately 9.7 Morgans (unpublished data) with heritability (h^2) known for all fatty acid traits (Purushothaman et al., 2025). The same training datasets (i.e., 80 % population or 427 individuals and full set of 39,780 SNP) used for cross-validation were created using PLINK (Purcell et al., 2007) and generated using genepop format by PGDSpider (Lischer and Excoffier, 2012). NeEstimator v2 (Do et al., 2014) calculated N_e with linkage disequilibrium method assuming random mating utilizing lowest minor allele frequency of 0.05.

3. Results

3.1. Overview and breakdown of fatty acid composition data

The 16 fatty acid compositions of the 534 samples have been

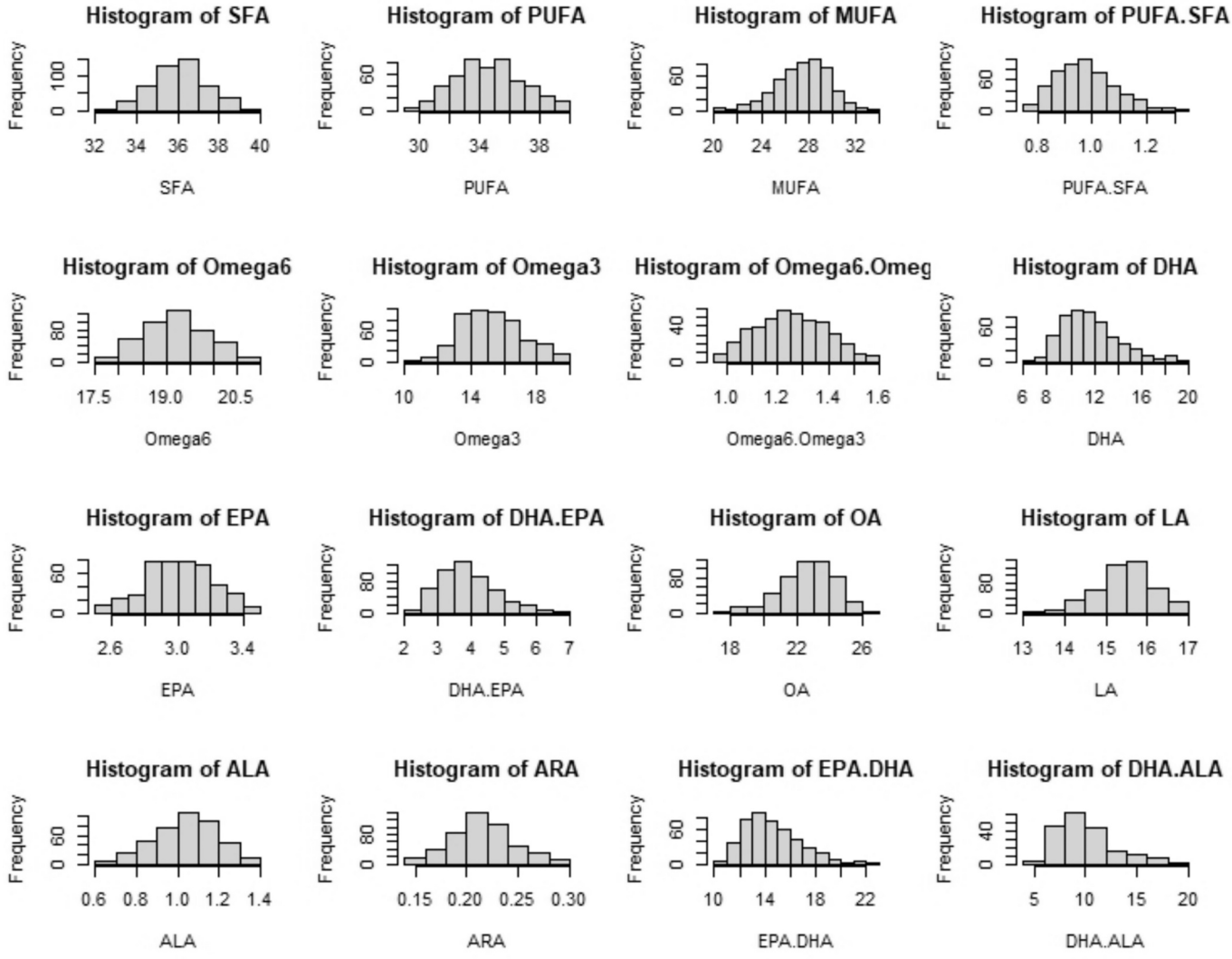

Fig. 1. Distribution of 16 fatty acid compositions for 534 Malabar red snappers.

previously described by Purushothaman et al. (2025), and its distributions ares presented in Fig. 1. Briefly, among the saturated, monounsaturated and polyunsaturated fatty acids, saturated fatty acids (SFAs) were the most abundant with an average content of 36.09 % (±1.33), followed by polyunsaturated fatty acids (PUFAs, 34.68 ± 2.21 %) and monounsaturated fatty acids (MUFAs, 27.48 ± 2.25 %). The PUFA/SFA ratio averaged 0.98 (±0.11), indicating a relatively balanced lipid profile. Within the Omega-3 fatty acids group, ALA, EPA and DHA accounted for 1.1 %, 3.0 % and 11.7 % of total fatty acids respectively, comprising a combined 15.3 % of Omega-3 fatty acids. Among the Omega-6 fatty acids, LA, ARA and total Omega-6 fatty acid levels were 15.5 %, 0.2 % and 19.2 %, respectively, resulting in an Omega-6/Omega-3 ratio of 1.3, reflecting either a balanced diet or genetic potential for Omega-3 deposition. LA was the predominant Omega-6 PUFA (15.5 %) while DHA was the most abundant n-3 PUFA, representing 33.7 % of total PUFAs. EPA and DHA altogether constituted 95.2 % of Omega-3 PUFAs, with a DHA/EPA ratio of 25.7. The overall fatty acid profile of the fish fillets consisted of 36.1 % saturated fatty acids (SFAs), 27.5 % monounsaturated fatty acids (MUFAs), and 34.7 % polyunsaturated fatty acids (PUFAs), yielding a PUFA/SFA ratio of 0.98. Oleic acid (OA) was the most abundant fatty acid, contributing 22.7 % of total fatty acids and 82.5 % of the total MUFAs.

3.2. Model fit diagnostics

All predicted values from validation test sets were collated and model fitness was visualized by plotting predicted phenotypes (GEBV) versus real phenotypes (**Supplementary Fig. S1**) and residuals

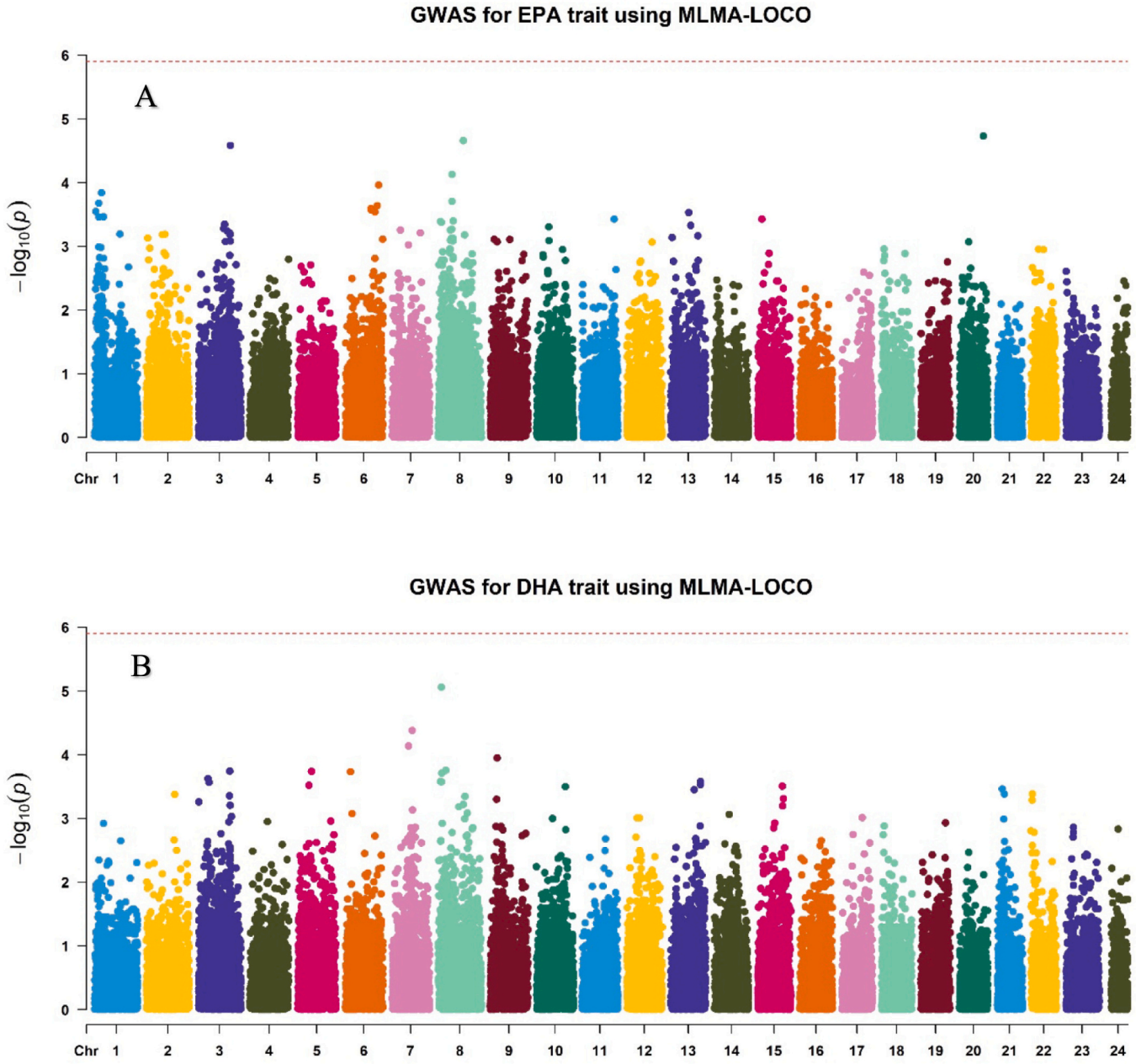

Fig. 2. Genome-wide association study for EPA (1A) and DHA (1B) using MLMA-LOCO (multi-locus mixed linear analysis with leave-one-chromosome-out) approach across 24 chromosomes. Each dot point is representative of a SNP effect (logarithm of P-value) to the trait of interest. No major QTLs were found to be associated with traits under study (below determined threshold – dotted red lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(**Supplementary Fig. S2**). In each test set, we subtracted the mean of real phenotype to individual GEBV, due to the absence of predicted residuals available for fish without phenotypes. Across 16 traits, GEBV explained a large proportion of phenotypic variance (R^2 = 0.47–0.86; e.g., ARA = 0.57, LA = 0.65, MUFA = 0.77, PUFA = 0.78, DHA/EPA = 0.86). The GEBV–phenotype scatterplots showed clear linear trends with limited scatter, indicating good predictive performance on unseen data. GEBV-residual plots are centered near zero, suggesting no major nonlinearity in which variance is approximately constant across the prediction range. Overall, these scatter plots support that the additive univariate GBLUP model provides an adequate fit and good generalization across traits. The same pattern was observed with the bivariate models.

3.3. GWAS analysis

The quality control procedure resulted in 39,780 SNPs and 534 phenotypes with SNP calling rate and minor allele frequency of 0.997 ± 0.003 and 0.34 ± 0.09, respectively. SNP density is uniformly high across 24 chromosomes (Supplementary Fig. S4), providing broad genomic coverage. Principal component analysis showed that two largest eigenvectors explained 31.9 % of total genetic variation (Supplementary Fig. S5), consistent with population structure arising from the inclusion of the three different hatchery stocks. Under AI-REML univariate models, we estimated heritability with and without fitting these two leading eigenvectors as fixed covariates, resulting in similar trends across most fatty acid traits studied (Supplementary Table S1). Consequently, we did not include the first two eigenvectors in the final GWAS and genomic prediction models.

Subsequently, we performed GWAS to identify possible loci associated with the 16 fatty acid traits and its effects across 24 chromosomes (as presented in Fig. 2 for EPA and DHA traits). The analysis employed the MLMA-LOCO (multi-locus mixed linear analysis with leave-one-chromosome-out) approach, which effectively accounted for population structure and relatedness while controlling for potential biases. Using the defined threshold (*P*-value <1.3E-6), as indicated by Bonferroni correction, no SNPs were found to have a significant effect across the 16 fatty acid traits studied.

Across the 16 fatty acid traits, we observed the same trend as seen for EPA and DHA (**Supplementary Fig. 2**). Genomic inflation factors (λ) were near unity across traits (median 1.084; range 0.999–1.122). Q-Q plots showed no early departure from expectation, indicating adequate control of population structure and relatedness without adding explicit principal components (Supplementary Fig. S6). The absence of large-effect and significant SNPs surpassing the defined threshold reflects the polygenic nature of these fatty acid composition traits. This further supported the suitability of the traditional GBLUP approach for genomic prediction of these traits.

3.4. Theoretical versus empirical genomic selection accuracy

The genomic prediction accuracies for 16 fatty acids were estimated using both univariate and bivariate GBLUP models and compared to theoretical expectations. Overall, prediction accuracy (Acc) varied across traits, ranging from 0.20 to 0.48 for bivariate models and from 0.29 to 0.48 for univariate models. The highest empirical accuracy was observed for Omega-3 and ALA (0.48), followed closely by EPA + DHA and DHA (0.47), indicating that these traits are more predictable using genomic information. Bivariate models exhibited predictive accuracy similar to univariate models, although minor reductions were noted in certain traits. Theoretical accuracies, computed based on training population parameters, were consistently lower than empirical values for most traits, reflecting possible overfitting or data structure limitations in empirical models. Traits such as DHA/EPA and PUFA/SFA showed strong agreement between empirical and theoretical accuracies (~0.44–0.45), suggesting a more reliable prediction for these. In contrast, complex ratio traits like DHA/ALA and Omega-6/Omega-3 exhibited large discrepancies, possibly due to low heritability or higher residual variance. RMSE and R^2 values showed considerable variation, with low R^2 observed in several traits (e.g., DHA/ALA: 0.01, Omega-6: 0.00), indicating challenges in accurately predicting ratio-based or low-heritability traits.

4. Discussion

Genomic prediction for fatty acid composition remains relatively scarce in aquaculture species, with only a limited number of studies investigating these complex traits (Houston et al., 2020; Yanez et al., 2023). In this study, we evaluated the potential of GS to improve fatty acid profiles in Malabar red snapper and found low to moderate prediction accuracies across 16 traits, particularly highest for key Omega-3 polyunsaturated fatty acids (PUFAs) such as EPA (0.43) and DHA (0.47). The genomic prediction accuracies ranged from 0.29 to 0.48, with traits like ALA, DHA, EPA, and combined EPA + DHA showing the highest predictability. These results are slightly higher than those reported in Atlantic salmon (*Salmo salar*, DHA = 0.41, EPA = 0.32) (Horn et al., 2020) and lower than that of rainbow trout (*Oncorhynchus mykiss*, DHA = 0.47, EPA = 0.53). This indicates that Malabar red snapper shows promising genomic prediction performance for fatty acids, falling between Atlantic salmon and rainbow trout in terms of DHA and EPA accuracy. This also highlights species-specific differences in genomic selection efficacy for these traits (Sonesson and Ødegård, 2016; Zhao et al., 2021).

The moderate to high accuracies observed for traits such as DHA (0.47 ± 0.16), EPA (0.43 ± 0.15), and Omega-3 (0.47 ± 0.19) support the possibility of applying GS in breeding programs to increase levels of these beneficial fatty acids in muscle. The combined prediction accuracy for EPA + DHA (0.47 ± 0.12) is particularly encouraging, as these long-chain Omega-3 PUFAs are critical not only for fish health and development, but also for human health (Tocher et al., 2019). Importantly, genomic prediction of these traits provides a pathway to improve nutritional value in farmed fish even under alternative feeding regimes, such as those incorporating plant-based ingredients (Napier and Betancor, 2023), which are becoming increasingly necessary in sustainable aquaculture.

Regarding model biases and reliability, we observed very low R^2 (0.00–0.10) and comparatively high RMSE (0.03–2.32) across all 16 traits studied (Table 1). These magnitudes are poorer calibrations compared to some studies with comparable sample sizes, for example survival trait in striped catfish (R^2 = 0.25–0.35, RMSE = 0.37–0.46) (Vu et al., 2021). This pattern indicates that the models can rank individuals better than random, which is useful for selection decisions, but their absolute predictions are poorly calibrated despite the positive accuracies obtained (0.29–0.48). Two features are likely driving this, firstly the compositional nature of fatty acid traits (sum-to-one constraints), as well as combined and ratio derivation, which inflate residual variance on the observed scale (Graeve and Greenacre, 2020). Secondly limited information content, given the modest training size (n = 534*0.8–427 individuals) for metabolically complex traits, likely constrains the proportion of phenotypic variance captured by the genomic predictors. For instance, Visscher and Goddard (2015) found that sampling variance is proportional to 1/N (N = samples size). In other words, heritability estimates can be upward-biased or imprecise with small sample sizes, further widening the gap between ranking accuracy and calibration.

The application of these findings in selective breeding programs involves identifying individuals with higher GEBVs for key fatty acid traits, and prioritizing them in broodstock selection. For traits with higher prediction accuracy such as DHA and EPA, the use of GS would allow earlier and more accurate selection of candidates, potentially accelerating genetic gain per generation (Allal and Nguyen, 2022; Song et al., 2023; Zenger et al., 2019). Over time, this could lead to cumulative improvements in muscle fatty acid content, contributing to

Table 1
Genomic prediction accuracy (Acc), RMSE, and R^2 for fatty acid traits using univariate, bivariate (with harvest body weight), and theoretical models (**$Acc_{theoretical}$**).

Trait	Acc_{uni} ± sd	RMSE/ R^2_{uni}	Acc_{biv} ± sd	RMSE/ R^2_{biv}	$Acc_{theoretical}$
ALA	**0.48 ± 0.17**	**0.14 / 0.07**	**0.47 ± 0.17**	**0.14 / 0.06**	**0.37**
ARA	*0.41 ± 0.25*	*0.03 / 0.02*	*0.39 ± 0.24*	*0.03 / 0.02*	*0.26*
DHA	**0.47 ± 0.16**	**2.32 / 0.06**	**0.46 ± 0.16**	**2.32 / 0.06**	**0.37**
DHA/ALA	0.30 ± 0.25	2.60 / 0.01	0.31 ± 0.26	2.60/ 0.01	0.43
DHA/EPA	0.44 ± 0.09	0.80 / 0.09	0.44 ± 0.09	0.81 / 0.09	0.45
EPA	0.43 ± 0.14	0.19 / 0.05	0.40 ± 0.15	0.20 / 0.04	0.36
EPA + DHA	**0.47 ± 0.12**	**2.06 / 0.10**	**0.47 ± 0.13**	**2.06 / 0.09**	**0.43**
LA	0.42 ± 0.17	0.65 / 0.03	0.40 ± 0.16	0.65 / 0.03	0.30
MUFA	0.43 ± 0.16	2.17 / 0.06	0.42 ± 0.15	2.17 / 0.06	0.39
OA	0.41 ± 0.19	1.59 / 0.04	0.40 ± 0.19	1.59 / 0.03	0.33
Omega-3	**0.48 ± 0.19**	**1.76 / 0.05**	**0.47 ± 0.19**	**1.76 / 0.05**	**0.34**
Omega-6	0.29 ± 0.24	0.65 / 0.01	0.20 ± 0.27	0.66 / 0.00	0.28
Omega-6/ Omega-3	0.44 ± 0.17	0.13 / 0.04	0.42 ± 0.15	0.13 / 0.04	0.32
PUFA	0.40 ± 0.18	2.15 / 0.05	0.39 ± 0.18	2.15 / 0.05	0.39
PUFA/SFA	*0.41 ± 0.11*	*0.10 / 0.06*	*0.41 ± 0.11*	*0.10 / 0.06*	*0.40*
SFA	0.35 ± 0.17	1.31 / 0.02	0.34 ± 0.18	1.31 / 0.02	0.33

Abbreviations: SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; OA: oleic acid; LA: linoleic acid; ALA: alpha-linolenic acid; ARA: arachidonic acid; RMSE: root mean square error; R2: = coefficient of determination. Traits indicated in bold denote greatest accuracies (0.47–0.48), and traits marked in italics & underlined denote both high accuracies (0.41) and lowest RMSE ~0.03–0.10 (i.e., good calibration).

healthier and more marketable fish products. Moreover, by integrating these predictions with economic trait selection indices, breeding programs could balance gains in nutritional quality with other important traits like growth in barramundi (Jerry et al., 2022; Massault et al., 2025) and disease resistance in barramundi and striped catfish (Shen et al., 2023; Vu et al., 2021).

Incorporating GS into breeding programs not only allows for the targeted improvement of Omega-3 PUFAs, but also aligns with the industry's demand towards sustainability (D'Agaro et al., 2021). By selecting fish that retain high levels of beneficial fatty acids, even when fed plant-based diets, the aquaculture sector can reduce its dependency on fishmeal and fish oil. This shift is critical for reducing the environmental footprint of aquaculture, while maintaining the high nutritional value of farmed fish. The application of GS could expedite this transition, enabling the selection of individuals better-adapted to plant-based feeds, without compromising their fatty acid profiles (Turchini et al., 2019).

In this study, genome-wide association analyses did not identify any significant QTLs associated with the 16 fatty acid traits tested. This lack of significant associations is consistent with the hypothesis that fatty acid composition is a highly polygenic trait, controlled by many loci each exerting small effects. The absence of significant associations likely reflects a combination of moderate sample size and limited SNP coverage, which together constrain the power to detect small-effect loci typical of polygenic traits. In addition, the stringent significance thresholds required to correct for multiple testing further reduce the likelihood of identifying genome-wide significant signals. The relatively sparse SNP density may also limit effective tagging of causal variants, leading to undetected associations. Together, these factors provide a parsimonious explanation for the lack of significant QTLs identified. Unlike in some aquatic species, the genome regions or genes related to fatty acid composition have been identified, such as in Atlantic salmon (*Salmo salar*) (Horn et al., 2018), rainbow trout (*O. mykiss*) (Blay et al., 2021) and oyster (*Crassostrea gigas*) (Meng et al., 2019). The absence of large-effect QTLs also supports the application of genomic selection, which leverages genome-wide marker information to capture the cumulative effects of many small-effect loci. While GWAS remains valuable for discovering candidate genes and understanding trait biology, it may have limited utility in directly improving selection for complex traits like fatty acid content. These results emphasize that GS, rather than single-marker approaches, would be better-suited for driving genetic gains in nutritionally- important traits in aquaculture species such as red snapper (Yanez et al., 2023; Yang et al., 2011; Yang et al., 2014).

Overall, this study provides valuable insights into the genetic architecture of muscle fatty acid composition in Malabar red snapper, and demonstrates the potential of GS to enhance these traits in breeding programs. Future research should focus on expanding the dataset, incorporating more diverse populations, and exploring gene-environment interactions to optimize the use of GS in improving the nutritional profile of farmed fish. Additionally, functional studies to identify key genes and pathways involved in fatty acid biosynthesis and deposition would further enhance our understanding of the genetic basis of these traits, and support the development of more targeted breeding strategies (Yanez et al., 2023; Yáñez et al., 2015). Another important area for future research is to explore gene-environment interactions. Environmental factors including diet, temperature, and rearing conditions can significantly influence fatty acid composition. Understanding how genetic factors interact with these environmental variables will provide critical insights for optimizing breeding strategies. Expanding GS models to incorporate gene-environment interactions would allow for the development of more robust and adaptive breeding programs that can maintain high nutritional quality across varying environmental conditions. Furthermore, functional genomics studies are needed to identify specific genes and pathways that regulate fatty acid metabolism. These studies could help refine the GS models by incorporating marker-assisted selection for key pathways related to fatty acid biosynthesis (López et al., 2015).

5. Conclusion

This study presents the first genomic analysis of muscle fatty acid composition in Malabar red snapper using GWAS and genomic selection approaches. By analyzing 16 fatty acid traits in 534 individuals with 39,780 SNP markers, we demonstrated moderate genomic prediction accuracies (0.29–0.48), especially for key Omega-3 fatty acid traits such as DHA (0.47), EPA (0.43), and EPA + DHA (0.47). Although no major QTLs were detected via GWAS, the polygenic nature of these traits supports the use of GS methods like GBLUP. Notably, traits like the DHA/ALA ratio and PUFA/SFA index also showed usable predictive power. The fatty acid profile of the population was nutritionally favorable, with high PUFA levels and balanced Omega-6/Omega-3 ratios. These findings support the application of genomic selection to enhance fatty acid composition in red snapper, contributing to both sustainable aquaculture and improved human nutrition. While further work is needed to refine prediction models, incorporate environmental interactions, and validate across populations, the study findings demonstrate a promising pathway for the genetic improvement of fish nutritional quality in aquaculture.

CRediT authorship contribution statement

Kathiresan Purushothaman: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nguyen Thanh Vu:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bing Liang:** Writing – review & editing, Methodology, Investigation, Data curation. **Saraphina Dianne Tneo Rwei Qing:** Writing – review & editing, Methodology, Investigation, Data curation. **Muhammad Hazim bin Mohamed:** Writing – review & editing, Methodology, Investigation, Data curation. **Rachel Ho Jia Wen:** Writing – review & editing, Methodology, Investigation, Data curation. **Xueyan Shen:** Writing – review & editing, Methodology, Investigation, Data curation. **David B. Jones:** Writing – review & editing, Methodology, Investigation, Data curation. **Grace Loo:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition. **Dean R. Jerry:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Jose A. Domingos:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition. **Shubha Vij:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.743398.

Data availability statement

The data used in this study will be made available upon reasonable request.

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