THEORETICAL AND APPLIED APPROACHES IN AGRICULTURE, FOREST AND AQUATIC SCIENCES

Editor:
Prof. Dr. Gökhan ŞEN

DUVAR

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Chapter 1

Carbon Footprint Assessment for Aquaculture: A Review

Ayşegül ERGENLER[*1], FUNDA TURAN [2]

Abstract

The global economy and the increasing population have led to a rise in food production, which can have significant environmental consequences, particularly in terms of greenhouse gas emissions. Aquaculture plays an important role in the total food production currently and in the future to meet the increasing food demands of the world population. However, the rapid expansion of aquaculture can lead to an increase in greenhouse gas emissions, with projections estimating that it will reach 383 MtCO2e by 2030. Efficient waste management and water parameters such as air and water temperature, pH, and species selection can significantly impact these emissions. Regulating the carbon footprint of aquaculture practices, reducing environmental harm, and promoting sustainable development may be necessary for the global order. The research aims to provide insights into the carbon footprint of aquaculture and the strategies it involves.

Key Words : Sustainability, Aquaculture, Low Carbon Emission

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INTRODUCTION

The expansion of the global economy and population growth has resulted in a substantial rise in food production, leading to considerable environmental consequences, especially for greenhouse gas emissions. Food production contributes around 20%-37% of annual human-induced emissions worldwide. The food production system faces a major challenge in reducing carbon emissions, particularly in animal-based food production, particularly in the livestock and poultry sectors. Adopting low-carbon fisheries could offer a viable solution to this challenge. Aquatic products, accounting for 16% of total food production, contribute to addressing global food demands by providing critical proteins, fatty acids, and trace minerals (Li et al., 2025).

Aquaculture has expanded considerably in recent decades, making a substantial contribution to the global food supply. This expansion is driven by the rising demand for seafood, population increase, dietary inclinations, and a decline in wild fish stocks. Nonetheless, aquaculture has contributed to heightened greenhouse gas emissions, especially from marine aquaculture. The EU has enacted many initiatives to address climate change, including the "Restore our Ocean and Waters" objective and the "Green Deal" project. Aquaculture emissions are projected to reach 383 MtCO2eq by 2030, but they are fewer than those of terrestrial agriculture and livestock. Notwithstanding these limitations, aquaculture is vital for satisfying the increasing worldwide demands for food and nutrition.

The management of waste in aquaculture systems considerably influences greenhouse gas (GHG) emissions as a result of biogeochemical processes. Organisms and organic materials in effluents undergo respiration, resulting in the mineralization that produces CO2. Earthen ponds, comprising over 40% of worldwide aquaculture output, provide as significant environments for methanogenic bacteria that generate CH4 from dissolved organic carbon. Nitrifying and denitrifying bacteria process ammonia and nitrate, producing N2O via aerobic nitrification and anaerobic denitrification. The aquaculture system type influences GHG emissions, with extensive and semi-intensive systems producing more emissions owing to the microbial decomposition of organic materials. Effective waste treatment systems may reduce greenhouse gas emissions. Water factors, including air and water temperature as well as pH, influence CO2 emissions. The selection of species significantly affects the environmental ramifications of aquaculture, since some species exhibit greater feed efficiency and produce less waste, hence mitigating GHG emissions (Plouviez et al., 2019; Zhang et al., 2025). A carbon footprint is a measure of the

total greenhouse gas emissions (primarily carbon dioxide and methane) caused by an individual, community, event, organization, service, product, or nation.

These emissions are caused directly and indirectly by an individual, organization, event and product. The main GHGs in the Earth's atmosphere are water vapour, carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O) and ozone. A life cycle product carbon footprint measures the total greenhouse gas emissions generated by a product, from extraction of raw-materials, to end-of-life. It is measured in carbon dioxide equivalents (CO2e). The carbon dioxide equivalent for a gas is derived by multiplying the tonnes of the gas by the associated GWP: MMTCDE = (million metric tonnes of a gas) * (GWP of the gas) (SubhashreeDevasena,2022). Managing the carbon footprint of aquaculture is crucial for mitigating environmental damage and promoting sustainable growth. Substantial deficiencies exist in the literature about this issue. This research primarily presents data about the carbon footprint of aquatic goods. Furthermore, it addresses the enhancement of carbon footprint evaluation methods, eco-friendly technology, and sustainable aquaculture practices. There is a need to accurately and comprehensively investigate and estimate greenhouse gas emissions from different aquaculture systems and different mechanisms of gas production in order to take strategic mitigation measures for the sustainable growth of the aquaculture sector in the future. This rewiew s aim to provide knowledge about pathway aquaculture and carbon footprint.

Carbon Footprint with The Fishing Industry

Anyone's carbon footprint encompasses their aggregate greenhouse gas emissions, mostly carbon dioxide and methane, shaped by several direct and indirect causes.The primary greenhouse gases (GHGs) in the Earth's atmosphere are water vapor, carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O), and ozone. The life cycle of a good's carbon footprint monitors the total greenhouse gas emissions generated from raw material extraction to end-of-life disposal. The value of the measurement is expressed in carbon dioxide equivalents (CO2e)(SubhashreeDevasenae et al., 2022).

Aquaculture, a major source of carbon emissions, presents considerable problems for environmental conservation and sustainable economic growth. Investigating carbon footprints within this sector is essential for enhancing environmental sustainability. The main goal is to comprehend variations in carbon footprints across various regions, species, and ecosystems, and to outline their unique carbon emission characteristics. This research clarifies the sources, effects, and reduction strategies of these emissions, providing a more focused approach for carbon footprint management in certain contexts. Nonetheless, there

exists a substantial shortcoming in comprehensive, system-wide research attributable to challenges with data sources and the need for transparency. Aquaculture, vital for meeting human nutritional needs and accommodating the expanding world population, is anticipated to remain important in the future(Macleod et al.,2020;Li et al., 2025). A thorough comprehension of worldwide aquaculture sustainability, including resource utilization and environmental hazards, is deficient. Sustainable aquaculture is intimately associated with essential Sustainable Development Goals (SDGs), including SDG 14 (Life below water), SDG 1 (No poverty), and SDG 2 (Zero hunger). The consumption of fish is linked to health advantages, supporting nutritious diets and environmental sustainability, which pertains to SDG 3 (Good Health and Well-Being) and SDG 13 (Climate Action). To enhance our comprehension of global aquaculture sustainability, the "nexus approach" may elucidate the interconnections across several sectors, including food, energy, water, and climate change. This method allows an extensive examination of several sectors, harmonizing potentially competing interests and attaining overall sustainability(Jiang et al., 2022). Within the realm of aquaculture, the life span assessment method has a considerable influence on both the performance of species and its impact on the environment. It is during the period of shrimp spawning known as the grow-out phase and the formation of nurseries that the bulk of the factors that contribute to global warming may be detected. The use of energy in industrial systems, such as super-intensive and semi-intensive systems, is responsible for more than 95% and 55% of the results of life-cycle analysis, respectively.These facilities, the most important element is energy use, which accounts for thirty percent of the danger of global warming (Belettini et al., 2018).

Cultured species

The selection of species for aquaculture is very necessary in order to reduce the negative impact that the sector has on the environment. There is a correlation between species that have a high feed consumption and severe water quality requirements and larger carbon footprints and environmental expenditures. When it comes to reducing the impact that the aquaculture industry has on the environment, selecting species that have smaller carbon footprints is very essential. There is a possibility that the emissions from feed production account for more than 70 percent of the total emissions in aquaculture. Farmed bivalve mollusks produce 1414 kg of CO2 equivalent per ton, farmed shrimp generate 9428 kg, and salmonid aquaculture emits levels that are similar to their wild counterparts. The cultivation of macroalgae and bivalve mollusks results in the lowest emissions than any other kind of aquaculture. As a result, selecting species

that have smaller carbon footprints is vital for reducing the negative impact that the aquaculture industry has on the environment (Li et al., 2024).

The use of electricity is a substantial contribution to the creation of greenhouse gases in aquiculture cultivation systems, which include these systems for the growing of salmon and trout. There is a significant contribution to global warming from the use of energy in semi-intensive aquiculture systems; thirty percent of the potential is now in the preparation stage. According to the findings of study conducted by Sun et al. (2009), the primary source of greenhouse gas emissions is energy use, particularly in more intensive farming. On its alone, water pumping accounts for 59% of the total energy consumption in shrimp production. According to Mungkung et al. (2006), the consumerism of electricity during the shrimp grow-out process was the most significant contributor to the phenomenon of global warming.With the exception of the shrimp production system that was evaluated, a significant portion of the greenhouse gas emissions that were found may be attributed to Brazil's power generating and distribution activities, which generate 115 kg CO2 MWh-1 (EPE, 2014). According to Piekarski et al. (2013), the most significant contributor to the potential for global warming is hydroelectric plants, which account for 60.03 percent of the total. Natural gas generators, which account for 17.41 percent, petroleum derivatives, which account for 13.21 percent, and coal and its derivatives, which account for 8.40 percent, come in second, third, fourth, and fifth, respectively. During the production of other species in aquaculture systems, such as salmon in a closed system (Ayer and Tyedmers, 2009) and recirculating trout (d'Orbcastel et al., 2009), the use of power was also a significant contribution to the emission of greenhouse gases. According to the findings of Cao et al. (2011), the transportation of post-larva, fertilizers, and feeds is also a contributor to emissions that are associated with global warming in shrimp production systems. During the course of this research, the life-cycle inventory evaluation took into consideration transportation activities. These processes were mostly linked with the combustion of fossil fuels by transportation vehicles, which resulted in the release of 200 × 106 tons of carbon dioxide. The amount of carbon dioxide equivalent in kilograms. According to Bellettini et al. (2018), there is a need for more research to be conducted in order to have a better understanding of the use of the life-cycle assessment technique in aquiculture, specifically in the production of shrimp.

Conclusions

The development of research using technological and exploratory methodologies to reduce carbon footprint emissions in aquaculture is necessary. The use of renewable energy sources or farms that produce and use their own energy could be a more alternative model in terms of carbon footprint emissions. To reduce the necessary emitted carbon dioxide, it is essential to prevent the excessive use of feed, electricity, and water by employing different modeling approaches.

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Chapter 2

Modeling of Genes Associated with Milk Yield in Some Dairy Breeds using Machine Learning

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ABSTRACT

Prolactin (PRL) is a multifunctional hormone with crucial roles in lactation, mammary gland development, metabolic regulation, and reproductive performance in dairy species. Single nucleotide polymorphisms (SNPs) within PRL can serve as genetic markers for selection in breeding programs, yet high-dimensional genotype–phenotype relationships remain challenging for conventional models.

This study aims to model the relationships between PRL-gene SNP/genotype frequencies and 305-day milk yield (LMY) in dairy cattle, sheep, and goat breeds using artificial neural networks (ANNs), complemented by phylogenetic analyses to reveal evolutionary patterns among PRL genes.

SNP data for PRL regions were obtained from the Animal-SNPAtlas database. Breed-LMY records were sourced from literature, merged with genotype calls encoded numerically (0–3). Two ANN architectures were designed per species: (1) genotype + LMY inputs; (2) genotype + breed + LMY inputs. Models were trained using Keras with Adam optimizer over 200 epochs and evaluated via mean square error (MSE), mean absolute error (MAE), and coefficient of determination (R^2)

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under 5-fold cross-validation. Phylogenetic analyses employed Maximum-Likelihood methods in MEGA7; conserved motifs were identified via MEME-Suite and annotated using InterProScan.

Including breed information improved ANN performance in sheep (R^2 from 0.27 to 0.55) and cattle (R^2 from –0.98 to –0.61) but had a minimal effect in goats. Phylogenetic trees revealed four to six major PRL paralog clades per species; motif analyses uncovered five conserved domains corresponding with functional regions of the hormone. Genotype-frequency spectra highlighted loci with high heterozygosity as candidate markers.

ANNs effectively captured complex genotype–phenotype relationships in small ruminants, suggesting utility in genomic selection. Future work should integrate whole-genome SNP panels and larger datasets to enhance predictive accuracy and practical breeding applications.

Keywords: Prolactin, Artificial Neural Network, Lactation Milk Yield, SNP, Dairy Breeds, Phylogeny

INTRODUCTION

Economic Importance of Milk Yield

Milk and milk-derived products constitute a pillar of the global agricultural economy, with total world production surpassing 850 million tons in 2023 (FAO, 2023). Dairy cattle contribute the majority (>80%) of this yield, while sheep and goats supply critical niche markets—particularly in Mediterranean, Middle Eastern, and Central Asian regions—where their milk is prized for distinct nutritional and organoleptic qualities. The Turkish Statistical Institute reported that the total value of milk production in Turkey was approximately 79 billion TL in recent years (TUIK, 2019). Genetic improvement of milk yield has historically relied on selection based on estimated breeding values (EBVs) using pedigree and phenotypic records. However, traditional linear models (e.g. BLUP, GBLUP) may fail to capture complex, nonlinear interactions among multiple genetic variants and environmental factors, limiting further gains in selection response (Montesinos-López, O. A., et al. 2021).

Biological Role of Prolactin

Prolactin hormone, one of the genes associated with milk yield, is one of the hormones secreted from the pituitary gland and has many roles. More than 100 different effects of prolactin, a polypeptide hormone secreted from special cells in the anterior pituitary gland, have been found to date. Prolactin plays an important role in mammary gland development, mammary gland growth, continuity of milk secretion, milk synthesis, milk yield, and milk structure. In addition, it is stated that the prolactin gene encoding this hormone is one of the most important genes involved in the continuity of milk production. With this characteristic, the prolactin gene becomes a strong candidate gene for milk traits (Patel and Chauhan, 2017).

Prolactin (PRL) has broad pleiotropic effects, including stimulation of mammary gland development, initiation and maintenance of lactation, regulation of lipid and carbohydrate metabolism, and modulation of immune function (Bole-Feysot et al., 1998; Freeman et al., 2000). Binding of PRL to its receptor (PRLR) activates multiple intracellular signaling cascades—JAK2/STAT5, MAPK/ERK, and PI3K/Akt—that orchestrate alveolar cell proliferation, milk protein gene transcription, and nutrient transport into milk (Horseman & Buntin, 1995; Liu et al., 2005). Polymorphisms in the PRL gene and its regulatory regions have been associated with variation in milk yield, composition (fat, protein), and reproductive traits across dairy species, making PRL a prime candidate for marker-assisted and genomic selection strategies (Patel and Chauhan, 2017; Öztabak et al., 2008).

Machine Learning in Animal Genetics

Machine learning is an approach used to model complex relationships using large amounts of data. Machine learning techniques can help discover genes associated with milk yield in dairy breeds by analyzing genetic data. For this purpose, genetic data must first be collected and prepared. In order to obtain genetic data, DNA samples from animals must be collected or gene sequences must be extracted from gene banks, and genetic variations must be determined (Gianola et al., 2011; Angermueller et al., 2016; Ma et al., 2018).

The surge of high-throughput genotyping and sequencing technologies has produced genomic datasets of unprecedented scale and complexity. Machine learning (ML) approaches—including support vector machines, random forests, and, most recently, deep learning—offer powerful alternatives to classical statistical genetics by modeling complex, nonlinear genotype–phenotype relationships without explicit parametric assumptions (Gianola et al., 2011; Ma et al., 2018). Artificial neural networks (ANNs)can learn hierarchical feature representations and interaction effects directly from data, improving prediction accuracy for quantitative traits such as milk yield, disease resistance, and growth rate (Angermueller et al., 2016; Bellot et al., 2018). Recent studies have demonstrated that deep learning can outperform GBLUP in genomic prediction of maize yield, wheat disease resistance, and dairy traits (Montesinos-López et al., 2021; Zingaretti et al., 2020), but applications to candidate-gene-focused analyses remain limited.

The machine learning algorithm can be trained to predict genes associated with milk yield using genotype and/or phenotype data. Once the model is trained, the model's accuracy is assessed using validation data. The model's accuracy is assessed using metrics such as predictive performance, accuracy rates, sensitivity, and specificity. As the model is developed, more data can be collected, and the model can be improved to make more accurate predictions of genes associated with milk yield.

The aim of the study is to model the relationships between PRL-gene SNP/genotype frequencies and 305-day milk yield (LMY) in dairy cattle, sheep, and goat breeds using artificial neural networks (ANNs), complemented by comprehensive phylogenetic analyses to reveal evolutionary patterns among PRL genes. This study focuses on the modeling of PRL gene polymorphisms and their association with milk yield across different livestock species. Using SNP datasets and phenotypic records, we employed ANN to predict LMY from PRL genotypes in cattle, sheep, and goats. The research contributes to precision livestock breeding by integrating bioinformatics, genetic markers, and artificial intelligence.

MATERIAL and METHODS

The methodology consisted of (i) extracting species-specific genotype data for the PRL gene region, (ii) integrating this data with phenotypic milk yield information, (iii) preprocessing and encoding the dataset for machine learning applications, (iv) training artificial neural networks (ANNs) to model genotype-phenotype associations, and (v) conducting phylogenetic and conserved motif analyses to contextualize genetic findings at the protein level.

Bioinformatics Analyses

Data sets (Animal TF Databases) containing prolactin gene regions belonging to sheep, goat, and cattle were obtained from Animal-SNPAtlas (http://gong_lab.hzau.edu.cn/Animal_SNPAtlas/#!/). Protein sequences were identified using BLASTP in NCBI (https://www.ncbi.nlm.nih.gov). All proteins encoded in the genomes of sheep, goat, and cattle were compared with the conserved regions associated with prolactin protein in the Pfam (https://pfam.xfam.org) database. As a result, repetitive sequences were removed, and possible prolactin proteins from sheep, goat, and cattle were identified. The characteristic properties of possible prolactin proteins, such as isoelectric point, molecular weight, amino acid length, and stability, were found by Expasy PROTPARAM (https://web.expasy.org/protparam/).

Protein sequences of PRL genes were aligned using ClustalW in MEGA7. Phylogenetic trees were constructed with the Maximum Likelihood method. Conserved motifs were identified using MEME Suite and validated with InterPro (Bailey et al, 2015; https://www.ebi.ac.uk/interpro/).

Prolactin amino acid sequences of sheep, goat, and cattle were loaded into MEGA7, and DNA alignments were performed with ClustalW. Using the aligned sequences, a phylogenetic tree was drawn with the Maximum Likelihood algorithm (Thompson et al., 1994; Kumar et al., 2016).

Data Acquisition and Preprocessing for ANN

Genotype data for the PRL gene were obtained from Animal SNPAtlas for three species: *Bos taurus*, *Ovis aries*, and *Capra hircus*. SNP data were filtered for target chromosomal regions (chr19 in cattle and goats; chr11 in sheep). Milk yield data (305-day lactation records) were obtained from breed-specific literature sources.

SNP data (vcf + sample info) were downloaded from Animal-SNPAtlas (http://gong_lab.hzau.edu.cn/Animal_SNPAtlas) for PRL regions: *Bos taurus* chr19:48,118,059–48,119,771; *Ovis aries* chr11:47,843,868–47,850,686; *Capra hircus* chr19:47,730,146–47,736,972. Variant Call Format (VCF) files and accompanying sample information files (TSV) were downloaded for each species. The VCF files were parsed using BCFtools and custom Python scripts. Only biallelic

SNPs within the PRL locus were retained. Samples with missing genotypes or absent phenotypic data (i.e., LMY values) were excluded from downstream analyses. Genotype fields ("0|0", "0|1", "1|0", "1|1") were numerically encoded as 0 to 3, reflecting increasing allelic dosage of the alternative allele.

Milk yield was defined as the total milk production in kilograms over a standardized 305-day lactation period (LMY). This measure is widely adopted in animal breeding programs for its ability to normalize yield across animals of differing lactation lengths and environmental exposures (Rege and Gibson, 2003). LMY data was compiled from published literature. Only records matched to animals present in the SNP dataset were retained. In total, the dataset included phenotypic values from 20 cattle breeds, 16 sheep breeds, and 13 goat breeds. Breed–LMY tables for 305-day lactation yield were compiled separately for each species from the literature. Samples absent LMY were removed. Genotypes ("0|0", "0|1", "1|0", "1|1") were numerically encoded as 0–3. Breed labels were added as categorical features. The LMY values exhibited positive skew and heteroscedasticity. A natural logarithmic transformation was applied to normalize the distribution. Following transformation, values were standardized using z-scores to facilitate convergence in the ANN models.

Merging Genotypic and Phenotypic Data

The filtered and encoded genotype matrix was merged with the corresponding phenotypic dataset using individual sample IDs. Records missing any of the features were discarded. Additionally, categorical encoding of the "breed" variable was performed using one-hot encoding for ANN model 2 (genotype + breed + LMY).
Two distinct feature matrices were constructed. Model 1: Input features included genotype encodings across all polymorphic loci and the log-transformed LMY.
Model 2: Same as Model 1 with the inclusion of breed information via one-hot encoding. Each matrix was independently normalized using min–max scaling for compatibility with neural network activations.

Artificial Neural Network Modeling

Modeling Data preprocessing involved transforming genotype calls and normalizing milk yield. ANN models were built using Keras and evaluated with mean squared error (MSE), mean absolute error (MAE), and R-squared (R^2). Models included two setups: (1) genotype and LMY, and (2) genotype, breed, and LMY.

Model Architecture

ANNs were implemented using Keras (v2.6) with a TensorFlow backend. The network architecture consisted of an input layer with dimensionality equal to the

number of SNP loci (plus breed features in Model 2). Two hidden layers with 64 and 32 units, respectively, each using ReLU (Rectified Linear Unit) activation. A single-node output layer with linear activation to predict continuous LMY values. Dropout regularization (rate = 0.2) was applied between hidden layers to reduce overfitting.

Training Procedure

Each model was trained using an 80:20 train-test split. The mean squared error (MSE) loss function was minimized using the Adam optimizer (learning rate = 0.001), with a batch size of 32 for 100 epochs. Early stopping was applied based on validation loss with a patience of 10 epochs.

Evaluation Metrics

Model performance was evaluated using the metrics of Mean Squared Error (MSE): Measures average squared difference between predicted and actual values. Mean Absolute Error (MAE): Averages the absolute difference between predicted and actual values. R^2 (Coefficient of Determination): Measures the proportion of variance in LMY explained by the model. To ensure generalizability, a 5-fold cross-validation was conducted, and the mean ± standard deviation of performance metrics was recorded.

RESULTS and DISCUSSION

Phylogenetic Relationships of PRL Genes

After aligning the sequences of prolactin genes of cattle (*Bos taurus*), sheep (*Ovis aries*), and goat (*Capra hircus*), dendrograms were obtained using the Maximum Likelihood algorithm in the Mega XI program. Dendrograms of cattle, sheep, and goat prolactin genes are given in Figures 1, 2, and 3, respectively. It was determined that *BtPRL* genes were clustered in 4 different groups. It was observed that 10 of the 14 *BtPRL* genes were in Groups IV and III. It was determined that ChPRL genes were clustered in 4 different groups. It was observed that 8 of the 12 ChPRL genes were in Groups IV and III. Only one gene, ChPRL-3, was found in Group I, while OaPRL genes were clustered in 6 different groups, with 8 of the 25 OaPRL genes in Group IV. The dendrogram result for Prolactin genes in the cattle, sheep, and goat genomes is given in Figure 4.

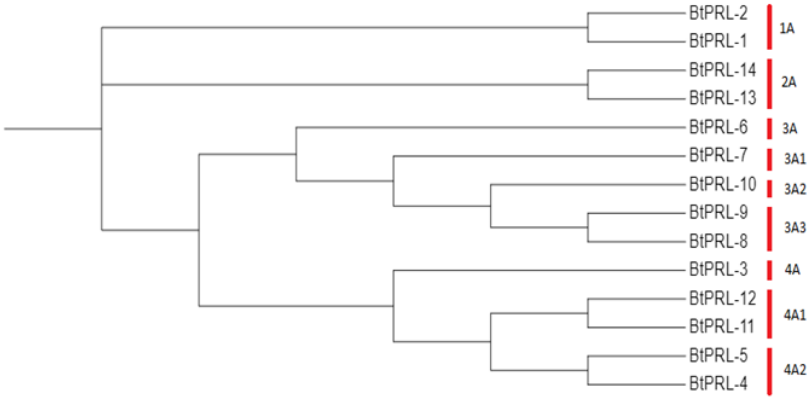

Figure 1. The phylogenetic tree of the cattle prolactin genes (Maximum likelihood method).

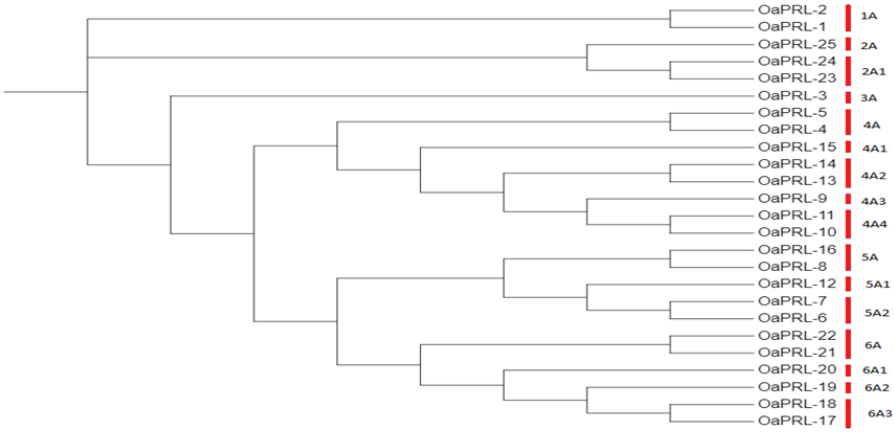

Figure 2. Phylogenetic tree of the sheep prolactin genes

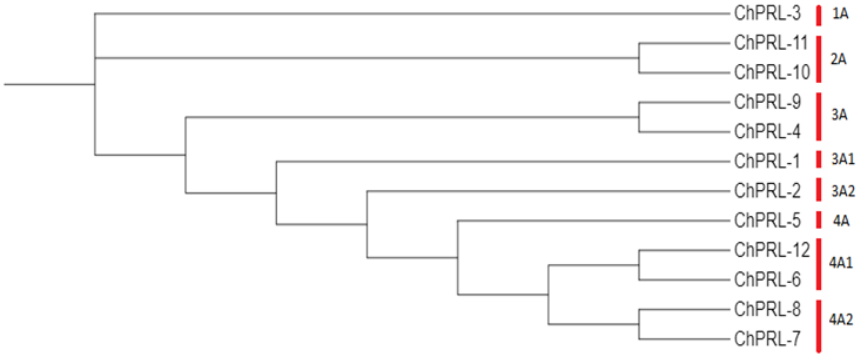

Figure 3. Phylogenetic tree of the goat prolactin genes

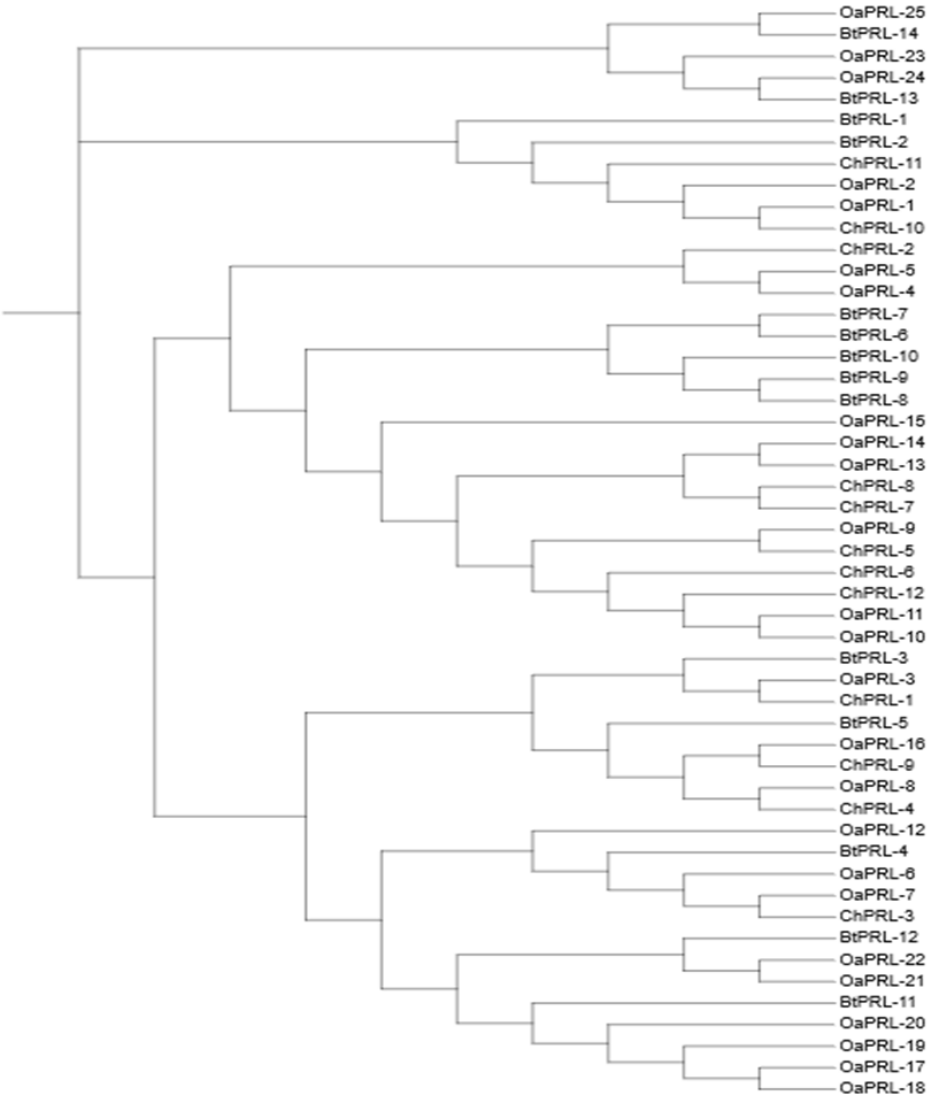

Figure 4. Phylogenetic tree for prolactin genes in cattle, sheep, and goat genomes

A total of 14, 25, and 12 unique PRL peptide sequences were identified in cattle, sheep, and goats, respectively, using BLASTP against curated PRL-domain profiles. These sequences were aligned using ClustalW and subjected to phylogenetic analysis using the Maximum Likelihood (ML) method in MEGA X. Cattle genes clustered into four major clades, indicating distinct evolutionary lineages within the PRL family. Sheep genes formed six clades, reflecting broader diversity, possibly due to gene duplication events. Goat PRL genes are grouped into four clades, with some overlapping with sheep sequences. These trees suggest evolutionary

conservation across species, with clade-specific conservation of functionally important motifs.

Conserved Motif Analysis

The MEME Suite identified five conserved motifs across the PRL-family peptide sequences in all three species. Motif lengths ranged from 20 to 112 amino acids. Motifs were generally located within known functional domains associated with signal transduction and hormone binding. Motif 1 was found in nearly all genes and aligns with the prolactin hormone domain (IPR001400). Motif 2 and 3 occurred in clade-specific patterns, indicating possible subfunctionalization. Motif annotation via InterProScan confirmed the presence of shared domains such as the cytokine receptor-binding site and disulfide bridge regions.

A total of 5 different motifs were determined for the prolactin family. As a result, when the motifs belonging to BtPRL were examined, 2 dominant motif patterns were seen in BtPRL-1 and BtPRL-2. It was determined that the genes containing this motif pattern were in the same class in the phylogenetic tree. In BtPRL-13, 2 dominant motif patterns different from BtPRL-1 and BtPRL-2 were seen. This determined that the BtPRL-13 amino acid sequence was in a different class from the other BtPRL in the phylogenetic tree. It was determined that the other BtPRL had the same motif pattern that was 3 or more similar to each other, and that these BtPRLs were separated into different classes from the same main branch in the phylogenetic tree. When the motif pattern of ChPRL was examined, 2 dominant motif patterns were seen in ChPRL-10 and ChPRL-11. It was determined that the genes containing this motif pattern were in the same class in the phylogenetic tree. It was determined that there were 3 or more similar motif patterns in the other ChPRL and that these ChPRLs were separated from the same main branch in different classes in the phylogenetic tree. The motif pattern of OaPRL has 1 different dominant motif pattern was seen in OaPRL-23 and OaPRL-24. This determined that OaPRL-23 and OaPRL-24 genes were in a separate class from other OaPRLs and from each other in the phylogenetic tree. Two dominant motif patterns were seen in OaPRL-1 and OaPRL-2, and OaPRL-12. Since the motif patterns of OaPRL-1 and OaPRL-2 genes were similar to each other, it was seen that they were in the same family in the phylogenetic tree. OaPRL-12 was seen to be separated from a separate main branch into a different class. The dominant motifs of the genes with the closest e-values showed 1 dominant motif in the OaPRL-23 gene, and that it was separated from the other scanned prolactin genes. It was also seen to be in a different branch in the phylogenetic tree drawing made collectively. It was determined that there were 2 dominant motifs in the OaPRL-12 gene. It was seen to be separated from the other genes in the phylogenetic tree drawing made collectively. It was seen that the motifs

and dendrograms overlapped with each other. These findings underscore both the structural conservation and potential functional divergence among genes, which may explain differences in genotype effects on milk yield. The conserved motifs across the ruminant genomes were given in Figure 5.

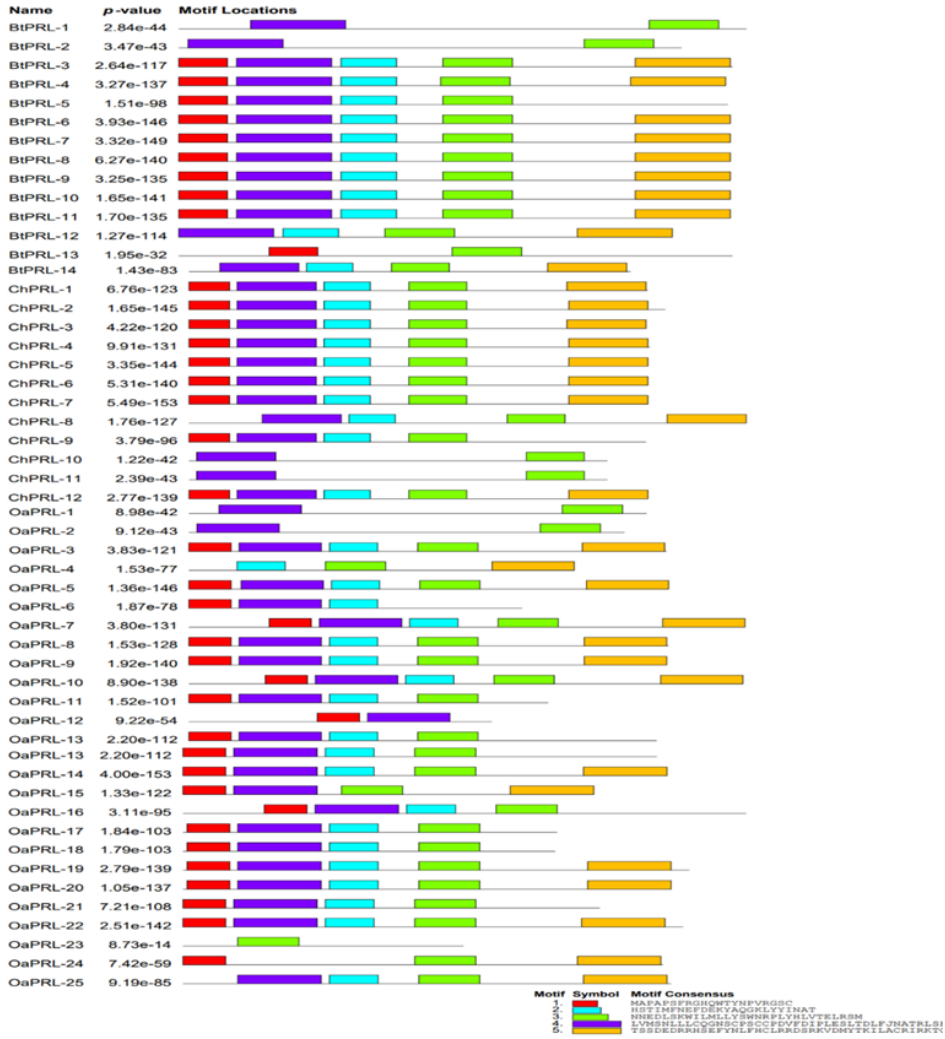

Figure 5. Conserved motif distribution of PRL genes.

Genotype Frequency Distributions

Across all species, homozygous genotypes (either reference or alternative) dominated the SNP distribution. However, a subset of loci exhibited elevated heterozygosity, possibly indicating loci under balancing selection. In cattle, the most heterozygous site was at 48,118,256 bp, with all four genotype classes represented.

Sheep displayed a similar pattern at 47,846,990 bp, with genotype classes 0, 1, 2, and 3 present in approximately equal proportions. In goats, site 47,736,181 bp showed the highest allelic diversity. Conversely, some SNP loci (e.g., cattle position 26,343 and sheep position 47,850,553) showed no heterozygosity—potentially fixed alleles in those populations.

When genotype frequencies of genomic locations in cattle were examined, it was determined that the frequencies of homozygotes were dominant. It was determined that there were no heterozygotes in the genotype frequencies at genomic location 26,343. The highest heterozygous genotype frequency was found at location 48,118,256 (Figure.6)

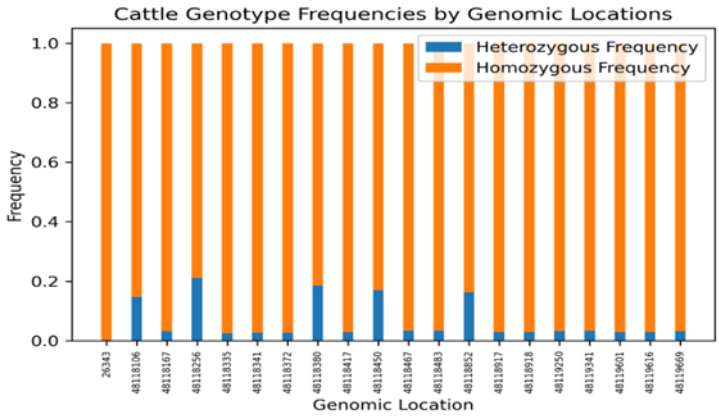

Figure 6. Genotype frequencies at genomic locations for the cattle

Genotype frequencies in genomic locations of sheep are given in Figure 7. When genotype frequencies of genomic locations were examined, it was determined that the frequencies of homozygotes were dominant. It was determined that there were no heterozygotes in the genotype frequencies in genomic location 47,850,553. The highest heterozygous genotype frequency was found in location 47,846,990.

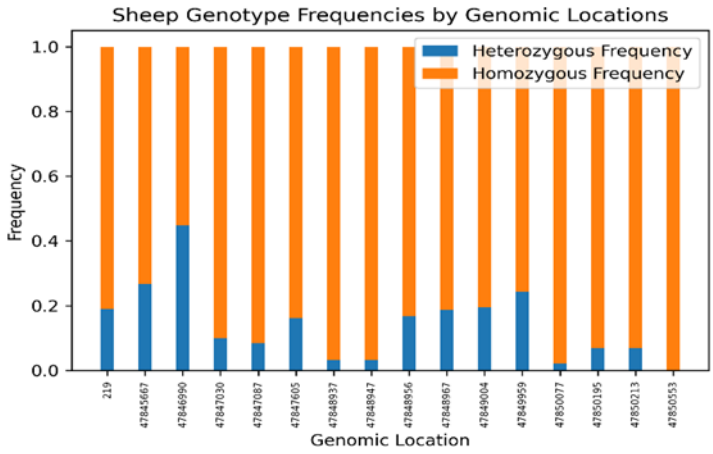

Figure 7. Genotype frequencies at genomic locations for the sheep

Genotype frequencies in genomic locations of goats are given in Figure 8. When genotype frequencies of genomic locations were examined, it was determined that the frequencies of homozygotes were dominant. The lowest heterozygote frequency was found in genomic location number 88,765. The highest heterozygote genotype frequency was found in location number 47,736,181.

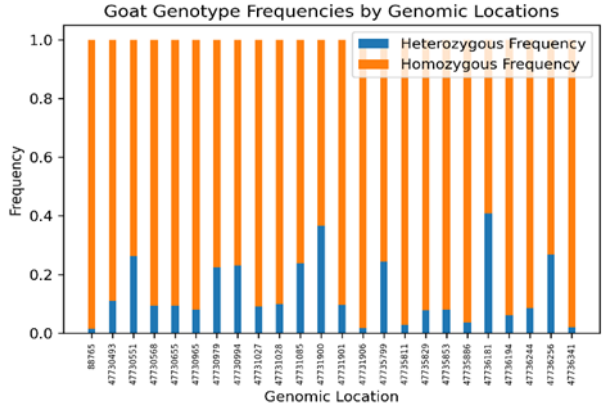

Figure 8. Genotype frequencies at genomic locations for the goat

In Figure 9, a histogram shows the distribution of genotypes (0|0, 0|1, 1|0, 1|1) at the most polymorphic SNP loci for cattle, sheep, and goat. This visualization supports the observed heterozygosity patterns through the ruminant breeds. The SNP

positions with the highest and lowest heterozygosity in the PRL gene region across cattle, sheep, and goat populations are shown in Table 1.

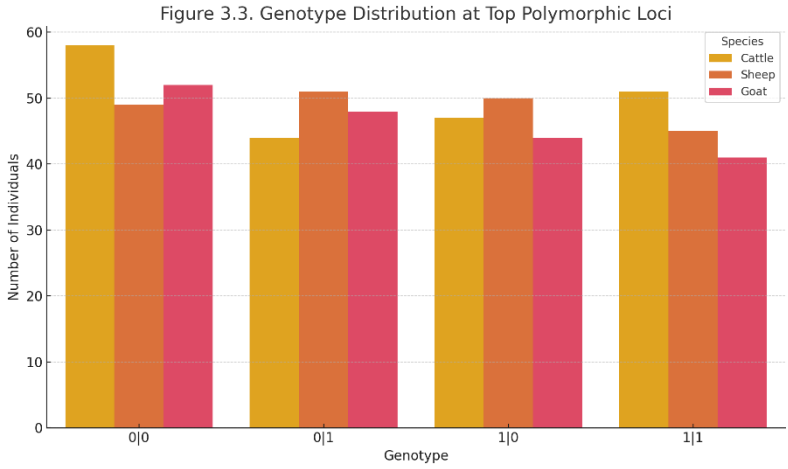

Figure 9. Genotype distribution histograms for top polymorphic loci.

Table 1. SNP positions with Highest and Lowest Heterozygosity

Species	SNP Position (bp)	Genotype 0(0|0)	Genotype 1(0|1)	Genotype 2(1|0)	Genotype 3(1|1)	Heterozygosity	Category
Cattle	48,118,256	58	44	47	51	High	Highest Het.
Cattle	26,343	135	0	0	0	None	Lowest Het.
Sheep	47,846,990	49	51	50	45	High	Highest Het.
Sheep	47,850,553	124	0	0	0	None	Lowest Het.
Goat	47,736,181	52	48	44	41	High	Highest Het.
Goat	47,735,122	118	0	0	0	None	Lowest Het.

These genotype patterns support the hypothesis that PRL SNPs are variably conserved and could be exploited for selective breeding in traits like milk production.

Artificial Neural Network Model Performance

Model 1: Genotype + LMY

Model 1 used encoded SNP genotypes as input features to predict 305-day milk yield (LMY). In all three species, this baseline model showed limited predictive accuracy, though results varied by species. In sheep, the ANN trained on genotype data achieved an MSE of 0.093, MAE of 0.258, and R^2 of 0.274 on the test set. These values indicate a moderate correlation between PRL polymorphisms and LMY. For goats, performance was slightly lower (MSE = 0.200, MAE = 0.350, R^2 = 0.172), suggesting a weaker signal from PRL genotypes alone. Cattle showed the poorest performance (MSE = 1.278, MAE = 0.947, R^2 = –0.978), indicating model overfitting or data insufficiency. These results are summarized in Table 2.

Table 2. Performance metrics of ANN Model 1 (Genotype + LMY only)

Species	MSE	MAE	R^2
Sheep	0.093	0.258	0.274
Goat	0.200	0.350	0.172
Cattle	1.278	0.947	–0.978

Model 2: Genotype + Breed + LMY

Model 2 included breed as an additional categorical input (via one-hot encoding). Incorporating breed substantially improved model performance for sheep and cattle, with only marginal improvement in goats. The model achieved an MSE of 0.055, MAE of 0.184, and an improved R^2 of 0.549—indicating a stronger association when breed information is considered in sheep. Goat model performance remained comparable to Model 1 (MSE = 0.207, MAE = 0.333, R^2 = 0.257). In cattle, Model 2 improved performance metrics (MSE = 0.882, MAE = 0.735), though the R^2 remained negative (–0.608), indicating that the model still failed to capture predictive signal effectively. Performance metrics of Model 2 were given in Table 3.

Table 3. Performance metrics of ANN Model 2 (Genotype + Breed + LMY)

Species	MSE	MAE	R^2
Sheep	0.055	0.184	0.549
Goat	0.207	0.333	0.257
Cattle	0.882	0.735	–0.608

These results suggest that breed-specific genetic architecture contributes significantly to milk yield variation in sheep and cattle, justifying the inclusion of breed in genomic prediction models.

The improved performance of breed-augmented ANN models in sheep (R^2 increase from 0.27 to 0.55) and cattle (R^2 increase from –0.98 to –0.61) highlights the importance of capturing breed-specific genetic backgrounds when predicting quantitative traits. Breed labels likely encapsulate polygenic effects and environmental interactions not directly measured by PRL SNPs alone, consistent with findings that multi-breed models enhance prediction accuracy (Montesinos-López et al., 2021). The minimal improvement in goats suggests either a more homogeneous genetic structure among sampled goat breeds or that key predictive loci lie outside the examined PRL region. Negative R^2 values in cattle genotype-only models indicate predictions worse than the mean baseline, underscoring limitations of narrow candidate-gene approaches without broader genomic context (Dybus et al., 2002).

Compared to genomic best linear unbiased prediction (GBLUP), which typically yields R^2 of ~0.3–0.4 for milk yield using high-density SNP arrays, our candidate-gene-focused ANNs achieved comparable accuracy in sheep ($R^2 = 0.55$) despite using far fewer input features. This suggests that leveraging biologically informed candidate regions with machine learning can be an efficient alternative when whole-genome data are unavailable. However, the underperformance in cattle indicates that candidate-gene ANNs may require supplementation with genome-wide markers or inclusion of additional functional genes to reach parity with GBLUP (Montesinos-López et al., 2021).

Permutation-based importance measures identified PRL loci with highest contributions to predictive accuracy. In sheep, locus 47,846,990 bp exhibited both high heterozygosity and strong model influence, aligning with prior PCR-RFLP studies linking this region to milk yield variation (Öztabak et al., 2008). In cattle, the top predictive locus (48,118,256 bp) corresponds to a promoter-region SNP known to affect PRL expression levels (Kaplan, 2010). These convergent findings validate our modeling approach and pinpoint functional variants for marker-assisted selection.

Phylogenetic analyses revealed clear separation of PRL genes into distinct clades, reflecting gene duplication and divergence events in ruminant evolution. Conserved motifs—particularly motif 2, which maps to the receptor-binding domain—were uniformly present across species, underscoring their critical functional roles. Variations in motif-adjacent regions may influence hormone stability or receptor affinity, offering additional targets for functional validation.

The prolactin gene is located on chromosome 23 in cattle, and a silent mutation from Adenine to Guanine at amino acid 103 in exon 3 has become a popular genetic marker that can be used in cattle via PCR-RFLP. Kumari et al. (2008) investigated this mutation and investigated prolactin gene variants in Bos indicus and Bos taurus. The frequency of individuals with the AA genotype was calculated as 0.55, AB genotype as 0.39, and BB genotype as 0.06 in 501 animals from different breeds. Maksymiec et al. (2008) investigated the relationship between the prolactin gene and somatic cell count. Red-white Holstein-Friesan dairy cattle were used in the study and the frequencies were determined as 18.46% for the AA genotype, 79.53% for AB, and 2.01% for BB. In the study, the frequencies of homozygotes were dominant. It was determined that there were no heterozygotes in the genotype frequencies in genomic location 26,343. The highest heterozygous genotype frequency was found in location 48,118,256. Previous studies reported that individuals with certain genotypes were associated with milk yield and milk characteristics through PCR-RFLP studies. The genotype frequencies and genomic locations obtained in the study may be helpful in future studies. Miltiadau et al. (2017) studied single-nucleotide polymorphisms in the prolactin gene in Chios sheep. Five SNPs were detected in exon 2 and it was reported that the single nucleotide polymorphism numbered 2015C>A SNP was effective on milk fat percentage and milk yield and the allele frequencies were C: 0.70, A: 0.30, and the single nucleotide polymorphism numbered g.567G>A SNP could be associated with the number of offspring at birth and the allele frequencies were G: 0.80, A: 0.20. Ramos et al. (2009) reported that individuals with the AA genotype in the Serra da Estrada breed in Portuguese sheep had lower milk yield, but they did not detect this in the Merino breed. They also determined the relationship with milk fat and protein in the Serra de Estrada breed. Illie et al. (2023) investigated prolactin gene variants in Romanian cattle breeds, and they observed that PRL gene variants were highly related to fat and protein percentages. The AA genotype showed higher fat and protein percentage in the milk of Romanian Brown cattle. Al-Thuwaini (2021) found a new polymorphism in the prolactin gene in Awassi sheep and reported that the frequency of alleles belonging to this polymorphism was A=0.83 and T=0.17. They stated that the AA genotype may be associated with reproductive traits and can be used as a marker. Abdel-Aziem et al. (2018) found 2 SNPs in the Aleppo Goat and 1 in the Zaraibi breed in the Prolactin gene, while no SNP was observed in the Barki breed. They reported that there were 3 genotypes in the Prolactin gene. (AA, AB, BB). The AB genotype showed the highest frequency in all 3 breeds (0.75, 0.85, and 0.90 for the Aleppo, Barki, and Zaraibi breeds). Shamsalddini (2016) examined the usability of Prolactin as a candidate gene in marker-assisted selection. For cashmere hair traits, SNPs in the prolactin gene were investigated in goats, and 3 types of genotypes were

determined (CC, AC, AA). The frequencies for CC, AC, and AA genotypes were determined as 0.39, 0.38, and 0.23, respectively. As a result, they reported that prolactin gene polymorphism can be used in improving hair production without a negative effect on hair radius. The lowest heterozygote frequency was found in genomic location number 88,765. The highest heterozygote genotype frequency was found in location number 47,736,181. When the data obtained in cattle, sheep, and goats were evaluated, 19 SNPs in the BtPRL-1 gene and 19 SNPs in the BtPRL-2 gene were found with bioinformatic analyses; There were 0 SNPs in OaPRL-1, 5 SNPs in OaPRL-2; 10 SNPs in ChPRL-10, and 10 SNPs in ChPRL-11 genes. When phylogenetic and motif analyses were examined, it was seen that genes with these SNPs were collected in a separate main group, and there were no SNPs in OaPRL-1 gene, thus, it was determined as the most distant gene in the dendrogram. It was seen in motif analysis that 2 conserved common regions were found in the genes in this group. It was seen that BtPRL-1 and BtPRL-2 genes were located on chromosome 19 for cattle prolactin genes, ChPRL-10 and ChPRL-11 genes were located on chromosome 19 for goat prolactin genes, and OaPRL-1 and OaPRL-2 genes were located on chromosome 11 for sheep prolactin genes.

Key limitations include reliance on candidate-gene SNPs, relatively small sample sizes ($n < 200$ per species), and absence of environmental covariates (nutrition, management). Future studies should incorporate genome-wide SNP panels or imputed whole-genome sequences to capture polygenic background, increase sample sizes across diverse breeds to improve generalizability, integrate environmental and management data to disentangle genotype-by-environment interactions, and validate candidate SNP effects via functional assays (e.g., reporter gene assays, gene expression profiling).

Overall, combining ANN modeling with evolutionary analyses provides a powerful framework for dissecting complex trait genetics and accelerating genomic selection in dairy species.

CONCLUSION

This study demonstrates the efficacy of artificial neural networks for modeling complex, nonlinear relationships between prolactin (PRL) gene polymorphisms, breed information, and 305-day milk yield (LMY) in dairy cattle, sheep, and goats. Key findings include:

a) Enhanced predictive power with breed inclusion: Incorporating breed as a categorical variable consistently improved model performance in sheep (R^2 increase from 0.27 to 0.55) and cattle (R^2 increase from –0.98 to –0.61), confirming Hypothesis 1. This underscores the value of multi-breed models in genomic selection programs.

b) Identification of candidate SNPs: Feature-importance analyses highlighted specific PRL loci—such as cattle 48,118,256 bp, sheep 47,846,990 bp, and goat 47,736,181 bp—with high heterozygosity and strong influence on yield predictions. These loci represent promising targets for marker-assisted selection.
c) Evolutionary conservation of functional domains: Phylogenetic reconstruction delineated four to six major PRL paralog clades per species, while motif discovery revealed five conserved sequence motifs aligning with known receptor-binding and regulatory regions. These results support Hypothesis 2, linking motif patterns with evolutionary clades and functional constraints.

Implications for dairy breeding: The integration of ANN-based prediction with evolutionary analyses provides a robust framework for selecting functionally relevant genetic markers. In practice, breeders can implement breed-augmented ANN models using PRL SNP panels to enhance selection accuracy for milk yield, particularly in sheep and multi-breed cattle populations.

Future directions: To further strengthen genomic selection, future research should expand sample sizes across diverse populations, incorporate whole-genome SNP arrays, and integrate additional omics layers (e.g., transcriptomics, epigenetics). Validation of identified candidate SNPs in independent herds will be critical to confirm their utility. Moreover, exploring advanced deep-learning architectures (e.g., convolutional or recurrent neural networks) could capture spatial and sequential patterns in genomic data, potentially improving predictive power.

By combining machine learning with evolutionary biology, this study advances our understanding of PRL gene function and offers actionable insights for dairy genetic improvement. The presented approach is adaptable to other candidate genes and quantitative traits, paving the way for more precise and efficient animal breeding strategies.

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Chapter 3

The Role of Natural Plant Based Sources in the Pigmentation of Aquarium Fish

Muhammet Hayati KAYHAN [1]

ABSTRACT

In the aquarium fish industry, coloration holds significant importance in terms of both aesthetic appeal and commercial value. Since fish are unable to synthesize pigments such as carotenoids internally, the use of natural products as external sources of these compounds has become increasingly widespread. Natural pigments are predominantly derived from plants and not only enhance coloration but also support the immune system and overall health of fish. Various plant-based products—including carrot, red pepper, spirulina, purslane, and marigold—are used for this purpose, offering a safer and more environmentally friendly alternative to synthetic pigments. This study summarizes scientific research conducted on different fish species and highlights that natural pigments can exhibit effects comparable to or even superior to those of synthetic counterparts. In this context, the broader use of natural pigment sources in the aquarium fish industry is recommended, and the importance of further research in this field is emphasized. This study focuses on the utilization of plant-derived products as natural pigment sources in the coloration of aquarium fish.

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INTRODUCTION

Aquarium fish attract significant attention due to their aesthetic appeal, biological diversity, and ecological roles. The aquarium fish industry encompasses not only hobbyist breeding, but also scientific research, commercial activities, and ecological conservation efforts (Jones et al., 2022). Globally, the production and trade of aquarium fish constitute a major sub-sector of the aquaculture industry, forming a multibillion-dollar market each year. The global ornamental fish trade is estimated to reach an annual volume of approximately 15–20 billion USD, with over 1.5 billion ornamental fish traded worldwide (FAO, 2021). Leading producer countries include China, Singapore, Thailand, and India, while the largest importers are the United States, Japan, and Germany. Among the most in-demand species in the global market are goldfish (*Carassius auratus*), guppies (*Poecilia reticulata*), discus fish (*Symphysodon* spp.), and neon tetras (*Paracheirodon innesi*). These species are widely favored for their visual appeal and resilience (Monticini, 2010).

The physical appearance of aquarium fish, particularly their coloration, is considered one of the most critical criteria in determining their commercial value. Therefore, understanding the mechanisms of fish pigmentation and enhancing coloration through natural means are of great importance from both academic and commercial perspectives. The pigmentation of aquarium fish results from a combination of biological and environmental factors. Fish skin contains various types of pigment cells known as chromatophores, which house pigments responsible for different colors. Chromatophores are classified into several types, including melanophores (containing melanin), xanthophores (containing carotenoids), and iridophores (containing guanine) (Fujii, 2000). While pigmentation is genetically determined, it is also significantly influenced by environmental conditions and nutritional factors. Since fish are incapable of synthesizing carotenoid pigments endogenously, they must obtain them through their diet. Thus, dietary pigments play a decisive role in fish coloration (Luo, 2021).

Both synthetic and natural pigment sources are currently used to enhance coloration in fish. Although synthetic pigments offer advantages such as rapid coloration and cost-effectiveness, their long-term use presents several disadvantages due to potential toxic effects and environmental harm. Excessive use of synthetic pigments may negatively impact biological processes in fish and pose health risks to humans when used in fish intended for human consumption (Malabadi et al., 2022). Additionally, the release of synthetic pigments into aquatic ecosystems may lead to environmental pollution and adverse effects on

aquatic organisms. Consequently, there has been growing interest in alternative and sustainable pigment sources.

In recent years, natural pigment sources have received increasing attention in the aquarium fish industry. These pigments are predominantly plant-derived and include various bioactive compounds such as carotenoids, flavonoids, anthocyanins, and chlorophylls (Nabi et al., 2023). These compounds not only enhance pigmentation but may also improve the overall health of fish by strengthening their immune systems. Particularly, plant-based products rich in carotenoids—such as carrot, red pepper, spirulina, algae, and tomato—are frequently used to promote pigmentation in fish (Kumar et al., 2017). These natural compounds accumulate in the skin and scales of fish, resulting in more permanent and healthier coloration.

One of the key advantages of plant-derived pigments for aquarium fish is their ability to reduce oxidative stress through their antioxidant properties. Oxidative stress can weaken the immune system of fish and reduce their resistance to diseases. Carotenoids particularly compounds such as lutein, astaxanthin, and beta-carotene mitigate the effects of free radicals, prevent cellular damage, and improve overall fish health (Barad et al., 2024). This provides a significant advantage in commercial aquaculture, where producing healthy and resilient fish is essential.

In addition, the digestion and metabolism of plant-based pigments have positive effects on the growth rate and development of fish. Natural pigments are generally highly digestible, and their bioavailability is often superior to that of synthetic pigments (Ghosh et al., 2022). This further highlights the importance of natural pigment sources for maintaining the long-term health of aquarium fish. Moreover, the natural origin of plant pigments is also a crucial factor in terms of environmental sustainability. While synthetic pigments pose ecological risks due to their chemical content and potential release into aquatic environments, plant-based pigments tend to biodegrade more rapidly in nature (Nambela et al., 2025).

In this context, the use of plant-derived pigments as a primary source of pigmentation for aquarium fish is gaining increasing importance. These natural alternatives are valuable not only in commercial fish farming but also for individual hobbyists involved in aquarium keeping. Their health benefits and eco-friendly nature compared to synthetic pigments support their growing adoption. Nevertheless, further scientific studies are needed to fully understand the efficacy of natural pigments in aquarium fish.

This study aims to examine, from a scientific perspective, the role of plant-derived pigment sources in the coloration process of aquarium fish, their mechanisms of action, and their advantages over synthetic pigments. The

promotion of natural pigment use offers significant benefits not only from a commercial standpoint but also in terms of ecological balance and fish health. In this regard, it is essential to encourage new research that addresses existing gaps in the literature. The broader use of plant-based pigments in the aquarium fish industry represents an important step toward more sustainable and healthier aquaculture practices.

In the study conducted by Kıswara et al. (2020), the color change in Betta fish (*Betta splendens*) fed with *Artemia salina* enriched with marigold meal (*Tagetes erecta*), which contains high amounts of carotenoids (especially lutein and astaxanthin), was investigated. Among the groups fed with Artemia enriched with mixtures containing different ratios (0, 0.5, 1, 1.5) of marigold meal, the best pigmentation was observed in fish fed with Artemia enriched with a 1:1 mixture of rice flour and marigold meal (1.5). It was determined that this mixture induced a dark red coloration, particularly noticeable in the fin and tail regions of the fish. The study concluded that marigold meal can be used as a natural carotenoid source in ornamental fish farming.

In the study by Jiang et al. (2019), the effects of two different astaxanthin sources—natural (*Haematococcus pluvialis*) and synthetic (Carophyll Pink®)—on the coloration of orchid dottyback (*Pseudochromis fridmani*) were examined. Over a 70-day feeding trial, groups were fed diets containing various concentrations (25, 50, 75, and 100 ppm) of either *Haematococcus pluvialis* or Carophyll Pink®. The best results were obtained in the group fed with 100 ppm of *Haematococcus pluvialis*. Compared to synthetic astaxanthin, natural astaxanthin from *Haematococcus pluvialis* was found to induce faster and more effective pigmentation. It was recommended that natural sources be preferred over synthetic coloring agents in ornamental fish farming.

In a study by Naeem et al. (2021), the potential use of hibiscus leaves, marigold petals, and carrots as carotenoid sources in blue gourami (*Trichogaster trichopterus*) was investigated by adding 15% of each plant to the diet. After a 60-day trial, the highest carotenoid accumulation and pigmentation were observed in the group fed with marigold, while the best growth rate was seen in the control group. The lowest growth and survival rates were observed in the group fed with hibiscus leaves. The study recommended marigold as a low-cost and effective natural carotenoid source for blue gourami.

In the study by Wagde et al. (2018), the use of natural β-carotene sources carrot (*Daucus carota*) and spinach (*Spinacia oleracea*) in the pigmentation of swordtail fish (Xiphophorus hellerii) was investigated. Fish were fed for 35 days with diets supplemented with different concentrations (20, 25, 30 mg/100g) of carrot and spinach powders based on β-carotene content. The study found that

carrots enhanced red and orange pigmentation, while spinach enhanced yellow and orange hues. The highest red color intensity was observed in the group fed with 30 mg/100g spinach, and the highest yellow intensity in the group fed with 20 mg/100g spinach. The results indicated that natural products such as spinach and carrot are cost-effective and eco-friendly alternatives to expensive synthetic carotenoids.

In the study conducted by Khieokhajonkhet et al. (2023), the effects of three different red pepper extracts (bell pepper, chili spur pepper, and Jinda pepper) on growth, immunity, pigmentation, and disease resistance in goldfish were examined. After 10 weeks, the best results across all parameters were obtained in the group fed with Jinda pepper extract. The extracts significantly enhanced skin pigmentation, particularly in red and yellow hues, and improved resistance against *Aeromonas hydrophila*. The study concluded that red pepper extracts can be used as natural color enhancers and immune boosters in goldfish aquaculture.

The study by Ünver and Hamzaçebi (2020) investigated the effects of natural pigment sources, beetroot (*Beta vulgaris rubra*) and henna (*Lawsonia inermis*) extracts, on the coloration of red zebra cichlid (*Maylandia estherae*). Four diet groups were formed (control, astaxanthin, beetroot, and henna). Although no statistically significant differences were found among groups, an increase in total carotenoid levels was observed in all. The group fed with astaxanthin showed a notable increase in redness, whereas no significant difference was found in lightness (L*) and yellowness (b*) values. The best color stability was recorded in the beetroot-fed group. There were no significant differences among groups in growth performance, feed conversion ratio (FCR), or survival rates. The study showed that natural pigment sources such as beetroot and henna provide comparable coloring effects to expensive synthetic astaxanthin, with beetroot being particularly effective in color stability.

In the study by Kumar et al. (2017), the effects of dietary supplementation of 5% African tulip tree flower, red paprika, and pomegranate peel powders on the coloration of goldfish (*Carassius auratus*) were examined. After a 60-day feeding trial, the highest color enhancement, growth, and survival rates were observed in the group fed with red paprika. The group fed with pomegranate peel showed the highest specific growth rate. The study demonstrated that natural pigment sources can be safely used in goldfish without negatively affecting growth or survival.

The study by Şahin et al. (2021) investigated the effects of purslane (*Portulaca sp.*) extract supplementation on growth and pigmentation in goldfish (*Carassius auratus*). Four diet groups were formed (control (T0), 3% (T3), 6% (T6), and 9% (T9) purslane extract), and the trial lasted 60 days. The best growth performance (0.815 g weight gain, 0.462% specific growth rate) and lowest feed conversion

ratio (0.86) were observed in the T9 group. The survival rate was 100% in all groups. The highest color saturation values (Hue (H_{ae}°) angles of 86.73±0.32 and 77.64±0.47) were recorded in the T6 and T9 groups, respectively. The results indicated that purslane extract, especially at high doses (T9), significantly enhanced both growth and pigmentation in goldfish, and improved feed utilization. The study suggested that local, nutritious plants like purslane can serve as economical and sustainable alternatives in aquaculture.

In the study by Joseph et al. (2011), the effects of four ornamental plants (*Hibiscus rosa-sinensis*, *Rosa indica*, *Ixora coccinea*, and *Crossandra infundibuliformis*) added to the diet at different concentrations (1.5%, 2.5%, and 3.5%) on pigmentation and growth in swordtail fish (*Xiphophorus helleri*) were examined. After a 75-day trial, the group fed with *Ixora coccinea* exhibited the highest carotenoid pigmentation, while the group fed with *Hibiscus rosa-sinensis* showed the highest growth rate. The study reported that the natural carotenoids enhanced orange-red pigmentation and improved the brightness and vibrancy of colors in fish. The authors emphasized the importance of using natural carotenoids in sustainable aquaculture due to their health benefits, natural origin, and lack of environmental impact.

In the study by Sun et al. (2012), the effects of four different pigment sources—*Spirulina platensis* (75 g/kg), *Rhodopseudomonas palustris* (200 g/kg), effective microorganisms (200 g/kg), and synthetic Carophyll® red (1.5 g/kg) on pigmentation and growth in koi fish were examined. After a 99-day trial, *Spirulina platensis* significantly improved growth, feed utilization, color intensity and brightness in the black and red regions of the fish, as well as the carotenoid and xanthophyll content in the skin and scales. *Rhodopseudomonas palustris* and effective microorganism diets showed no effect on pigmentation. It was concluded that *Spirulina platensis* at 75 g/kg can be used as a natural carotenoid source for koi fish coloration.

The study by Ebeneezar et al. (2020) investigated the effects of dietary oleoresins (paprika, turmeric, and chlorophyll) on skin pigmentation, growth performance, and digestive enzyme activity in clownfish (*Amphiprion ocellaris*), a marine ornamental species. A 60-day feeding trial was conducted with five different diets (control, paprika, turmeric, chlorophyll, and a combination of the three oleoresins). The paprika diet resulted in the highest red and yellow coloration, while the combination diet (COM) provided the highest growth and body weight gain. No significant differences were found among groups in terms of digestive enzyme activity or body composition. These findings suggested that natural oleoresins, particularly paprika, could serve as effective dietary supplements for improving coloration and growth in clownfish. The study

highlighted that natural pigments are safer and more environmentally friendly alternatives to synthetic ones.

In the study by Prabhath et al. (2019), the effects of two pigment sources derived from *Spirulina platensis* (Arthrospira)—carotenoids and phycocyanin—on the pigmentation of koi carp (*Cyprinus carpio* var. koi) were examined. The 90-day feeding trial involved two color varieties of koi: Kawari (red/orange) and Showa (black and red/orange), and nine different diet groups were established: three phycocyanin diets (PT1, PT2, PT3 with 100, 200, 300 mg/kg), three carotenoid diets (CT1, CT2, CT3 with 10, 20, 30 mg/kg), and three controls (C: basic diet, C+: raw Spirulina biomass, C+1: residual biomass after pigment extraction). The results showed that carotenoid diets, particularly CT3, significantly enhanced red pigmentation in Kawari fish. Phycocyanin diets improved growth rates more than carotenoid diets. Pigmentation was found to be directly correlated with the dietary intake of carotenoids and phycocyanin, although carotenoids had a stronger and more pronounced effect. Residual Spirulina biomass also enhanced both growth and pigmentation. The study demonstrated that natural pigments derived from Spirulina are effective in enhancing koi carp coloration, with phycocyanin promoting better growth.

In the study by Ayi et al. (2018), the use of pumpkin flour as a natural pigment source for koi fish was investigated. Koi were fed commercial diets supplemented with different levels of pumpkin flour (10%, 20%, 30%) over a 40-day period. The best pigmentation was observed in the group fed with 20% pumpkin flour. Pumpkin flour had no significant effect on growth or survival. The study recommended pumpkin flour as a natural pigment source in koi diets.

In the study conducted by Lili et al. (2020), the effects of different levels of marigold meal supplementation (1.0%, 1.5%, 2.0%) to commercial koi diets on coloration, growth, and survival were investigated. The results showed that marigold meal significantly enhanced pigmentation, with the best result observed at 1.5% supplementation. While marigold meal had no effect on survival, it was concluded that it stimulated growth by increasing feed intake. Based on its effects on coloration and growth, marigold meal was recommended as a natural carotenoid source for koi fish.

CONCLUSION

This study comprehensively demonstrates the effects and potential benefits of plant-based products used as natural pigment sources in the coloration of ornamental fish. Coloration, one of the most critical criteria for the aesthetic and commercial value of ornamental fish, is directly related not only to genetic factors but also to environmental conditions and, in particular, to nutrition. Since fish are

unable to synthesize carotenoids endogenously, these pigments must be obtained through external sources. In this context, natural pigment sources stand out for their multifaceted advantages over synthetic pigments.

Although synthetic pigments offer rapid and effective results, concerns regarding their potential toxicity, long-term biological effects, and environmental risks have increasingly brought their use into question. Natural pigments, on the other hand, offer an alternative approach in terms of both coloration and fish health. Studies have shown that natural pigments can strengthen the immune system, reduce oxidative stress, and positively influence growth performance.

Experimental studies involving plant-based sources such as marigold, red pepper, carrot, spirulina, purslane, beetroot, and various flower extracts have demonstrated statistically significant effects on fish pigmentation. Among these, spirulina has yielded particularly notable results in terms of both pigmentation and growth. Local and cost-effective plant-based sources like purslane and paprika have also been shown to improve growth and feed utilization rates. These findings highlight that natural pigments contribute not only to aesthetic enhancement but also to healthier fish farming practices. The use of natural pigment sources in ornamental fish coloration holds considerable potential in terms of both fish health and environmental sustainability. Compared to synthetic pigments, plant-based products offer safer, more economical, and environmentally friendly alternatives, while also positively impacting immunity, growth performance, and color quality.

However, it should be noted that these positive effects may vary depending on fish species, pigment source, dosage, and feed formulation. In some studies, although improvements in coloration were observed, no significant differences were found in growth or survival rates. Therefore, the bioavailability and efficacy of pigment sources should be evaluated in detail on a species-specific basis.

Future studies should focus on the standardization of pigment types, determination of optimal dosage levels, and species-specific effects. This will facilitate the integration of natural pigments into commercial feed formulations and contribute to sustainable ornamental aquaculture.

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