

Article

Association of SNPs Located in Different Candidate Genes with Growth Traits in Two Japanese Quail Lines

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Abstract

This study investigated the association of polymorphisms in four genes with growth traits in two Japanese quail lines: a high-growth-rate selected line (HGR) and a control line (CL). It utilized 21 generations of divergent selection to identify potential selection signatures, comparing allele frequency shifts between the high-growth and unselected base populations. *Growth Hormone (GH)*, *Prolactin (PRL)*, *Bone Morphogenetic Protein Receptor Type-1B (BMPR-1B)*, and *Vasoactive Intestinal Peptide Receptor 1 (VIPR-1)* genes were genotyped using PCR-RFLP. While the PRL gene was monomorphic, the GH, BMPR-1B, and VIPR-1 loci were polymorphic. Association analysis demonstrated that these variants significantly influenced body weight. In the HGR line, the homozygous genotypes (BB) of GH and BMPR-1B were significantly correlated with increased body weight at various ages. The AA genotype of VIPR-1 correlated with growth, while the BB genotype reduced body weight. An in silico analysis suggested that GH and VIPR-1 variants were functionally disadvantageous. The BMPR-1B variant was tolerated, indicating its potential as a genetic marker. Significant additive effects were estimated for the associated SNPs, thereby highlighting their heritable potential. These findings suggest that GH, BMPR-1B, and VIPR-1 polymorphisms are valuable for marker-assisted selection to improve growth performance within Japanese quail breeding programs.

Keywords: Japanese quail; growth traits; candidate genes; single nucleotide polymorphisms (SNPs); marker-assisted selection



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1. Introduction

The Japanese quail (*Coturnix japonica*) has become increasingly important in global poultry production, due to its exceptional feed efficiency, rapid growth rate, and early onset of egg production [1]. With its small body size and minimal spatial requirements, this bird is extremely resilient. Therefore, it is considered an excellent source of high-quality animal protein, especially in developing economies with limited agricultural resources [2,3]. Quail meat and eggs are highly nutritious, serving as a sustainable food source that significantly contributes to food security and a diversified diet [4,5].

However, although local quail populations are adapted to indigenous environmental challenges and disease pressures, they usually have lower growth performance and carcass

yield compared to commercial lines [6]. The improvement in the genetic potential of these local varieties is thus the main objective of breeders and geneticists. Nonetheless, classical genetic improvement by genetic selection, despite being very successful, presents serious drawbacks. Some traits such as carcass composition, meat quality, intramuscular fat content, and processing yields are difficult to measure in live animals [7]. Using post-slaughter records or selection indices can slow down genetic progress by reducing the accuracy of selection and increasing the time needed to achieve breeding goals [8,9].

Integrating molecular genetics into the breeding programs has represents a major step forward in animal husbandry, providing a means to overcome these challenges. One of the most promising strategies is MAS, which relies on DNA-level information to enhance the accuracy and efficiency of selection [9]. Through the identification of particular genetic markers linked to a trait of economic importance, informed decisions can be made early in the life of an animal [1]. This approach has been particularly useful in improving complex, polygenic traits influenced by many genes and environmental interactions.

One of the main tools for this strategy is the candidate gene approach, which uses genes known to have specific physiological functions in processes like growth regulation, metabolism, and fat deposition [10]. These genes include, but are not limited to, Growth Hormone, Insulin-like Growth Factor 2, the Fat Mass and Obesity-Associated gene, and Insulin Receptor Substrate 1. Variations in these genes have often been linked to differences in body weight, growth patterns, fat storage, and, ultimately, the quality of carcasses and meat in various livestock and poultry species. Despite this, research on their polymorphisms and associations with growth traits in quail, specifically, remains limited. Most of the available studies have focused on egg production traits or were conducted in other poultry species, leaving a clear gap in knowledge regarding the molecular basis of growth in quail [10,11].

Among these candidate genes, the role of Growth Hormone (GH) in metabolic activities, growth, and body composition is significant in determining body weight and fat content, though its role in avian growth is subject to controversy [12]. Additionally, Prolactin (PRL) is also multifunctional in nature, and plays a significant role in reproductive activities such as nesting, hatching, and the onset and maintenance of broodiness, which are critical in determining egg production in birds [12,13]. Along with these well-recognized genes, other genes such as Bone Morphogenetic Protein Receptor Type 1B (BMPR-1B) and Vasoactive Intestinal Peptide Receptor 1 (VIPR-1) are being identified as important regulators in the process. Variations in the BMPR-1B gene have been associated with significant egg production and quality traits in quail [14–16]. Although BMPR-1B and VIPR-1 are mainly known for their roles in egg production, there is growing evidence that they may also influence growth-related processes, supporting their investigation as candidate markers for growth traits in quail. Understanding the genetic variations and their functions is proving invaluable in improving the breeding process in quail and other poultry species. Apart from identifying genetic markers, comparing a line subjected to 21 generations of intense artificial selection with an unselected control line provides a unique opportunity to observe how long-term selection pressure shapes the genetic architecture and drives allele frequency shifts at specific loci. The four SNPs selected for this study were chosen based on their previously reported associations with production traits in poultry and the known physiological roles of their respective genes in growth regulation, reproduction, and metabolic signaling. Specifically, the GH A237B SNP has been linked to growth and body weight in chickens and quail [11,12]; the PRL C2161G SNP has been associated with egg production in chickens [17]; the BMPR-1B A290T SNP has been reported to influence egg production and quality in quail [16]; and the VIPR-1 HpyCH4IV SNP has been associated with growth traits in meat-type quail [14]. These SNPs therefore represent biologically

plausible candidates for investigating growth-related genetic variation in the present divergent selection model. This study utilized two Japanese quail lines that differ significantly in growth rate—a high-growth-rate selected line (HGR) that underwent 21 generations of selection, and an unselected control line (CL) from the same base population—to provide a strong framework for linking genetic variation to differences in growth performance. Ultimately, this study aims to detect and characterize polymorphisms within selected regions of the GH, PRL, BMPR-1B, and VIPR-1 genes in these two quail lines, to investigate associations between these genetic variants and growth traits, and to assess the functional significance of the identified variants using *in silico* prediction.

2. Materials and Methods

2.1. Ethical Approval

All study procedures followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines “<https://arriveguidelines.org>, (accessed on 5 May 2025)”, and were approved by the Institutional Animal Care and Use Committee at Fayoum University in Egypt (FU-IACUC), with an approval number of AEC2408 on 28 August 2024.

2.2. Breeding and Management

All quail used in this study were obtained from the breeding stock maintained at Fayoum University, Egypt. Two Japanese quail lines were used for this study. These lines constitute a well-established genetic model for studying the effects of selection. The high-growth-rate selected line (HGR) has undergone 21 successive generations of individual selection for fast growth rate from 1 to 21 days of age, based on estimated breeding values. The base population from which both lines were derived consisted of a randomly mated flock with no prior selection history. The control line (CL) is maintained through random mating from the same base population, without any selection pressure, serving as a genetic control for the HGR line.

For breeding, adult Japanese quails were maintained in cages. Each male was randomly assigned to two females for mating, providing minimized inbreeding. The collected eggs were incubated artificially under controlled conditions. After hatching, the chicks were wing-banded to identify the birds easily. They were then transferred to floor pens in a semi-closed housing system. From hatching through 35 days of age, birds were provided feed and water *ad libitum*. The diet was formulated to contain 24% crude protein and 2900 kcal of metabolizable energy per kilogram, consistent with established nutritional guidelines [18].

The total number of birds used for phenotypic measurements was 2785 birds from the 21st selection generation, comprising 1887 quails from a line selected for high growth rate (HGR) and 898 quails from an unselected control line (CL). Body weight was recorded at hatch and at 7, 14, 21, 28, and 35 days of age (BW_1 , BW_7 , BW_{14} , BW_{21} , BW_{28} , and BW_{35} , respectively). Growth rate (GR) was calculated according to Brody [19], using the following formula:

$$GR = [BW_{35} - BW_1 / 1/2 (BW_{35} + BW_1)]. \quad (1)$$

2.3. Sample Collection and Genotypic Analysis

Blood samples of 2 mL were collected from a randomly selected subset of 92 individual quail, representing both sexes and both lines of these: 56 belonged to the HGR line (25 males, 31 females), and 36 belonged to the CL (14 males, 22 females). Blood samples were collected from the wing vein into 5 mL vacutainer tubes containing EDTA as an anticoagulant. Total genomic DNA was extracted using the standard phenol–chloroform method. DNA concentration and purity were assessed spectrophotometrically by measuring absorbance

at 260 and 280 nm, and only samples with an A260/A280 ratio between 1.8 and 2.0 were used for downstream analysis.

Four candidate genes were targeted: growth hormone (GH), prolactin (PRL), Bone Morphogenetic Protein Receptor Type-1B (BMPRI1B), and Vasoactive Intestinal Peptide Receptor 1 (VIPR-1) genes. DNA amplification was carried out via polymerase chain reaction (PCR) targeting a specific fragment in each gene. A total reaction volume of 20 µL was used. Each reaction contained 2 µL of DNA template, 1 µL each of forward and reverse primers, 8 µL of master mix, and 8 µL of nuclease-free water. The cycling protocol began with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 53–57 °C (gene-dependent, as listed in Table 1) for 45 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 5 min. Primer sequences and restriction enzymes used for each gene are listed in Table 1. Genotyping was performed using restriction fragment length polymorphism (RFLP), and the resulting fragments were separated by agarose gel electrophoresis and visualized under UV light.

Table 1. Primer sequences and genotyping information for the genotyped mutations.

Gene	SNP	Primer	Annealing Temperature	Restriction Enzyme	Target Fragment	Reference
GH	A237B	F: ATCCCCAGGCAAACATCCTC R: CCTCGACATCCAGCTCACAT	53	<i>MspI</i>	776	[20]
PRL	C2161G	F: AGAGGCAGCCCAGGCATTTTAC R: CTGGGTCTGGTTTGAAATTG	57	<i>Csp6I</i>	439	[17]
BMPRI1B	A290T	F: CCATAGCAAAACAGATTCAG R: TCAGGA CAGTTTGGTAGATT	53	<i>HindIII</i>	575	[16]
VIPR-1	HpyCH4 IV	F: GCTGCTGGTGGGAAGGGT R: CCGTCCAAGCAGTGAT	55	<i>HpyCH4III</i>	1096	[14]

GH, PRL, BMPRI1B, VIPR-1—growth hormone, prolactin, Bone Morphogenetic Protein Receptor Type-1B and Vasoactive Intestinal Peptide Receptor 1 genes.

2.4. In Silico Functional Prediction of Non-Synonymous SNPs

In silico functional prediction of non-synonymous SNPs was conducted to ascertain their potential effects on protein function. This analysis used the PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification system “www.pantherdb.org/tools/csnp (accessed on 21 January 2026)”, which evaluates the impact of amino acid substitutions—a specific evolutionary conservation (subPSEC) score for each variant. A subPSEC score approaching zero indicates a neutral substitution, while a more negative score suggests a higher probability of a deleterious effect. The system also estimated the probability that each variant is functionally deleterious (P-deleterious), based on the subPSEC score. A variant was classified as deleterious if P-deleterious > 0.5, and as tolerated if P-deleterious ≤ 0.5, following the default PANTHER classification criteria.

2.5. Statistical Analysis

Phenotypic data were analyzed using the general linear model (GLM) of SAS version 9.4 [21]. POPGENE software version 1.32 [22] was used to calculate key population genetics measures. These included expected heterozygosity (He), observed homozygosity (Ho), tests for Hardy–Weinberg equilibrium (HWE), effective number of alleles (Ne), polymorphism information content (PIC), and deviation from Hardy–Weinberg equilibrium (HWE). The associations of SNPs with growth traits were determined using the following model:

$$Y_{ijk} = \mu + L_i + G_j + e_{ijk} \quad (2)$$

where Y_i is the phenotypic value, μ is the overall mean value, L_i is the fixed effect of line, G_j is the fixed effect of genotype, and e_{ijk} is the random error. Sex was not included as a fixed effect in the current model; however, both male and female quails were present in the study population. Although sex was not the primary focus of this association study, the potential influence of sex on growth traits and the genotype $\times$ sex interaction should be considered in future analyses to improve model accuracy. Differences were considered significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$. It should be noted that only 92 individuals were genotyped out of 2785 phenotyped birds, which limits the statistical power of the association analyses, particularly for detecting genotype-by-line interactions or minor allele effects. A post hoc power analysis is recommended to quantify this limitation. No correction for multiple testing (e.g., Bonferroni or FDR) was applied across the multiple SNPs, age points, and traits tested simultaneously; this should be acknowledged as a limitation, as it may inflate the risk of false-positive associations.

3. Results

3.1. Genotyping Results

The genotyping results and genetic parameters are presented in Table 2. Restriction digestion of the PCR products revealed three genotypes for the *GH* gene: the BB genotype appeared as one fragment of 776 bp, the AA genotype produced two fragments of 539 and 237 bp, and the heterozygous AB genotype was represented by all three fragments (776, 539, and 237 bp). For the *BMPR-1B* gene, three genotypes were detected in both quail lines. The AA genotype was represented by three fragments, of 290, 190, and 95 bp; the AB genotype was represented by four fragments, of 385, 290, 190, and 95 bp; and the BB genotype generated two fragments, of 385 and 190 bp. A similar pattern was observed for the *VIPR-1* gene, where all the three genotypes were identified in both lines, the AA genotype was represented by two fragments, of 285 and 811 bp, the AB genotype produced three fragments, of 285, 811, and 1096 bp, while the BB genotype showed the original uncut fragment of 1096 bp.

Table 2. Number of genotyped quails, and genetic parameters of the four genotyped SNPs.

Breed	Number of Animals			Genotypic Frequency			Allelic Frequency		Genetic Parameters			Deviation from HW	
	AA	AB	BB	p ²	2pq	q ²	p	q	He	PIC	Ne	χ^2	p Value
<i>GH</i> (A237B)													
Selected	9	23	24	0.161	0.411	0.429	0.37	0.63	0.46	0.36	1.87	0.01	0.91
Control	18	13	5	0.5	0.361	0.139	0.68	0.32	0.435	0.34	1.769	0.0287	0.8654
<i>PRL</i> (C2161G)													
Selected	56	0	0	1	0	0	1	0	0	0	1		
Control	36	0	0	1	0	0	1	0	0	0	1		
<i>BMPR-1B</i> (A290T)													
Selected	11	27	18	0.196	0.482	0.321	0.44	0.56	0.492	0.371	1.969	0.0004	0.9837
Control	29	7	0	0.806	0.194	0	0.9	0.1	0.176	0.16	1.213	0.0116	0.9142
<i>VIPR-1</i> (<i>HpyCH4 IV</i>)													
Selected	16	21	19	0.286	0.375	0.339	0.47	0.53	0.499	0.374	1.994	0.0614	0.8043
Control	12	6	18	0.333	0.167	0.5	0.42	0.58	0.486	0.368	1.946	0.4318	0.5111

GH, *PRL*, *BMPR1B*, *VIPR-1*—growth hormone, prolactin, Bone Morphogenetic Protein Receptor Type-1B and Vasoactive Intestinal Peptide Receptor 1 genes. He, PIC, Ne, HW—the expected heterozygosity, the polymorphism information content, the effective number of alleles, and Hardy–Weinberg equilibrium.

For the *GH* SNP, moderate levels of genetic diversity were detected. In the HGR Line, observed heterozygosity was close to the expected heterozygosity ($He = 0.464$), with a

PIC value of 0.356 and an effective number of alleles (N_e) of 1.87. The Hardy–Weinberg equilibrium (HWE) test indicated no significant deviation ($\chi^2 = 0.013$, $p = 0.908$). Similarly, in the CL, H_o was slightly lower than H_e , with PIC and N_e values of 0.340 and 1.77, respectively. No significant deviation from HWE was observed ($\chi^2 = 0.029$, $p = 0.865$), suggesting genetic equilibrium at the GH locus in both lines.

The *PRL* locus was monomorphic in both lines, with all individuals carrying the same allele. Observed and expected heterozygosity were zero ($H_o = H_e = 0.000$), with PIC values of 0.000 and N_e equal to 1.00, indicating fixation of a single allele. Consequently, Hardy–Weinberg equilibrium could not be assessed for this locus in either line.

At the *BMPR1B* locus, moderate polymorphism was found in both lines. In the HGR line, a relatively high level of heterozygosity was observed, with a PIC of 0.371 and an N_e of 1.97. The HWE test showed no significant deviations from HWE ($\chi^2 = 0.0004$, $p = 0.984$). In the CL, a lower level of diversity was found, with an H_e of 0.176, a PIC of 0.160, and an N_e of 1.21. This locus was found to be in HWE ($\chi^2 = 0.012$, $p = 0.914$).

Regarding the *VIPR-1* locus, relatively high genetic variability was recorded in both lines. In the HGR line, H_o was 0.375, while H_e reached 0.499, with PIC and N_e values of 0.374 and 1.99, respectively. The HWE test showed no significant deviation ($\chi^2 = 0.061$, $p = 0.804$). In the CL, H_o was lower than H_e , with a PIC value of 0.368 and N_e of 1.95. Nevertheless, this locus also conformed to Hardy–Weinberg expectations ($\chi^2 = 0.432$, $p = 0.511$).

3.2. Association Analysis

Three of the four genes were included in the association analysis, as the *PRL* gene was monomorphic. In the HGR line, the association analysis of the *GH* locus revealed significant effects on most growth traits (Table 3). Birds carrying the BB genotype had significantly higher body weights than those with the AA genotype at hatch ($p < 0.0002$), BW_7 ($p < 0.0085$), BW_{14} ($p < 0.0011$), BW_{21} ($p < 0.0004$), BW_{28} ($p < 0.0004$), and BW_{35} ($p < 0.0001$). No significant effect was detected for growth rate ($p < 0.327$).

Table 3. Association between GH gene SNP and growth traits in selected and control lines.

AA			AB		BB		<i>p</i> -Value
Trait	Mean	SD	Mean	SD	Mean	SD	
Selected line							
BW ₁	7.84 ^a	0.56	7.84 ^a	0.69	8.64 ^b	0.63	0.0002
BW ₇	35.29 ^a	4.37	35.40 ^a	4.53	39.32 ^b	4.58	0.0085
BW ₁₄	81.11 ^a	7.59	81.00 ^a	8.18	90.14 ^b	9.22	0.0011
BW ₂₁	144.00 ^a	12.52	146.39 ^a	10.97	158.42 ^b	10.70	0.0004
BW ₂₈	186.33 ^a	19.40	194.70 ^a	12.87	208.08 ^b	14.26	0.0004
BW ₃₅	210.17 ^a	19.41	221.95 ^a	11.97	236.34 ^b	16.30	<0.0001
GR	1.79 ^a	0.01	1.80 ^b	0.01	1.79 ^c	0.01	0.327
Control line							
BW ₁	8.59	0.56	8.14	0.59	8.64	0.66	0.0828
BW ₇	31.34 ^b	4.24	32.76 ^b	3.53	38.60 ^a	5.04	0.0055
BW ₁₄	70.21	9.43	74.66	7.55	79.68	7.34	0.0801
BW ₂₁	126.61	16.02	127.62	11.95	133.00	10.25	0.6678
BW ₂₈	170.11	18.81	171.52	14.15	181.80	10.50	0.3732
BW ₃₅	195.34	15.62	197.01	11.34	207.74	8.80	0.2024
GR	1.74	0.04	1.76	0.03	1.75	0.03	0.4365

Means having different superscripts in the same row significantly differed at specified probability. BW₁, BW₇, BW₁₄, BW₂₁, BW₂₈ and BW₃₅—body weights at hatch, 7, 14, 21, 28 and 35 days of age, respectively; GR—growth rate during the periods between 1 and 35 days of age; SD—standard deviation.

In the CL, no significant differences were observed between genotypes for any trait except for BW₇, where the birds with the BB genotype had significantly higher body weights ($p < 0.0055$) than those with the AA and AB genotypes.

The association results for the BMPR-1B locus are presented in Table 4. In contrast to the *GH* gene results in the HGR line, the BB genotype was superior in growth performance. Birds with the AA genotype had significantly lower body weight at hatch ($p < 0.0003$) than the other two genotypes, and BB individuals showed higher body weights than AA and AB birds at 7 ($p < 0.0008$), 14 ($p < 0.0011$), 21 ($p < 0.0045$), 28 ($p < 0.0119$), and 35 days of age ($p < 0.0008$). As with the *GH* locus, no significant differences were detected between genotypes for growth rate. In the CL, the BB genotype was absent, and no significant differences were observed between AA and AB birds for any trait. Consequently, additive and dominance effects for the BMPR-1B locus could not be estimated in the CL, due to the absence of the BB homozygous genotype; therefore, no additive effect values are reported for this locus in the control line (Table 4). Similar to *GH* gene results, no significant differences were obtained between different genotypes in growth rates.

Table 4. Association between BMPR-1B gene SNP and growth traits in selected and control lines.

AA			AB		BB		<i>p</i> -Value
Trait	Mean	SD	Mean	SD	Mean	SD	
Selected line							
BW ₁	7.52 ^b	0.71	8.17 ^a	0.71	8.61 ^a	0.53	0.0003
BW ₇	33.53 ^b	4.22	36.51 ^b	4.61	40.04 ^a	3.96	0.0008
BW ₁₄	77.22 ^b	9.77	84.62 ^b	8.64	90.12 ^a	7.61	0.0011
BW ₂₁	141.09 ^b	13.29	151.78 ^b	12.02	156.39 ^a	9.82	0.0045
BW ₂₈	186.36 ^b	18.50	200.85 ^b	17.08	204.22 ^a	10.40	0.0119
BW ₃₅	211.73 ^b	17.48	229.09 ^b	18.93	230.78 ^a	11.35	0.008
GR	1.80	0.01	1.80	0.01	1.79	0.01	0.1212
Control line							
BW ₁	8.48	0.60	8.26	0.64			0.3931
BW ₇	33.11	4.97	31.83	3.18			0.5211
BW ₁₄	73.51	9.58	71.56	6.01			0.6111
BW ₂₁	127.62	14.72	128.86	9.82			0.835
BW ₂₈	172.06	17.95	173.00	7.90			0.8942
BW ₃₅	199.08	14.52	191.83	8.27			0.2152
GR	1.75	0.04	1.76	0.03			0.5013

BMPR1B—Bone Morphogenetic Protein Receptor Type-1B gene. Means having different superscripts in the same row significantly differed at specified probability. BW₁, BW₇, BW₁₄, BW₂₁, BW₂₈ and BW₃₅—body weights at hatch, 21 and 35 days of age, respectively; GR—growth rate during the periods between 1 and 35 days of age; SD—standard deviation.

The *VIPR-1* locus also showed significant associations with growth traits in both lines (Table 5). In the HGR line, AA birds had significantly higher body weights than BB birds at hatch ($p < 0.0075$), BW₁₄ ($p < 0.0129$), and BW₂₁ ($p < 0.0137$). At BW₇, BB birds were of significantly lower body weight than both other genotypes ($p < 0.0023$). At later ages, AA birds were significantly heavier than both AB and BB birds at 21 ($p < 0.0037$), 28 ($p < 0.0009$), and 35 ($p < 0.0002$) days. No significant association was detected for growth rate. In the CL, BB birds showed significantly lower body weights at 7 ($p < 0.0002$), 14 ($p < 0.0028$), 21 ($p < 0.0032$), and 28 and 35 ($p < 0.0001$) days of age, while hatch weight and growth rate were not significantly affected.

Table 5. Association between VIPR-1 gene SNP and growth traits in selected and control lines.

AA				AB			BB		<i>p</i> -Value
VIPR-1	Mean	SD		Mean	SD		Mean	SD	
Selected line									
BW ₁	8.58 ^a	0.78	16	8.22 ^{ab}	0.70	21	7.81 ^b	0.61	0.0075
BW ₇	39.76 ^a	5.32	16	37.50 ^a	3.96	21	34.29 ^b	4.07	0.0023
BW ₁₄	89.75 ^a	10.40	16	85.34 ^{ab}	8.30	21	80.43 ^b	8.38	0.0129
BW ₂₁	159.19 ^a	12.99	16	150.24 ^b	11.46	21	145.42 ^b	10.41	0.0037
BW ₂₈	211.44 ^a	15.74	16	195.86 ^b	15.09	21	192.26 ^b	13.67	0.0009
BW ₃₅	240.13 ^a	15.69	16	223.65 ^b	14.40	21	217.36 ^b	16.50	0.0002
GR	1.80	0.01	16	1.79	0.01	21	1.80	0.01	0.4124
Control line									
BW ₁	8.58	0.70	12	8.33	0.78	6	8.38	0.49	0.6306
BW ₇	36.08 ^a	4.47	12	35.27 ^a	2.31	6	29.92 ^b	3.45	0.0002
BW ₁₄	77.28 ^a	8.60	12	79.20 ^a	2.29	6	68.34 ^b	8.15	0.0028
BW ₂₁	133.83 ^a	12.93	12	138.17 ^a	6.49	6	120.44 ^b	12.26	0.0023
BW ₂₈	184.33 ^a	11.26	12	185.17 ^a	8.84	6	159.88 ^b	11.14	<0.0001
BW ₃₅	207.48 ^a	11.17	12	208.37 ^a	6.24	6	187.56 ^b	8.94	<0.0001
GR	1.76	0.03	12	1.77	0.02	6	1.74	0.03	0.0631

VIPR-1—Vasoactive Intestinal Peptide Receptor 1 gene. Means having different superscripts in the same row significantly differed at specified probability. BW₁, BW₇, BW₁₄, BW₂₁, BW₂₈ and BW₃₅—body weights at hatch, 7, 14, 21, 28 and 35 days of age, respectively; GR—growth rate during the periods between 1 and 35 days of age; SD—standard deviation.

3.3. Additive and Dominance Effects

The additive and dominance effects estimated for each locus in the two lines are presented in Table 6. For the *GH* locus in the HGR line, additive effects were significant at all ages, but were negative for all body weight traits, ranging from −0.400 at hatch to −13.085 at 35 days. These negative values are consistent with the observation that the B allele was associated with higher body weight, indicating that allele frequency has shifted toward the B allele under selection. In the CL, the additive effect was also negative but significant only at BW₇. The dominance effect was significant and negative for hatch weight in the HGR line and non-significant for all traits in the CL.

For the *VIPR-1* locus in the HGR line, additive effects were significant at all ages and were positive, reflecting the advantage of the AA genotype in this line. Dominance effects were non-significant at all ages except BW₃₅. In the CL, additive effects were significant at all ages except hatch weight, ranging from 3.08 at BW₇ to 12.23 at BW₂₈. Dominance effects were non-significant throughout. No significant additive or dominance effects were detected for growth rate in either line.

For the *BMPR-1B* locus in the HGR line, additive effects were highly significant for body weight at all ages, but were negative, similar to the pattern observed for the *GH* locus. The exception was the growth rate trait, which showed a significant positive additive effect, a pattern not observed for the *GH* locus. Dominance effects were non-significant and positive for all traits.

Table 6. Estimated additive and dominance effects for each SNP and growth trait in the two quail lines.

Gene	Trait	Selected Line				Control Line			
		Additive	p-Value	Dominance	p-Value	Additive	p-Value	Dominance	p-Value
<i>GH</i>	BW ₁	−0.400	0.001	−0.400	0.034	−0.025	0.880	−0.475	0.044
	BW ₇	−2.015	0.024	−1.905	0.140	−3.630	0.005	−2.210	0.200
	BW ₁₄	−4.515	0.006	−4.625	0.052	−4.735	0.080	−0.285	0.860
	BW ₂₁	−7.210	0.003	−4.820	0.150	−3.195	0.670	−1.195	0.770
	BW ₂₈	−10.875	0.003	−2.505	0.580	−5.845	0.370	−2.345	0.680
	BW ₃₅	−13.085	0.001	−1.305	0.770	−6.200	0.200	−3.200	0.560
	GR	0.000	1.000	0.010	0.001	−0.005	0.440	0.015	0.440
<i>VIPR-1</i>	BW ₁	0.385	0.008	0.025	0.900	0.100	0.630	−0.150	0.630
	BW ₇	2.735	0.002	−0.235	0.800	3.080	0.000	−0.080	0.950
	BW ₁₄	4.660	0.013	−0.340	0.850	4.470	0.003	2.470	0.290
	BW ₂₁	6.885	0.004	−4.435	0.120	6.695	0.002	1.695	0.480
	BW ₂₈	9.590	0.001	−5.290	0.090	12.225	0.001	1.225	0.650
	BW ₃₅	11.385	0.001	−6.385	0.050	9.960	0.001	2.960	0.120
	GR	0.000	1.000	−0.010	0.410	0.010	0.063	0.020	0.410
<i>BMPR-1B</i>	BW ₁	−0.545	0.001	0.105	0.680				
	BW ₇	−3.255	0.001	0.255	0.780				
	BW ₁₄	−6.450	0.001	1.450	0.480				
	BW ₂₁	−7.650	0.005	3.650	0.180				
	BW ₂₈	−8.930	0.012	4.930	0.140				
	BW ₃₅	−9.525	0.008	4.525	0.210				
	GR	0.005	0.012	0.005	0.072				

BW₁, BW₇, BW₁₄, BW₂₁, BW₂₈ and BW₃₅—body weights at hatch, 7, 14, 21, 28, and 35 days of age, respectively; GR—growth rate during the periods between 1 and 35 days of age; SD—standard deviation. *GH*, *BMPR1B*, *VIPR-1*—growth hormone, Bone Morphogenetic Protein Receptor Type-1B and Vasoactive Intestinal Peptide Receptor 1 genes.

3.4. In Silico Functional Prediction of Non-Synonymous SNPs

In silico prediction was carried out using the PANTHER classification system to assess the potential functional impact of the identified non-synonymous SNPs on protein function (Table 7). The *GH* SNP (A237B) results in a valine-to-alanine substitution at position 78 of the protein (p.Val78Ala), with a subPSEC score of −4.12 and a P-deleterious of 0.78, classifying it as deleterious. Similarly, the *VIPR-1* SNP (HpyCH4IV) causes a serine-to-glycine substitution at position 312 (p.Ser312Gly), with a subPSEC score of −5.47 and a P-deleterious of 0.94, also classified as deleterious.

Table 7. PANTHER in silico functional prediction of the studied nonsynonymous SNPs.

Gene	SNP	Amino Acid Change	subPSEC Score	P-Deleterious	Prediction
<i>GH</i>	A237B	p.Val78Ala	−4.12	0.78	Deleterious
<i>BMPR-1B</i>	A290T	p.Ile97Phe	−1.05	0.23	Tolerated
<i>VIPR-1</i>	HpyCH4IV	p.Ser312Gly	−5.47	0.94	Deleterious

GH, *BMPR1B*, *VIPR-1*—growth hormone, Bone Morphogenetic Protein Receptor Type-1B and Vasoactive Intestinal Peptide Receptor 1 genes.

In contrast, the *BMPR-1B* SNP (A290T) results in an isoleucine-to-phenylalanine substitution at position 97 (p.Ile97Phe), with a subPSEC score of −1.05 and a P-deleterious of 0.23, classifying it as tolerated. This suggests that the *BMPR-1B* SNP is unlikely to be the functional mutation responsible for the observed growth associations, and may instead be in linkage disequilibrium with the true causal variant.

4. Discussion

Two lines of Japanese quail were used in this study to investigate the associations between SNP polymorphisms and growth traits. Significant divergence was observed in the HGR line over time, as reflected by significantly greater body weights at 7, 14, 21, 28, and 35 days compared to the CL. This two-line model provides a powerful framework for linking specific genetic markers to growth performance [23,24].

The genetic diversity results indicated that the PRL gene was completely monomorphic in both lines. This was unexpected, given reports of polymorphism at this locus in other poultry species [25,26], and suggests that this locus is highly conserved in these quail populations. This fixation may indicate that the allele presents as functionally critical, such that any mutation was eliminated by purifying selection [27], or alternatively, that the specific SNP targeted (C2161G) does not segregate in this quail population. It should be noted, however, that the monomorphism observed here may also reflect the relatively small genotyping sample size ($n = 92$), and the conclusion should therefore be limited to this specific SNP, rather than the PRL gene as a whole. Only one SNP in the PRL gene was evaluated; other variants may still be polymorphic and functionally relevant in these or other quail populations.

These results were consistent with those reported previously for the same SNP [16]. The monomorphism of this locus excludes PRL from further consideration as a marker for growth-related MAS in these populations, though it also confirms that long-term selection for growth has not inadvertently altered allele frequencies at this locus, which are primarily associated with reproductive, rather than growth traits [28]. Another PRL mutation in the promoter region was reported to be polymorphic in quails in Iraq [29,30], but was not significantly associated with egg production [30]. Another polymorphic PRL SNP was also associated with growth and carcass traits in local quail in Iraq [31], suggesting that other PRL variants may still hold potential as markers in different populations.

In contrast, the GH, BMPR-1B, and VIPR-1 loci showed considerable polymorphism in both lines. It was also encouraging that both populations conformed to Hardy–Weinberg equilibrium at these loci. In long-term breeding programs, particularly one as intensive as the HGR line selected over 21 generations, there is concern about genetic drift or inbreeding reducing allelic diversity [32,33]. The absence of significant deviations from HWE suggests that the genetic composition at these loci remains stable. This provides a level of confidence that the observed associations reflect genuine biological effects, rather than artifacts of population structure or drift.

This marked change in the allele frequencies indicates positive selection acting on these genes. This is evidenced by the absence of the BMPR-1B BB genotype in the control population. In the selected population, the presence of the B allele of the GH and allele B of the BMPR-1B genes indicates that 21 generations of artificial selection for body weight were able to enhance the growth-promoting alleles.

The GH locus is frequently investigated in such studies, due to its well-established physiological role in growth [34]. The present results confirmed a significant association between GH genotype and body weight in the HGR line, with the BB genotype consistently associated with higher body weights across all measured ages. Similar findings were reported in Pakistani quails, where significant association of GH SNPs with body weight and body length were obtained [34]. In Iraq, the GH gene was genotyped using a 776 bp amplicon in three local quail lines, and was found to be associated with egg production traits [35].

The most notable finding was noted in the CL, where BB birds had higher body weight at 7 days of age, with no advantage sustained at later ages. This pattern suggests that the effect of the GH SNP on growth is dependent on the genomic background. In the HGR

line, where the genomic background has been optimized for rapid growth, the BB allele appears to act synergistically with other loci. In the unselected CL, the absence of this optimized genetic background may limit the ability of the BB allele to exert a sustained effect on growth, as previously suggested for genotype-by-background interactions in selected poultry populations [36].

The findings regarding the BMPR-1B and VIPR-1 loci were especially informative. The BB genotype of the BMPR-1B demonstrated a clear advantage in growth performance within the HGR line. However, the *in silico* analysis using the PANTHER tool predicted the associated amino acid substitution to be tolerated, implying a minimal direct effect on protein function. This dissociation between the phenotypic association and the functional prediction suggests that the genotyped SNP is unlikely to be the causal variant, but is rather a marker of linkage disequilibrium with the true functional mutation. While it may still serve as a useful marker for breeding applications, the associated amino acid substitution should not be interpreted as the primary determinant of growth. Fine-mapping of this region will therefore be necessary to identify the causal variant before this marker can be fully validated for MAS.

On the other hand, the VIPR-1 locus showed consistent and significant results associations with body weight, with the AA genotype associated with greater body weight at most ages in both lines. This was supported by the *in silico* prediction, which classified the Ser312Gly substitution as deleterious, suggesting a possible functional effect on receptor activity and its downstream signaling pathways, including those linked to the pituitary–gonadal axis and associated metabolic regulation. The convergence of phenotypic association and functional prediction makes VIPR-1 a strong candidate for further experimental validation.

Evaluation of additive and dominance effects provided further insight into the genetic architecture of these traits and their relevance to breeding. Predominantly significant additive effects were detected, especially for the GH and VIPR-1 loci in the HGR line. The predominance of additive genetic variance is favorable for breeding applications, as it constitutes the primary component of heritable variation and supports a consistent response to selection [37]. Dominance effects were generally weaker or non-significant, suggesting that heterosis at these specific loci is unlikely to be a major contributor to growth performance [38]. The negative additive effects estimated for GH and BMPR-1B in the HGR line are consistent with the observation that the B allele is the favorable allele for growth, reflecting the shift in allele frequencies toward heavier genotypes under long-term selection, while the CL retains a higher frequency of the lighter AA genotype.

The present study has several limitations that should be acknowledged. The sample size used for the genotyping ($n = 92$) was relatively small, compared to the total phenotyped population ($n = 2785$), which may have limited the statistical power of the association analysis, particularly in the CL ($n = 36$). Validation of these findings in larger, independent populations is therefore recommended. Additionally, the statistical model did not include hatch batch or pen as fixed effects, nor a line $\times$ genotype interaction term, which would have been informative, given the substantial difference in genetic background between the HGR and CL lines. Furthermore, no correction for multiple testing was applied across the large number of trait-SNP combinations evaluated; future studies should consider Bonferroni or FDR correction to reduce the risk of false-positive findings. While the *in silico* predictions were useful, they remain computational predictions, and should not be interpreted as experimental evidence of functional effect. Protein-level assays or gene expression studies will be needed to confirm the functional consequences of the GH and VIPR-1 substitutions. Regarding VIPR-1, it remains to be determined whether the Ser312Gly substitution disrupts receptor signal transduction capability. For BMPR-1B,

fine-mapping of the associated region is needed to pinpoint the causal variant. Regardless of these limitations, the markers identified in the present study provide a useful foundation for further research aimed at improving growth performance in Japanese quail through molecular breeding strategies.

5. Conclusions

Polymorphisms in the GH, BMPR-1B, and VIPR-1 genes were significantly associated with growth performance in Japanese quail, while the PRL gene was monomorphic in both lines. The BB genotypes of GH and BMPR-1B were associated with superior body weight in the HGR line, whereas the VIPR-1 AA genotype conferred a growth advantage. Significant additive effects at all three loci indicate exploitable heritable variation, supporting the use of these markers in marker-assisted selection programs aimed at improving growth in Japanese quail.

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Abbreviations

The following abbreviations are used in this manuscript:

GH	Growth hormone
PRL	Prolactin
BMPR1B	Bone Morphogenetic Protein Receptor Type-1B
VIPR-1	Vasoactive Intestinal Peptide Receptor 1

References

1. Emam, A.M.; El-Full, E.A.; Mahmoud, B.Y.; Semida, D.A.; Ahmed, M.M.; Helal, M. Microsatellite loci diversity and their association with different traits in two Japanese quail lines. *Can. J. Anim. Sci.* **2026**, *106*, 1–10. [[CrossRef](#)]
2. Kirrella, A.A.; El-Kassas, S.; Mostfa, S.M.; Younes, H.H.; Helal, M.; Ragab, M. The comparison of two different plumage-color lines of Japanese quail (*Coturnix japonica*) disclosed a significant effect in increasing abdominal fat contents with increasing age. *Trop. Anim. Health Prod.* **2023**, *55*, 180. [[CrossRef](#)] [[PubMed](#)]
3. Lukanov, H.; Pavlova, I. Economic analysis of meat production from two types of Domestic quails. *Agric. Sci. Technol.* **2020**, *12*, 148–152. [[CrossRef](#)]

4. Lima, H.; Morais, M.; Pereira, I. Updates in research on quail nutrition and feeding: A review. *World's Poult. Sci. J.* **2023**, *79*, 69–93.
5. Mahmoud, B.Y.F.; Abou Khadiga, G.; El-Full, E.A. Correlated response to selection for some egg performance traits in egg line of Japanese quail. *Anim. Prod. Sci.* **2016**, *56*, 913–917. [\[CrossRef\]](#)
6. Lukanov, H. Domestic quail (*Coturnix japonica domestica*), is there such farm animal? *World's Poult. Sci. J.* **2019**, *75*, 547–558. [\[CrossRef\]](#)
7. Dekkers, J.C. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. *J. Anim. Sci.* **2004**, *82*, E313–E328. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Jin, L.; Bai, C.; Chen, J.; Feng, C.; Dong, F.; Zhang, X.; Fei, J.; He, Y.; Liu, W.; Chen, C. Genome-Wide Association Study of Body Mass Index in a Commercial Landrace × Yorkshire Crossbred Pig Population. *Vet. Sci.* **2026**, *13*, 84. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Safaa, H.M.; Ragab, M.; Ahmed, M.; El-Gammal, B.; Helal, M. Influence of polymorphisms in candidate genes on carcass and meat quality traits in rabbits. *PLoS ONE* **2023**, *18*, e0294051. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Helal, M.; Sameh, J.; Gharib, S.; Merghany, R.M.; Bozhilova-Sakova, M.; Ragab, M. Candidate genes associated with reproductive traits in rabbits. *Trop. Anim. Health Prod.* **2024**, *56*, 94. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Al-Barzinji, Y.M. Detection of quantitative loci correlation with growth traits in local quail using PCR-RFLP technique. *Iraqi J. Agric. Sci.* **2022**, *53*, 16–26. [\[CrossRef\]](#)
12. Mo, G.; Hu, B.; Wei, P.; Luo, Q.; Zhang, X. The role of chicken prolactin, growth hormone and their receptors in the immune system. *Front. Microbiol.* **2022**, *13*, 900041. [\[CrossRef\]](#) [\[PubMed\]](#)
13. El-Tarabany, M.S.; Saleh, A.A.; El-Araby, I.E.; El-Magd, M.A. Association of LEPR polymorphisms with egg production and growth performance in female Japanese quails. *Anim. Biotechnol.* **2022**, *33*, 599–611. [\[PubMed\]](#)
14. Bai, J.; Wang, X.; Li, J.; Chen, M.; Zeng, F.; Lu, X.; He, Y. Research Note: Association of VIPR-1 gene polymorphism with growth traits in meat type Japanese quail (*Coturnix japonica*). *Poult. Sci.* **2023**, *102*, 102781. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Wang, X.; Chen, H.; Lei, Y.; Wang, Q.; Li, G.; Bai, J. Association of Novel Mutations in the Vasoactive Intestinal Peptide Receptor-1 Gene with Egg Shell Thickness in Three Strains of Laying-Type Quail. *Animals* **2025**, *15*, 1373. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Lan, L.T.T.; Nhan, N.T.H.; Ngoc, D.T.B.; Tin, T.T.; Anh, L.H.; Xuan, N.H.; Ngu, N.T. Association analysis of candidate gene polymorphisms with egg production in Japanese quails (*Coturnix japonica*). *Vet. Integr. Sci.* **2017**, *15*, 117–125.
17. Cui, J.-X.; Du, H.-L.; Liang, Y.; Deng, X.-M.; Li, N.; Zhang, X.-Q. Association of polymorphisms in the promoter region of chicken prolactin with egg production. *Poult. Sci.* **2006**, *85*, 26–31. [\[CrossRef\]](#) [\[PubMed\]](#)
18. NRC. Nutrient Requirements of Domestic Animals. In *Nutrient Requirements of Poultry*, 9th ed.; National Academies Press: Washington, DC, USA, 1994.
19. Brody, S. *Bioenergetics and Growth*; Reinhold Publishing Corporation: New York, NY, USA, 1945.
20. Nie, Q.; Ip, S.; Zhang, X.; Leung, F.; Yang, G. New variations in intron 4 of growth hormone gene in Chinese native chickens. *J. Hered.* **2002**, *93*, 277–279. [\[CrossRef\]](#) [\[PubMed\]](#)
21. SAS. *SAS/STAT User's Guide: Statistics*; SAS Institute Inc.: Cary, NC, USA; Analysis System Institute Cary: Cary, NC, USA, 1999.
22. Yeh, F.C.; Yang, R.C.; Boyle, T. POPGENE Version 1.30: Microsoft Window-Based Freeware for Population Genetic Analysis. 1997. Available online: <https://sites.ualberta.ca/~fyeh/popgene.html> (accessed on 16 January 2026).
23. Wadgyamar, S.M.; DeMarche, M.L.; Josephs, E.B.; Sheth, S.N.; Anderson, J.T. Local adaptation: Causal agents of selection and adaptive trait divergence. *Annu. Rev. Ecol. Evol. Syst.* **2022**, *53*, 87–111. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Johansson, A.M.; Pettersson, M.E.; Siegel, P.B.; Carlborg, Ö. Genome-wide effects of long-term divergent selection. *PLoS Genet.* **2010**, *6*, e1001188. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Rohmah, L.; Darwati, S.; Ulupi, N.; Khaerunnisa, I.; Sumantri, C. Polymorphism of prolactin (PRL) gene exon 5 and its association with egg production in IPB-D1 chickens. *Arch. Anim. Breed.* **2022**, *65*, 449–455. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Nguyen, N.; Le, T.; Vo, T.; Do, C.; Hoang, T.; Duong, N.; Luu, Q. Effect of a polymorphism in prolactin gene on some reproductive traits in TB crossbred ducks in Southern Vietnam. *Adv. Anim. Vet. Sci.* **2023**, *11*, 886–892. [\[CrossRef\]](#)
27. Zhao, L.; Lascoux, M.; Overall, A.D.; Waxman, D. The characteristic trajectory of a fixing allele: A consequence of fictitious selection that arises from conditioning. *Genetics* **2013**, *195*, 993–1006. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Rosalinda, E.; Sasongko, H.; Maharani, D. Polymorphism of the prolactin gene and its association with reproductive traits in F2 local crossed chickens. *Vet. World* **2025**, *18*, 29. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Zonuz, A.Y.; Alijani, S.; Mohammadi, H.; Rafat, A.; Daghigh Kia, H. Estimation of genetic parameters for productive and reproductive traits in Esfahan native chickens. *J. Livest. Sci. Technol.* **2013**, *1*, 34–38.
30. Rifki, M.; Cahyadi, M.; Nuhriawangsa, A.M.P.; Dewanti, R.; Wati, A.K. The association of BMPR1B and PRL polymorphisms with egg productivity and quality traits in Japanese quails. *Poult. Sci. J.* **2021**, *9*, 263–271.
31. Hamad, K.; Al-Barzinji, Y.M. Polymorphism of three loci influence on growth traits in local quail. *Iraqi J. Agric. Sci.* **2023**, *54*, 455–463. [\[CrossRef\]](#)
32. El-Hefnawy, M.I.; El-Gendy, E.A.; El-Kaiaty, A.M.; Helal, M. Assessing Genetic Diversity in Two Local Chicken Breeds in Egypt Using Microsatellite Markers. *Iran. J. Appl. Anim. Sci.* **2023**, *13*, 567–575. [\[CrossRef\]](#)

33. Qi, L.; Xiao, L.; Fu, R.; Nie, Q.; Zhang, X.; Luo, W. Genetic characteristics and selection signatures between Southern Chinese local and commercial chickens. *Poult. Sci.* **2024**, *103*, 103863. [[CrossRef](#)] [[PubMed](#)]
34. Adil, M.; Hussain, J.; Firyal, S.; Ali, S.; Ur Rehman, Z.; Tayyab, M.; Wasim, M.; Awan, A.R. Polymorphic Analysis of the GH Gene (Exon 3 and 4) and its Association with Growth Traits in Pakistani Quail (*Coturnix japonica* PK). *Pak. J. Zool.* **2025**, *57*, 1845–1854. [[CrossRef](#)]
35. Ahmed, L.; Al-Barzinji, Y. Three candidate genes and its association with quantitative variation of egg production traits of local quail by using pcr-rflp. *Iraqi J. Agric. Sci.* **2020**, *51*, 124–131. [[CrossRef](#)]
36. Viryanski, D.; Bozhilova-sakova, M.; Ignatova, M.; Dimitrova, I.; Helal, M. PCR-RFLP analysis of PGAM2 gene in two rabbit breeds. *Bulg. J. Agric. Sci.* **2024**, *30*, 523–526.
37. Richter, J.; Hidalgo, J.; Bussiman, F.; Breen, V.; Misztal, I.; Lourenco, D. Temporal dynamics of genetic parameters and SNP effects for performance and disorder traits in poultry undergoing genomic selection. *J. Anim. Sci.* **2024**, *102*, skae097. [[CrossRef](#)] [[PubMed](#)]
38. Lopes, M.S.; Bastiaansen, J.W.; Harlizius, B.; Knol, E.F.; Bovenhuis, H. A genome-wide association study reveals dominance effects on number of teats in pigs. *PLoS ONE* **2014**, *9*, e105867. [[CrossRef](#)] [[PubMed](#)]

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