

Association of vasoactive intestinal peptide receptor 1 gene polymorphism with layer economic traits in Rhode Island Red chicken

AMIYA RANJAN SAHU¹✉ and SANJEEV KUMAR²

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

The research work was conducted to determine allelic polymorphism of vasoactive intestinal peptide receptor-1 (*VIPR-1*) gene and its association with layer traits in Rhode Island Red (RIR) chicken. Blood samples were collected from pullets (n=111) belonging to five hatches. Data on body weight at 20 weeks of age (BW20) and layer economic traits, viz. age at sexual maturity (ASM), egg weight at 28 (EW28) and 40 weeks (EW40) of age, and egg production up to 40 weeks of age (EP40) were recorded. Genomic DNA was isolated by phenol chloroform extraction method. Samples were amplified for product sizes of 364, 434 and 486 bp of *VIPR-1* gene. Amplified regions were digested by *TaqI*, *HhaI* and *TaqI* restriction enzymes. Digested fragments of *TaqI* and *HhaI* revealed monomorphism, whereas *TaqI* showed polymorphism. Allelic frequencies for polymorphic locus were 0.98 (A) and 0.02 (B) and genotypic frequencies for AA and AB were 0.96 and 0.04, respectively. The BW20 and layer traits (ASM, EW28, EW40 and EP40) were analysed by least-squares analysis of variance taking sire as random and hatch as fixed effect. Average ASM and egg production up to 40 weeks of age were 135.19 ± 1.15 days and 124.55 ± 1.94 eggs, respectively. Least-square analysis of variance had revealed significant effect of hatch on ASM, EW28 and EP40. However, effect of *TaqI*-RFLP genotypes was non-significant on layer traits. Further investigations on a larger sample basis are suggested to confirm effect of this mutation on layer traits as molecular marker in RIR chicken.

Keywords: Layer economic traits, Polymorphism, Restriction enzyme, RIR chicken, *VIPR-1* gene

The most widely domesticated species, chicken, plays significant role in ensuring food-security. Total poultry population of India is 851.81 million, which is an increase of 16.81% over previous livestock census (20th Livestock Census 2019). The annual egg production is 103.32 billion pieces bringing the country at 3rd position by contributing 5.65% globally (BAH and FS 2019). However, the per capita availability is only 79 eggs per year against ICMR recommendation of 180 eggs annually (Borah and Halim 2014). Rhode Island Red (RIR) is a dual-purpose poultry breed more popular in rural areas due its adaptability to the local environmental conditions, disease resistance and is preferred by small flock owners. It has gained more appreciation among consumers due to its brown shelled eggs and better egg producing ability. Since 1979, RIR is being maintained at ICAR-Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh. The selection programme based on 40 weeks part-period egg production was initiated and selected strain of RIR has undergone 35 generations of selection attaining present annual egg production of 214 eggs (CARI, Annual Report 2016-17).

DNA based molecular markers have been used as

efficient tools for a large number of applications, including phylogenetic analysis, assessment of genetic diversity for accelerated breeding, selection of hybrid parents, studying population structure and marker-assisted selection (Collard *et al.* 2005). PCR-RFLP is also used for identification of genetic disorders, gene localization and parentage analysis (Emara and Kim 2013). In commercial breeding programs, it is used not only to select for a preferred genotype, but also to remove unwanted genotypes. Studies have been carried out to know the effect of candidate genes like vasoactive intestinal peptide receptors (*VIPRs*) genes on sexual maturity, egg productivity and reproduction performance in exotic chicken breeds and quail (Xu *et al.* 2011a, Xu *et al.* 2011b, Ngu *et al.* 2015, Pu *et al.* 2016). Vasoactive intestinal peptide (*VIP*) gene regulates GnRH secretion in poultry (Li *et al.* 2009), and effect of *VIP* gene on the body depends upon vasoactive intestinal peptide receptors (*VIPR-1* and *VIPR-2*). *VIPR-1* gene is considered as an indicator to improve egg quality (Zhou *et al.* 2008), and have association with lower age at first egg (AFE) and higher egg production at 300 days of age (EP300) (Xu *et al.* 2011a, 2011b). Hence, the investigation was conducted to genotype the egg-production associated *VIPR-1* gene polymorphism in sampled population of the selected strain of RIR chicken and to determine the association between polymorphic markers and layer production traits.

Present address: ¹ICAR-Central Coastal Agricultural Research Institute, Goa. ²Bihar Animal Sciences University, Patna, Bihar.
✉Corresponding author email: dramiyavet@gmail.com

MATERIALS AND METHODS

Compliance with ethical standards: The samplings from experimental birds were done in accordance with the ethical standards approved by Institute Animal Ethical Committee.

Sample collection and DNA isolation: Blood sample (0.5 ml) was collected in heparinized (5 IU/ml) centrifuge tubes from experimental RIR hens (n=111) of more than 40 weeks age and 1.5 kg weight belonging to five hatches from ICAR-Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India. Genomic DNA was isolated by the Phenol: Chloroform extraction method (Sambrook and Russell 2001). Concentration and purity of genomic DNA were assessed by spectrophotometer using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., U.S.A.). The samples showing absorbance ratio (260/280) of ~1.8 (between 1.7 and 1.9) were considered as of satisfactory purity and used in further analysis. Quality of extracted DNA was assessed on 0.7% horizontal submarine Agarose Gel Electrophoresis and samples showing intact bands without smearing were considered good and used in further analysis.

PCR amplification: Three sets of primers were identified from database as reported by Xu *et al.* (2011b). Forward and reverse primers were obtained from Genei Laboratories Private Limited, Bengaluru. The nucleotide sequences of the primers, corresponding optimized annealing temperatures and amplicon sizes are given in Table 1. The amplification reaction was carried out in 0.2 ml microfuge tubes using thermal cyclers (Eppendorf Mastercycler ep gradient S and Applied Biosystems 2720 models). The PCR reaction was carried out having total reaction mixture of 20 µl using 10 µl master mix (Ampliqon), 0.8 µl each of forward and reverse primers (10 pmol/µl), 1.5 µl template DNA (50 ng/µl) and 6.9 µl of nuclease-free water (NFW). The amplification reactions were carried out with a program of 5 min denaturation at 95°C followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 58.2°C for 45 sec and extension at 72°C for 45 sec, with a final extension for 5 min at 72°C. The primer sequences used for the amplification is presented in Table 1. The PCR products were checked by 2% agarose electrophoresis and visualized under UV light in gel documentation system (Bio-rad Laboratories, USA).

PCR-RFLP and genotyping: The PCR products were digested overnight by three restriction enzymes, viz. *Tai*I, *Hha*I and *Taq*I incubating at temperature of 65°C, 37°C

and 65°C, respectively. The restriction digestion mixture consisted of 10 µl of PCR product, 2 µl of 10X RE buffer, 0.2 µl respective restriction enzyme (10 units/µl) and 8 µl of NFW. The digested products were checked in 2% agarose gel with fast ruler low-range DNA ladder (GeneRuler™, Fermentas, USA) supplied by ThermoFisher Scientific, Mumbai, India. The genotypes were assigned on the basis of band pattern of the PCR products. Number of alleles were recorded and analysed for their molecular sizes with the Quantity One® software (Bio-Rad Laboratories Inc., U.S.A.). The allele and genotype frequencies were calculated by standard formula (Falconer and Mackay 1996).

Statistical analysis: Data recorded on layer economic traits were analysed for the association with genotypes of *VIPR-1* gene by *Taq* I-RFLP. The influence of genotypes of *VIPR-1* gene on layer traits were determined by the least squares analysis of variance (Harvey 1990) using following statistical model:

$$Y_{ijkl} = \mu + S_i + H_j + G_k + e_{ijkl}$$

where, S_i , effect of i^{th} sire; H_j , effect of j^{th} hatch; G_k , effect of k^{th} genotype of *VIPR-1* gene on layer economic traits of Y_{ijkl} , and e_{ijkl} , error associated with mean zero and variance σ^2_e .

RESULTS AND DISCUSSION

The allelic profiles of *VIPR-1* candidate gene at three loci were performed using restriction enzymes. The details of PCR amplifications and product size of restriction fragments obtained have been presented in Table 1. Allelic profiles at egg production associated candidate gene loci have been presented in Fig. 1, Fig. 2 and Fig. 3. The restricted digested fragments showed that *VIPR-1* gene was monomorphic for *Tai*I and *Hha*I RFLP and polymorphic for *Taq*I RFLP. The allelic frequency for the polymorphic locus was 0.98 (A) and 0.02 (B). The genotypic frequencies for AA and AB were 0.96 and 0.04, respectively. The number of alleles and their frequencies at respective loci of the gene have been presented in Table 2. Xu *et al.* (2011a) observed the polymorphisms at all three loci, viz. A1661691G, C1704887T and C1715301T of *VIPR-1* gene by *Tai*I, *Hha*I and *Taq*I restriction enzymes. They had reported genotypes GG, AG and AA by *Tai*I RFLP; CC, TC and TT by *Hha*I RFLP; and CC, TC and TT by *Taq*I RFLP. Ngu *et al.* (2015) had identified the variations by restriction digestion as C>T transition mutations at locus C1715301T by *VIPR-1/Taq*I for 486 bp and at locus C1704887T by *VIPR-1/*

Table 1. Details of the primer amplification and restriction digestion of *VIPR-1* gene

SNP	Primer Sequences	Annealing temp (°C)	Amplicon size (bp)	Restriction enzymes	Restriction digests (bp)
A1661691G	F: TGAAAGCCCCCA GGATCT R: AGCAAAACAAAA CCCAAATCA	58.2	364	<i>Tai</i> I	268/ 96
C1704887T	F: CCCCGTTAAACT CAGCAGAC R: CCCAAAGTCCCA CAAGGTAA	58.2	434	<i>Hha</i> I	253/181
C1715301T	F: CTCCTCAGGCAG ACCATCATG R: CTTGCACGTATCCTT GGGTAGC	58.2	486	<i>Taq</i> I	310/176

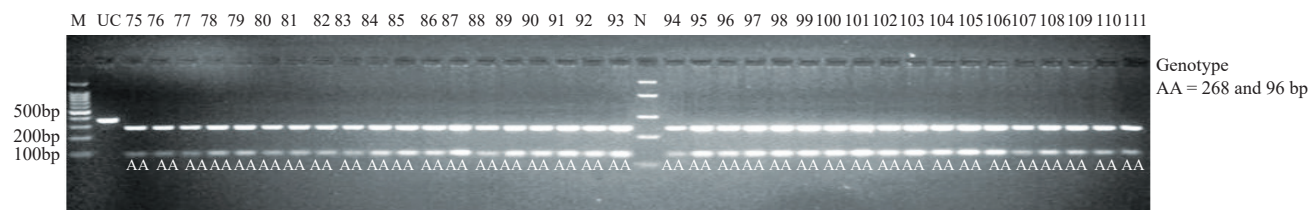


Fig. 1. *TaiI* PCR-RFLP of *VIPR-1* gene in RIR chicken. Lanes – M: 100 bp DNA ladder and N: low range DNA ladder; UC: Uncut PCR product (364 bp); 1-37: Samples.



Fig. 2. *HhaI* PCR-RFLP of *VIPR-1* gene in RIR chicken. Lanes – M: 100 bp DNA ladder; UC: Uncut PCR product (434 bp); 75-111: Samples.

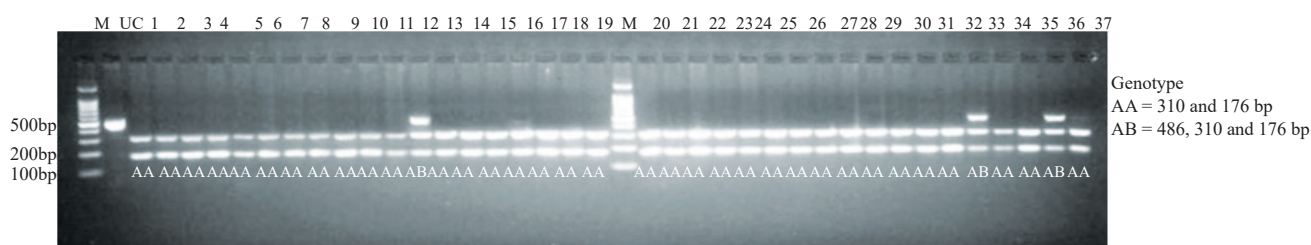


Fig. 3. *TaqI* PCR-RFLP of *VIPR-1* gene in RIR chicken. Lanes– M: 100 bp DNA ladder; UC: Uncut PCR product (486 bp); 1-37: Samples.

HhaI 434 bp in the Noi chicken of Vietnam ($n=236$). Pu *et al.* (2016) had detected two mutations in *VIPR-1* gene by sequencing and PCR-RFLP at loci G373T (*BsrDI*) and A313G (*HpyCH4IV*) in quails ($n=398$).

Table 2. Number of alleles and their frequencies at regions of *VIPR-1* gene in RIR chicken

PCR-RFLP	Genotypes	Genotype frequency	Alleles	Gene frequency
<i>Tai</i> I-RFLP	AA	1	A	1
<i>HhaI</i> -RFLP	AA	1	A	1
<i>Taq</i> I-RFLP	AA	0.96	A	0.98
	AB	0.04	B	0.02

Production performance: The least squares analysis of variance of body weight at 20 weeks of age (BW20) and various layer economic traits, viz. age at sexual maturity (ASM), egg weight at 28 (EW28) and 40 (EW40) weeks of age and egg production up to 40 (EP40) weeks of age

presented in Table 3. Sire had no significant effect on all the studied layer traits. Hatch had significant effects ($P \leq 0.05$) on ASM, EW28 and EP40. The least squares mean for BW20 and layer economic traits, viz. ASM, EW28, EW40 and EP40 have been presented in Table 4. The overall least-squares mean of ASM, EW28, EW40 and EP40 were 135.19 ± 1.15 days, 42.49 ± 0.27 g and 48.19 ± 0.42 g, and was 124.55 ± 1.94 eggs, respectively. Least-squares means of body weight revealed that the birds of 1st hatch showed highest BW20 (1382.28 ± 52.46 g) followed by 3rd (1376.72 ± 47.61 g), 4th (1351.97 ± 32.71 g), 5th (1340.05 ± 35.72 g) and 2nd (1284.59 ± 52.21 g) hatch. Least squares mean of layer economic traits also revealed that pullets of 5th hatch showed highest EW28 (44.51 ± 0.62 g), and 2nd hatch showed highest EW28 (49.99 ± 1.15 g), and 1st hatch showed highest EP40 (139.01 ± 5.29). Although the same management practices were followed for all the hatches, still there was a significant hatch effect. It might

Table 3. Least squares analysis of variance for hatch effect and *VIPR-1/TaqI* genotypes on layer traits in RIR chicken

Source of variation	df	Mean sum of squares				
		ASM	BW20	EW28	EW40	EP40
Sire	44	123.04	19845.82	6.041	14.03 (42)	295.47 (42)
Hatch	4	384.73*	11339.26	20.16*	21.53	531.01*
<i>VIPR-1</i> (<i>TaqI</i>) Genotype	1	154.89	0.974	2.36	2.21	5.35
Error/ Remainder	60	114.63	22024.97	6.59 (58)	10.17 (55)	207.51 (55)

df, Degrees of freedom; *, $P \leq 0.05$.

Table 4. Least squares mean $\pm$ S.E of layer traits in Rhode Island Red chicken

Factor		Least squares mean $\pm$ standard errors					
		N	ASM (days)	BW20 (g)	EW28 (g)	EW40 (g)	EP40
Overall		110	134.98 $\pm$ 1.30	1343.12 $\pm$ 17.65	42.54 $\pm$ 0.32 (87)	48.15 $\pm$ 0.56 (87)	121.47 $\pm$ 2.44 (87)
Hatch	1	15	140.97 ^a $\pm$ 3.84	1382.28 $\pm$ 52.46	41.62 ^b $\pm$ 0.91	47.59 $\pm$ 1.17	139.01 ^a $\pm$ 5.29
	2	14	132.75 ^{cd} $\pm$ 3.82	1284.59 $\pm$ 52.21	42.58 ^b $\pm$ 0.90	49.99 $\pm$ 1.15	124.24 ^b $\pm$ 5.23
	3	14	140.07 ^{ab} $\pm$ 3.48	1376.72 $\pm$ 47.61	41.39 ^b $\pm$ 0.82	47.293 $\pm$ 1.05	120.54 ^b $\pm$ 4.78
	4	31	134.98 ^{bc} $\pm$ 2.40	1351.97 $\pm$ 32.71	42.32 ^b $\pm$ 0.56	46.772 $\pm$ 0.75	120.72 ^b $\pm$ 3.40
	5	36	127.20 ^d $\pm$ 2.62	1340.05 $\pm$ 35.72	44.51 ^a $\pm$ 0.62 (34)	49.289 $\pm$ 0.87 (29)	118.25 ^b $\pm$ 3.95 (29)
Genotype	BB	106	135.64 $\pm$ 3.31	1347.16 $\pm$ 44.12	42.54 $\pm$ 0.76 (104)	48.24 $\pm$ 1.13 (99)	124.46 $\pm$ 5.23 (99)
	AB	4	126.13 $\pm$ 6.29	1346.40 $\pm$ 86.28	41.37 $\pm$ 1.49	47.10 $\pm$ 1.95	126.23 $\pm$ 8.89

N, Number of observations; Means with same superscript in a column do not differ significantly ($P \leq 0.05$); Figures within parentheses denote number of observations.

be due to micro-environmental variability as well as fast environmental fluctuations among the hatches, which were beyond the human control. Similar findings were reported for significant hatch effect on early egg weights (Smith and Bohren 1975), on egg production (King and Henderson, 1954) and on ASM in the coloured Punjab Broiler-II (PB2) dam line (Madapurada 2001, Nwague *et al.* 2007, Das 2013, Debnath *et al.* 2015).

Association of VIPR-1/TaqI genotypes with layer traits: All the experimental birds were genotyped for polymorphic *VIPR-1* locus by *TaqI* enzyme. Least squares analysis of variance was carried out to determine the effect of RFLP genotypes on layer economic traits wherein RFLP genotype was taken as one of the independent factors. The least square means of various traits are given in Table 4 and the effect of *Taq I*-RFLP genotypes was found to be non-significant on layer traits.

Zhou *et al.* (2008) had associated mutations at loci A-284G, A+457G, C+598T, D+19820I, C+37454T, C+42913T, and C+53327T with broodiness. There were significant associations ($P < 0.05$) between C+598T in intron 2 and broody frequency (%), and C+53327T and duration of broodiness, in which allele C was positive for duration of broodiness. Xu *et al.* (2011a) reported significant association ($P < 0.05$) of SNP A1661691G with age at first egg, whereas other two SNPs at loci C1704887T and C1715301T did not have any significant association. Xu *et al.* (2011b) had reported highly significant association of C1704887T ($P < 0.001$) and significant association of C1715301T with egg number at 300 days of laying (EN300) in Ningdu Sanhuang Chinese native chicken. Ngu *et al.* (2015) had identified variations at locus C1715301T by *VIPR-1/TaqI* in 486 bp, at locus C1704887T by *VIPR-1/HhaI* in 434 bp amplicons, and reported significant associations between genotypes and egg numbers ($P < 0.05$) in 20 weeks of laying (28-47 weeks of age) in Noi chicken ($n=111$ for *VIPR-1/TaqI* and $n=125$ for *VIPR-1/HhaI*) of Vietnam. Pu *et al.* (2016) identified two mutations in *VIPR-1* gene by sequencing and PCR-RFLP at loci G373T (*BsrDI*) and A313G (*HpyCH4IV*) and were found to be significantly ($P < 0.05$) linked with egg weight ($n=398$) in quails.

The age at sexual maturity in the studied population, followed regular trend over the generations. *VIPR-1* gene was monomorphic for *TaiI* and *HhaI* RFLP, but polymorphic for *TaqI* RFLP. The genotypes of the polymorphic locus had no significant associations with layer economic traits in the studied samples. Further investigations on large sample basis may impart the possible use of this mutation as a potential molecular marker on layer economic traits in RIR chicken.

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