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SNPs detection in PRLHR, GHSR and SALL3 genes and their association analysis with egg production in chicken

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Abstract
The main reason to carry out the present study was to detect SNPs (Single Nucleotide Polymorphisms) in the coding sections of the genes GHSR, SALL3, and PRLHR and to evaluate their relationship with the chicken egg production at the age of 64 weeks. Egg production data and amplicon data of 48 ABWLH and 48 ASWLH were obtained and analysed. Total 69 SNPs (46 previously published and 23 novel) were found in 96 samples. As per the association study, rs316357564 and rs317267520 of the GHSR can be used as selection markers linked to egg number 64 in chickens.

Keywords: ABWLH, ASWLH, Egg production, GHSR, polymorphism, PRLHR, SALL3

1. Introduction
In the breeding of chicken, characteristics related to egg production have significant economic significance. Accurate selection may be possible through the identification and usage of single-nucleotide polymorphisms (SNPs) linked to these features; additionally, marker-assisted selection (MAS) has been applied in breeding programmes to promote quick genetic improvement (Uemoto *et al*., 2011) [1]. Egg weight (EW), recruited dominant follicle count (DRC), and hen-housed egg production (HHEP) are all components of laying performance in chicken. The genetic foundation of laying performance has been thoroughly researched in recent time due to the advancement of molecular biological techniques. It is challenging to determine the extent of genetic advancements because the trait is polygenic and regulated by minimum two genes with low to moderate heritability (Venturini *et al*., 2013) [2]. It has previously been documented that a candidate gene approach is an affordable way to look at the relationships between gene polymorphisms and quantitative trait loci that account for differences in traits of interest (Mu *et al*., 2016) [3]. SNPs, on the other hand, have been used in MAS to find putative candidate genes and related genotypes, which may improve the selection of more profitable features in chicken breeding operations (Xu *et al*., 2010) [4].
The PRLHR gene, also known as the Prolactin-Releasing Hormone Receptor gene, plays a crucial role in regulating various physiological processes, particularly in relation to reproductive functions, in both mammals and birds, including chickens. In chickens, the PRLHR gene is associated with the regulation of prolactin secretion, which is important for various aspects of reproduction such as egg production, broodiness (the tendency to sit on and incubate eggs), and maternal Behaviors. Research indicates that mutations or variations in the PRLHR gene can influence reproductive traits in chickens. Understanding the genetic variations within the PRLHR gene can therefore provide insights into the reproductive biology of chickens and may have implications for breeding programs aimed at improving reproductive traits in poultry (Omori *et al*., 2015) [5].
The development of chickens, including body weight, egg production, carcass weight, feed conversion ratio and energy balance at various ages, is significantly influenced by somatotropic axis genes (Nie *et al*., 2005) [6]. Important components of the hypothalamus-pituitary growth axis, also known as the somatotropic axis, include thyroid hormones, ghrelin, growth hormone, GHSR, leptin, insulin-like growth factors (IGF-I and II), insulin and various receptors that basically belongs to them (Kadlec *et al*, 2011) [7].

Tanaka *et al*., (2003) [8] identified the GHSR gene, also known as the Growth Hormone Secretagogue Receptor or ghrelin receptors. This 4.1 kb gene, which is found on chromosome 9, has two exons split by one intron and is responsible for producing 347 amino acids.

The SALL3 gene, which encodes a sal-like C2H2-type zinc-finger protein, is part of a family of evolutionarily conserved genes discovered across a range of species, including vertebrates, C. elegans, and Drosophila. According to recent research, the transcription factors sal-like 1 (SALL1) and SALL3 genes regulate the growth, maturity, and ovulation of ovarian follicles in humans and mice, which may have an impact on the production of eggs in birds (Li, 2011) [9]. Another potential degree of regulation of protein activity is provided by the cytoplasmic protein SALL3, which has the ability to extract full-length spalt protein from the nucleus. (Sweetman *et al*., 2003) [10].

The present study was carried out with the objective to detect the SNPs (Single nucleotide polymorphisms) in the genes viz., SALL3, GHSR and PRLHR from amplicon sequenced data to detect SNPs in Anand Bantamized White Leghorn and Anand Synthetic White Leghorn chicken which are the two strains of the famous breed "White Leghorn". In this study, we mainly searched the SNPs present in these three genes, and analysed their effect on the egg production at the age of 64 weeks.

2. Materials and Methods

2.1 Experimental birds

Four distinct industrial strain-crosses viz., B.H.78 chicken, Hisex white, Shaver Star cross-280 and BV-300—were crossed to create the Anand Synthetic White Leghorn (ASWLH) line. The current ASWLH birds used for research were created by selecting for egg weight and quantity at 40 and 56 weeks, respectively, for 12 generations. Following this, they were chosen based on egg weight and quantity at age of 64 we. The Anand Bantamized White Leghorn (ABWLH) line, which has 6.25% Bantam heredity, was created by crossing Bantam chicken with IWN and IWP strains of the famous breed "White Leghorn". Each of the chosen birds was kept in a separate cage within a three-tiered cage system, with standard procedures for feeding, caring for, and administering care. To optimise genetic variation, the Poultry Research Station, Kamdhenu University, Anand, generated full populations of ASWLH and ABWLH chickens, consisting of 24 top-producing birds and 24 low-producing birds, were chosen for the current study.

2.2 Amplicon sequenced data

Amplicon sequenced data of ASWLH and ABWLH chicken already generated using Illumina MiSeq sequencer at Department of Animal Biotechnology of the College were utilized for the present study. Summary of the amplicon targets and corresponding primer sequences employed for amplifying the target regions is given in Table 1.

2.3 Collection of production data

The data on egg production of 24 high egg producer and 24 low egg producer from each population of ABWLH and ASWLH birds, whose amplicon sequenced data were available, were collected from Poultry Research Station, Kamdhenu University, Anand. Noteworthy aspects of the production performance for both ASWLH and ABWLH breeds are presented in Table 2.

2.4 Bioinformatics analysis

FastQC v0.11.9 was utilised to check the quality of files obtained in Fastq format (Figure 1) (https://www.bioinformatics. babraham.ac.uk/projects/fastqc/). PRINSEQ standalone lite v0.20.4 was utilised to filter the data having minimum quality mean of 35 (https://sourceforge.net/projects/prinseq/ files/). Burrows Wheeler Alignment (BWA v0.7.17) was utilised to map the filtered Fastq files genes obtained from Gal_gal4 (https://sourceforge.net/ projects/bio-bwa/files/). Picard tool (v2.25.6) was utilised to align the metrics of reads stored in the BAM files (https://broadinstitute.github.io/picard/). SAMTools (v1.12) was utilized to call different variants in form of VCF files (https://www.htslib.org/ doc/samtools.html). SnpEff was utilized to annotate and filter the detected variants in the VCF files (https://snpeff.sourceforge.net/SnpEff. html) based on read depth of 20 and QUAL score of 30 and SnpSift (v5.0e) (https://snpeff.sourceforge.net/ SnpSift.html). PLINK (v1.07) was utilized to associate the identified variants with egg production at the age of 64 weeks was performed using (https://zzz.bwh.harvard.edu/plink/). The desired features, such as easing the assumptions regarding the Hardy-Weinberg equilibrium and normality of continuous phenotypes, handling small sample sizes and rare alleles, were made possible by the permutation within cluster that was used to generate the significance level.

Results and Discussion

Greater exon lengths required the use of multiple amplicons to cover the full exon; the bespoke amplicon panel contained 29 amplicons altogether, with a maximum amplicon length of 375 bp using two primer pools. 96% of the raw data produced by the sequencing run on the Illumina Miseq platform had clusters passing the filter, and 90.6% of the raw data had a Q-Score of 30 or higher, at least 99.9% accuracy in base calling.

Table 1: Production performance of both ASWLH and ABWLH breeds

Sr. No.	Production Traits	ASWLH	`ABWLH
1.	Average age at first egg (days)	148.77±1.66	144.25±1.07
2.	Average body weight (g) at 20th weeks	1221.07±58.48	1309.04±26.38
3.	Average no. of eggs produced up to 64th weeks	233.95±1.88	252.34±4.51
4.	Average no. of eggs produced up to 72nd weeks	279.14±4.17	302.38±6.51
5.	Average egg weight (g) at 40th weeks	54.50±0.61	50.42±0.63
6.	Average egg weight (g) at 64th weeks	58.24±0.90	52.63±0.55
7.	Average egg weight (g) at 72nd weeks	59.14±0.76	53.76±0.49

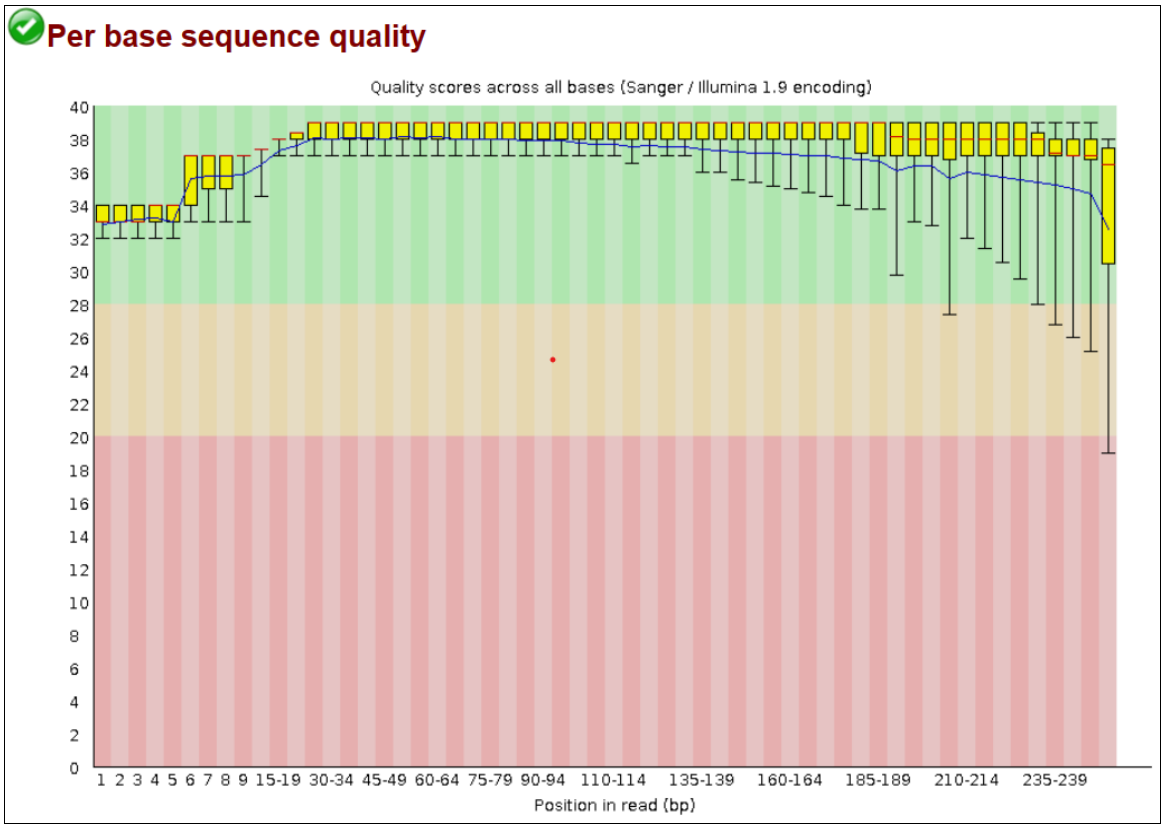

Fig 1: An illustrative representation of the data quality assessment conducted by FastQC

3.1 Variant Calling

A total of 109 SNPs in PRLHR, GHSR and SALL3 genes were detected from all 96 samples. 40 SNPs gets discarded by filtering them based on read depth of 20 and QUAL score of 30. Out of the remaining 69 SNPs, 46 were previously reported in database of dbsnp145 and 23 SNPs were found to be novel. Details regarding these SNPs are given in Table 3.

Transversion and transition can both change sequence of the and ultimately that will lead to the change in the resultant protein; however, transversion has a greater effect on DNA regulation mechanism due to the binding of transcription factors that are unique to alleles and transcription factors that bind to motifs (Guo *et al.*, 2017) [11]. A transition to transversion ratio of 4.9 was obtained in the exon regions following the identification of a total of 49 transitions and 10 transversions. The results of Pal *et al.* (2023) [12] and Weng *et al.* (2020) [13], who similarly noted that transitions occurred more frequently than transversions, have been supported by these data.

3.2 Association between Identified SNPs and egg production: Total two SNPs on GHSR gene's exons were significantly (p<0.05) associated with the egg production in the higher egg producing birds of Anand Bantamized White Leghorn (ABWLH) population. However, no SNP on PRLHR and SALL3 were significantly (p<0.05) associated with the egg production. Information regarding the significantly associated SNPs with egg production are given in Table 4.

Table 2: Information about significantly associated SNPs

Gene	Region	SNP	P value
GHSR	Exon_1	rs316357564	0.01
	Exon_2	rs317267520	0.04

Missense variations are relatively more concerning since they alter the translated protein's amino acid sequence, which may alter the phenotypic i.e., the generation of eggs. After annotation, we discovered that an alanine-to-valine shift is caused by the GHSR gene variant rs316357564. It is commonly known that synonymous SNPs can change the fitness, translation rate, protein folding, protein solubility, protein or nucleic acid binding sites and mRNA stability (Durosaro *et al.*, 2021) [14]. The rs317267520 of GHSR was found to be synonymous SNP after performing the annotation. Total 17 SNPs were identified in exonic region of PRLHR gene. 15 were previously reported and 2 of them were found to be novel. No SNP was found to be significantly associated with EN64. The present finding is in contrast with Zhang *et al.* (2022) [15], Isa *et al.*, (2024) [16] and Liu *et al.* (2019) [17] who reported no significant association between PRLHR nucleotide polymorphism and the egg production.

A total of 23 SNPs were found in GHSR gene. Out of which 8 SNPs were found to be novel, while 15 were previously reported. The present findings are matched exactly with the observations by Tanaka *et al.* (2003) [8], Abdul-Kareem *et al.* (2006) [18] and Choi *et al.* (2016) [19] as they also reported significant association of GHSR gene nucleotide polymorphism with the egg production, however, the variants observed differed from those in the current study.

Total 29 SNPs were identified in exonic region of PRLHR gene. 16 were previously reported and 13 of them were found to be novel. No SNP was found to be significantly associated with EN64. The present finding is in contrast with Zhu *et al.* (2018) [20] and Zhu *et al.* (2019) [21] as they have also reported no significant association between SALL3 nucleotide polymorphism and egg production.

Table 3: Summary of the amplicon targets and corresponding primer sequences employed for amplifying the target regions

Gene	Ch	Exon	Start	End	Primer Sequence
			Co-ordinate	**Co-ordinate**	
GHSR	9	Exon_1	18772903	18773232	F: CGCTCTGCGGCGAGTTTAGTCCGAC
					R: TTGCATCTTGGCAGGAGCGCGGTAA
		Exon_1	18773242	18773560	F: ACCACCAGCATGGTCATCAGGTTGC
					R: TTAGTCCGACGTGCGCGCTCCGCTC
		Exon_1	18773548	18773737	F: GTGCAGCTCTCGCTGATGAACTGGA
					R: CGACGAAGAGGAGGACGCAGGCGAC
		Exon_1	18773726	18774056	F: GCTGTACAGCACCGTGAGGCAGAAT
					R: AGGAGATCTCCGAAGTTCCAGGGCC
		Exon_1	18774045	18774278	F: GTAGGAAAGGTTTTAAAACAATGCT
					R: AAAGAAGATGCTGGAGATCCACACC
		Exon_2	18776465	18776764	F: GAAGAGGACAAAGGACACCAAGTTG
					R: AATTGATTGTACCTGGGCAGCCTGG
		Exon_2	18776753	18777064	F: ACGTGACATCTCCCAGCAAATCCAG
					R: GATCACTGCTATCTCCAAGGATCCA
		Exon_2	18777053	18777283	F: TTTCTTCCTAACGCCCAACCTAAAA
					R: AGTAGAGCTTTCCTTGCCGATTTGT
PRLHR	6	Exon_1	29293666	29293981	F: ACAAGCAGTACTTCAATTTCATCCA
					R: AAGGAGAGGCAGAGATCATATCCCC
		Exon_1	29293970	29294231	F: GCTTACAGCACTCTCATCATCACCT
					R: ACTGGTTTGCCATGATGTCTGCTTG
		Exon_1	29294220	29294470	F: TTGTGTCTGTCTTCACCTTGACTGT
					R: TTGGCTGTCATCTCCCTGTCCTACC
		Exon_1	29294459	29294738	F: CAGTCCTTCAAGCCACTCATCATCC
					R: ATAGGTATTACGCCACGGTGTACCC
		Exon_1	29294727	29294959	F: TGCTTGTTTACTATCCATGTGGCAA
					R: CTAGTAGTTTTTGTTGGTGTCATTG
SALL3	2	Exon_4	57012895	57013202	F: AAATGGACTTGCCATGAAGAACAAT
					R: TCCTGACCTAAATGCATAGGTTCCC
		Exon_4	57013191	57013481	F: CAAACAATACTGTAAGTAACAGGGT
					R: CATACAGAACGGAGGCATTCCCCAG
		Exon_3	57014323	57014644	F: CATGATCAAAATGGAAGTGAATGGT
					R: TTGAACCTGGCCAGTTTTAGGGCTA
		Exon_3	57014633	57014777	F: CCACTATGGGTAATTTAAAACAACA
					R: GATCTCTTTGGGTGAGGTTCCCTCG
		Exon_2	57014873	57015201	F: GCTGTTGGTGATCTCGAAAGCCAGA
					R: ATTGGAAATTCACTACCGCAGCCAT
Gene	**Ch**	**Exon**	**Start**	**End**	***Primer Sequence***
			Co-ordinate	Co-ordinate	
SALL 3	2	Exon_2	57015189	57015377	F: TCTCTTACTCTGGGTCATGTCCTTC
					R: CTGCAATGTCAGAGTCTTCTTCCTC
		Exon_2	57015366	57015691	F: CAAAGGCAATCTAAAAACGCATTTT
					R: CTGTGATCTCCAGTATCGCTGCTTT
		Exon_2	57015680	57016002	F: ACATCTTCAATTCCAACCATTGTTA
					R: AGCAAAGCCACCACTTAGAGTACAG
		Exon_2	57015991	57016310	F: TACCGACCATTCCAACTTCCATTGG
					R: GTTTCAACAAGCCTCCCAAACCCTG
		Exon_2	57016291	57016600	F: CCGATTTTGTGCCAAGGTCTTTGGA
					R: ATTCCTGGTGTGAACAGTTACGGAG
		Exon_2	57016589	57016909	F: GATTACATCTGTAACACCTGTTTCA
					R: TTTACAAATCCACCTCCGCTCGCAC
		Exon_2	57016898	57017211	F: CGCAGCCAGGTGGCCATGATGAACC
					R: TACCATCAGTGCTTCACAGCCCCAG
		Exon_2	57017163	57017418	F: AGCTATACCATGCCAAACACGAATG
					R: TAACAGGGCAGGCCTCAAACCAGCT
		Exon_2	57017407	57017721	F: GAGCACAAGAAGAACTGCACAAAAA
					R: ACTCTGCTGAGCACTAAAGTGGCGG
		Exon_2	57017710	57017976	F: CTGGGACCTCTGAAATGTTGAAGTG
					R: CTGATCGTGAATGAAGATGAGGCAG
		Exon_1	57032149	57032472	F: TTTATTTGCGAACAGTTATTTCCAG
					R: GAACTGAGAAGTTCGCAGTCCAGCC

Table 4: Details regarding all SNPs found in three candidate genes

Gene	Region	rsID	Gene	Region	rsID
PRLHR	Exon_1	rs737397322	GHSR	Exon_2	rs732663917
	Exon_1	rs316370160		Exon_2	rs315776434
	Exon_1	rs14591249		Exon_2	g.18776889A>C
	Exon_1	rs315936089		Exon_2	rs316634191
	Exon_1	rs317876095		Exon_2	rs312927035
	Exon_1	rs312416911	SALL3	Exon_4	rs735157440
	Exon_1	g.29294299G>A		Exon_4	rs315009185
	Exon_1	rs313255214		Exon_4	rs316034336
	Exon_1	g.29294345A>G		Exon_4	rs741029902
	Exon_1	rs731932497		Exon_4	rs313513462
	Exon_1	rs14591250		Exon_2	rs732661101
	Exon_1	rs14591251		Exon_2	g.57015793C>T
	Exon_1	rs315373567		Exon_2	g.57015863C>T
	Exon_1	rs14591252		Exon_2	rs15102763
	Exon_1	rs312281773		Exon_2	rs315196627
	Exon_1	rs315379960		Exon_2	rs312469397
	Exon_1	rs313824654		Exon_2	rs15102765
GHSR	Exon_1	g.18772976C>T		Exon_2	rs734737219
	Exon_1	g.18773004G>A		Exon_2	rs731943141
	Exon_1	rs739711087		Exon_2	rs16001044
	Exon_1	rs733281422		Exon_2	rs734006453
	Exon_1	g.18773209G>A		Exon_2	g.57017383C>T
	Exon_1	g.18773522C>T		Exon_2	rs317669237
	Exon_1	g.18773546T>C		Exon_2	g.57017385G>A
	Exon_1	rs318127492		Exon_2	g.57017386C>T
	Exon_1	rs736336822		Exon_2	g.57017387C>T
	Exon_1	rs734030123		Exon_2	g.57017390T>C
	Exon_1	g.18773889C>T		Exon_2	g.57017391T>C
	Exon_1	rs315570740		Exon_2	g.57017544T>C
	Exon_1	rs741300747		Exon_2	g.57017607T>G
	Exon_1	rs314897308		Exon_2	rs738974634
	Exon_1	rs316357564		Exon_1	g.57032234C>A
	Exon_2	g.18776524G>A		Exon_1	g.57032239A>C
	Exon_2	rs312859032		Exon_1	g.57032248C>T
	Exon_2	rs317267520			

Conclusion

This study is the first in-depth use of amplicon sequenced data to find polymorphisms in the genes viz., GHSR, SALL3, and PRLHR and their relationship to the production of eggs in Anand Bantamized White Leghorn (ABWLH) and Anand Synthetic White Leghorn (ASWLH) layer lines. We have identified two SNPs, viz., rs316357564 and rs317267520 of GHSR gene's exon region which were significantly associated with egg production at the age of 64 weeks (EN64). Our conclusion is that marker-assisted selection studies aimed at producing chicken eggs can benefit from the utilisation of these SNPs.

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Conflict of Interest: None.

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