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Genetic variability in egg production-associated microsatellites in Rhode Island Red chicken

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ABSTRACT

Present investigation was carried out in 114 birds belonging to selected strains of Rhode Island Red chicken maintained at institute experimental layer farm with the objective to analyze polymorphism in egg production associated microsatellite loci and to determine various population genetics statistics based on allelic polymorphism. Genomic DNA samples were isolated from all experimental birds and PCR was performed using primers for ten microsatellite loci, reported to be associated with egg production traits in chicken. Alleles were separated on 3.4% MetaPhore^{TM} agarose and their sizes were determined by Quantity One software. Allelic data were analyzed by POPGENE. Allele numbers varied from 2 to 5 and average number of alleles per locus was 4.00±0.37 (N_a). Allele sizes ranged from 99–280 bp. Allele frequency per locus ranged from 0.0225–0.8919. Nei's heterozygosity, Botstein's polymorphic information content (PIC) and Wright's fixation indices at each locus were estimated. All studied microsatellite loci were polymorphic and estimated PIC ranged from 0.19 (ADL0273) to 0.72 (MCW0110). Seven loci were moderate to highly polymorphic (PIC>0.50). Nei's heterozygosity per locus ranged from 0.20 (ADL0273) to 0.77 (MCW0110). Averaged effective number of alleles (N_e), Shannon's Information index (I) and Wright's fixation indices were 2.71±0.26, 1.0654±0.1046 and 0.5126±0.0757, respectively. Average observed (H_o) and expected (H_e) heterozygosities were 0.3036±0.0625 and 0.5930±0.0505, respectively. Study revealed prevalence of heterozygosity as the N_e was lesser than the N_a. It further revealed that the population was under Hardy-Weinberg disequilibrium as (H_e) was more than (H_o).Chi square and G-square estimates were significant, which suggested that the studied microsatellite loci might have some association with ongoing selection for 40-week part-period egg production in RIR chicken.

Key words: Chicken, Egg production, Genetic variability, Heterozygosity, Microsatellites, PIC, Rhode Island Red

Microsatellite markers, by virtue of their co-dominant nature and having multiple alleles have proved to be efficient in genetic diversity studies in various poultry species including chicken (Alyethodi and Kumar 2010, Das *et al.* 2015a, 2015b and Das *et al.* 2016).They provide a powerful tool for MAS, QTL research, genome scanning and genetic clustering analysis (Sewalem *et al.* 2002).

Rhode Island Red (RIR) chicken is gaining more and more appreciation due to its good egg producing ability and brown shelled eggs, which are preferred by the consumers. RIR is being maintained at CARI, Izatnagar since 1979 as a closed stock. A selection programme initiated based on part-period egg production up to 40 weeks and 30 generations have already been generated. The annual egg production is ~225 eggs (Das 2013). Selected strain of RIR (RIR^{S}) population is showing positive genetic response in egg production, which however has slowed down in the recent past due to loss of variation in population due to long-term selection program. Faster genetic progress is possible through genomics, which will have a significant impact on future layer breeding (Albers and Van Sambeek 2002). Scanty information is available on molecular genetic characterization of RIR using microsatellite markers and their association with egg production traits (Das *et al*. 2016). Therefore, present investigation was carried out with the objectives of analyzing allelic profiles of some egg-production associated microsatellite loci, average heterozygosity and polymorphic information content (PIC) at these loci, to evaluate Hardy-Weinberg disequilibrium and determine Wright's fixation indices in selected strain of Rhode Island Red (RIR^{S}) chicken.

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MATERIALS AND METHODS

Experimental birds: Straight run chicks (286), progeny of 11 sires, belonging to selected strain of RIR chicken, were produced through molecular breeding of RIR chicken

based on the genotypes at ADL0176 microsatellite locus. The parent population belonged to 30th generation of selected strain of RIR chicken undergoing selection based on 40 week part-period egg production. All straight-run chicks were maintained at Experimental Layer Farm (ELF) of institute under standard management conditions from December 2014 to July 2015 and 114 pullets of RIR chicken were raised at ELF for this study.

DNA isolation, quality and purity checking and quantitation: Genomic DNA was extracted from 0.1 ml venous blood collected from jugular vein by Phenol Extraction method (Kagami *et al.* 1990) followed by quality checking on 0.7% horizontal submarine agarose gel electrophoresis, purity checking and quantity determination using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., USA). Samples showing intact DNA band and optical density ratio (260/280 nm) between 1.7 and 1.9 were used in subsequent experiments. PCR ready DNA samples were prepared at a final concentration of 50 ng/µl.

Primers: Ten microsatellite markers reportedly having association with egg production traits in various chicken breeds were identified from published literature (Chatterjee *et al.* 2008a,b, Chatterjee *et al.* 2010 and Radwan *et al.* 2014). Forward and reverse primers were procured from M/S Xcelris Genomics Labs Ltd., Ahmedabad (India) and screened for their use in this present study. Annealing temperature for each of primer was optimized as per Wimmers *et al.* (2000) (Table 1).

PCR reaction mix and amplification programme: PCR was carried out in 0.2 ml nuclease free PCR tubes (Axygen Scientific, Inc. USA) in a final volume of 25 µl in programmable thermal cycler (PTC-200 DNA Engine® thermal cycler, Bio-Rad, USA). Reaction mixture per genomic DNA sample was prepared by gently mixing 2.5 µl of 10× Taq buffer with $MgCl_2$, 2.5 mM each of dNTP mix, 0.8 µM each of forward and reverse primers, 0.75 U Taq DNA polymerase and 50 ng template DNA into nuclease free water. PCR amplification was carried out using PCR programme as initial heat inactivation at 94°C for 5 min, followed by 30 cycles of (i) denaturation at 94°C for 1 min, (ii) annealing at optimized temperature for each microsatellite primer pair for 45 sec and (iii) extension at 72°C for 45 sec, followed by a final extension at 72°C for 5 min and then 4°C forever.

Gel electrophoresis: The molecular sizes of amplified products were adjudged for their probable sizes through 2% horizontal agarose gel electrophoresis by loading approx. 10 µl of PCR product along with 5 µl of 100 bp DNA ladder (Bangalore Genei, India) as molecular marker for identification of desired product. The electrophoresis was done at 2–5 volts/cm for 60 min. and examined under UV light in Gel Documentation system (Biorad Laboratories, USA) and documented. The microsatellite alleles were then identified by running the amplified products on horizontal MetaPhore™ Agarose gel electrophoresis (3.4% MAGE). In parallel, 25 bp GeneRuler low range DNA ladder (Thermo scientific, USA) was also loaded on the marker lane. Electrophoresis was carried out @ 6–8V/cm for 2 h 30 min and gel was visualized in Gel-doc system (Bio-Rad Laboratories Inc., USA) and microsatellite allelic patterns were photographed for further genotyping. The molecular sizes (in bp) of all the alleles were determined with the help of Quantity One® software 4.6.8 (Bio-Rad Laboratories Inc., USA). Genotypes of all the birds were determined on the basis of presence of microsatellite alleles.

Statistical analysis of population genetics data: Data on genotype of all experimental birds at ten microsatellites were compiled and analyzed using POPGENE® 3.1 software (Yeh *et al.* 1999) for their population genetics parameters. The primary data on genotype was subjected to co-dominant marker diploid data analysis to estimate observed and expected genotypic frequencies, Hardy-Weinberg (HW) equilibrium status, allele frequency, observed and effective number of alleles, percentage of polymorphic loci, observed and expected homozygosity and heterozygosity, and Shannon's index.

Average heterozygosity and Polymorphic Information Content (PIC) at each microsatellite locus were estimated according to Nei (1978) and Botstein *et al.* (1980) as has also been used by Das *et al*. (2015b).

Table 1. Details of microsatellite markers

Marker	Forward (F) and reverse (R) primers sequence	T_a (°C)	Map location
ADL0023	F-CTTCTATCCTGGGCTTCTGA R-CCTGGCTGTGTATGTGTTGC	61	5
ADL0158	F-TGGCATGGTTGAGGAATACA R-TAGGTGCTGCACTGGAAATC	52	E29
ADL0176	F-TTGTGGATTCTGGTGGTAGC R-TTCTCCCGTAACACTCGTCA	54	2
ADL0273	F-GCCATACATGACAATAGAGG R-TGGTAGATGCTGAGAGGTGT	55	Z
MCW0044	F- AGTCCGAGCTCTGCTCGCCTCATA R-ACAGTGGCTCAGTGGGAAGTGACC	63	2
MCW0069	F-GCACTCGAGAAAACTTCCTGCG R-ATTGCTTCAGCAAGCATGGGAGGA	55	26
MCW0110	F-CATCTGTGTTACTGTCACAG R-TCAGAGCAGTACGCCGTGGT	58	3
MCW0103	F-AACTGCGTTGAGAGTGAATGC R-TTTCCTAACTGGATGCTTCTG	55	E48
MCW0145	F-ACTTTATTCTCCAAATTTGGCT R-AAACACAATGGCAACGGAAC	55	1
MCW 258	F-TTCTTAGTCCTTGCCAGAGGC R-CTGCAGGAGGATGTGTCCTAG	55	Z

Ta, annealing temperature.

RESULTS AND DISCUSSION

Microsatellite allele profile: Various alleles at ten egg production associated microsatellite loci along with their molecular sizes are presented in Table 2. The molecular sizes ranged from 99 bp at MCW0110 microsatellite (MS) locus to 280 bp at MCW0069 MS locus. The observed alleles and their sizes in RIR were comparable to the previous reports. Das *et al*. (2015a) screened 24 MS loci including ADL0158, ADL0176 and MCW0044 by 6% urea-PAGE and documented quite similar alleles at MCW0044 but variable alleles at ADL0158 and ADL0176 in 24 number of RIR^{S} population. Deshmukh *et al.* (2015) studied 25 microsatellite loci including ADL0176, ADL0273, MCW0069 and MCW0103 in 76 birds of five chicken breeds namely Hill fowl (20), Rhode Island Red (14), Kadaknath (14), White Cornish and White Leghorn (14) on 3.4% Metaphor™ Agarose and revealed dissimilar allelic patterns at ADL0176, ADL0273, MCW0069 and MCW0103 microsatellites loci. El-sayed *et al*. (2011) studied fifteen microsatellite loci including ADL0273, MCW0145 and MCW0258 on 8% and 10% PAGE and reported dissimilar alleles at ADL0273, MCW0145 and MCW0258 in each Egyptian chicken breed of Fayoumi and Dandrawi, respectively. Chatterjee *et al.* (2010) evaluated five loci viz., ADL0023, ADL0158, ADL0176, MCW0044 and MCW0110, which were also explored in present investigation along with other microsatellite in two indigenous native breed, Kadaknath and Aseel, and three different chicken lines, viz. Vanaraja male line, Vanaraja female line and Gramapriya female line using 8% non-denaturing PAGE and reported quite comparable alleles at ADL0176, MCW0044 and MCW0110 but variable alleles at ADL0023 and ADL0158. Bao *et al.* (2008) studied MCW0069 and MCW0103 loci along with other microsatellite loci in 14 Chinese indigenous chicken breeds and red jungle fowl using 8% PAGE and reported variable alleles at MCW0069 and quite similar alleles at MCW0103 locus. However, the variation in allele sizes and numbers with present investigation might be attributed to difference in breed, line or strain as well as to the methodologies adopted for their resolution and size estimation in different studies.

In this study, total number of alleles ranged from 2 to 5 at different loci and average number of alleles per locus was 4.00±0.37 (Table 2). Das *et al.* (2015a) reported that the number of alleles at various microsatellite loci ranged from 2 to 7 and the average number of alleles per locus at these MS loci were 4.04±0.23, which was quite in accordance to the present findings, and also reported varied number of observed alleles i.e. 3 alleles at MCW0044, 4 alleles at ADL0158 and 3 alleles at ADL0176 in RIR^{S} chicken. However, Deshmukh *et al.* (2015) reported 2 to 3 alleles at polymorphic locus and average number of alleles per locus across the breeds was 2.41 in Hill fowl, Rhode Island Red, Kadaknath, White Cornish and White Leghorn, and also reported varied numbers of observed alleles, i.e. 3 alleles at ADL0176, 1 allele at ADL0273, 2 alleles at MCW0069 and 1 allele at MCW0103 in Rhode Island Red chicken. El-sayed *et al*. (2011) reported similar number of alleles at ADL0273 in Egyptian chicken breed of Fayoumi and at MCW0145 of Dandrawi, but varied number of alleles at MCW0258 microsatellite loci in both Egyptian chicken breed of Fayoumi and Dandrawi. Chatterjee *et al.* (2010) reported the observed numbers of alleles in two indigenous native breed (Kadaknath and Aseel) as well as three different chicken lines (Vanaraja male and female line and Gramapriya female line) for loci ADL0023, ADL0158, ADL0176, MCW0044 and MCW0110 which were close to those observed in the present investigation.

In the present study, a total of 40 alleles were documented

Table 2. Number of alleles, their molecular sizes and frequencies at various egg production associated microsatellites loci in pure strain of RIR chicken

MS loci	No. of alleles	Allele code	Allele size (bp)	Allele frequency
ADL0023	5	A	232	0.0225
		B	218	0.0225
		C	186	0.0586
		D	177	0.3874
		E	168	0.5090
ADL00158	5	A	247	0.0225
		B	219	0.0676
		C	207	0.1216
		D	196	0.4505
		E	189	0.3378
ADL0176	5	A	225	0.0586
		B	210	0.0225
		C	202	0.4324
		D	196	0.3964
		E	187	0.0901
ADL0273	3	A	160	0.0450
		B	147	0.8919
		C	141	0.0631
MCW0044	2	A	144	0.3829
		B	134	0.6171
MCW0069	4	A	183	0.1126
		B	174	0.1216
		C	165	0.4685
		D	158	0.2973
MCW0103	3	A	280	0.1081
		B	273	0.3964
		C	267	0.4955
MCW0110	5	A	119	0.1036
		B	113	0.1351
		C	107	0.3378
		D	102	0.2613
		E	99	0.1622
MCW0145	3	A	226	0.1351
		B	209	0.4099
		C	203	0.4550
MCW0258	5	A	216	0.2342
		B	201	0.0315
		C	168	0.1126
		D	161	0.2162
		E	147	0.4054
Mean ± SE	4.00±0.365			

in the ten MS loci studied with their allele frequencies ranging from 0.0225 to 0.8919 and the most frequent allele was 147 bp sized allele at ADL0273 with frequency of 89.19% (Table 2). Das *et al.* (2015a) observed a total of 97 alleles in 24 MS loci and the allele frequencies ranged from 0.042 to 0.883 in RIR^S chicken population which were quite close to present findings. Parmar *et al.* (2007) studied twenty-five chicken specific microsatellite loci along with ADL0023, ADL0158 and ADL0176 in three different varieties of Kadaknath chicken and reported the overall allele frequencies ranged from 0.009 to 0.704.

Average heterozygosity and polymorphic information content (PIC): In present study, Nei's heterozygosity at various microsatellite loci ranged from 0.20 (ADL0273) to 0.77 (MCW0110). Eight microsatellite loci, viz. ADL0023 (0.57), ADL0158 (0.67), ADL0176 (0.65), MCW0069 (0.67), MCW0103 (0.59), MCW0110 (0.77), MCW0145 (0.61) and MCW0258 (0.72) demonstrated heterozygosity more than 0.50 and two microsatellite loci, ADL0273(0.20) and MCW0044 (0.47) demonstrated heterozygosity less than 0.50; the average heterozygosity pooled over all polymorphic loci being 0.59±0.05 in RIR^S chicken (Table 3). Similar to the present findings, Vijh and Tantia (2004) studied four native chicken breed, viz. Nicobari, Miri, Aseel and Kashmir Favorolla and Pandey *et al.* (2005) studied Ankaleshwar chicken and reported higher Nei's heterozygosity at ADL0023 and ADL0176 but lower heterozygosity for ADL0158 MS loci than present study. Pandey *et al*. (2005) also reported higher mean Nei's heterozygosity than the present investigation. Das *et al*. (2015b) also reported lower Nei's heterozygosity at ADL0158 (0.5972), ADL0176 (0.5800) and high at MCW0044 (0.7222) loci in selected strain of RIR chicken. Rahim (2015) reported that Nei's heterozygosity ranged from 0.0997 (ADL0210) to 0.7421 (MCW0069) and the average heterozygosity pooled over all polymorphic loci was 0.4119±0.2475 in selected RIR chicken, which was lower than that reported in the present investigation. It was also reported that Nei's heterozygosity at ADL0023 (0.4211) and MCW0103 (0.3878) were lower and at ADL0176 (0.6606) and MCW0069 (0.7421) loci, it was higher than the present estimates.

Polymorphic information content (PIC) is a parameter which is indicative of the degree of informativeness of a marker (Parmar *et al*. 2007, Rahim 2015). All ten microsatellite loci under study were found to be polymorphic. The estimated mean PIC value was 0.50±0.06 and it ranged from 0.19 (ADL0273) to 0.72 (MCW0110). Deshmukh *et al*. (2015) evaluated same four MS loci, viz. ADL0176, ADL0273, MCW0069 and MCW0103 and reported high PIC value at ADL0176 (0.656) and low at MCW0069 (0.250). Suh *et al.* (2014) reported higher PIC value for ADL01766 (0.703) and MCW0145 (0.787) and lower for MCW0103 (0.258) in 6 Korean native chicken and 3 imported (White Leghorn, Rhode Island Red and Cornish) breeds than present study. Chatterjee *et al*. (2010) reported higher PIC value at ADL0023 (0.712), ADL0176 (0.694) and lower PIC value at ADL0158 (0.598) and MCW0044 (0.384) in two indigenous native breed (Kadaknath and Aseel) and three different chicken lines (Vanaraja male line, Vanaraja female line and Gramapriya female line), than present study. Hu *et al*. (2010) reported the PIC value at MCW0258 locus was 0.847 in Chinese Silkies chicken, which was higher than present estimate. Parmar *et al*. (2007) reported higher PIC values for ADL0023 (0.602), ADL0158 (0.703) and ADL0176 (0.589) than present investigation. Bao *et al*. (2008) reported low PIC value for MCW0103 (0.25 and 0.29) and quite comparable for MCW0069 (0.6 and 0.63) in Chinese chicken breeds and red jungle fowl than the present study. The differences in polymorphic information content of various microsatellite loci may be due to the differences in

Table 3. Average heterozygosity and polymorphic information content (PIC), observed and expected heterozygosities, number of observed and expected alleles, Shannon's index, Wright's fixation index statistics of Chi-square (χ^2) and likelihood ratio (G-square) test for Hardy-Weinberg equilibrium at egg production associated microsatellite loci in pure strain of Rhode Island Red chicken

MS loci	df	Nei's H	PIC	H_o	H_e	N_a	N_e	I	F_{IS}	Chi square	G square
ADL0023	10	0.57	0.51	0.0901	0.5891	5	2.42	1.0481	0.8464	215.883***	191.882***
ADL0158	10	0.67	0.60	0.4595	0.6661	5	2.97	1.2496	0.3071	97.314***	112.634***
ADL0176	10	0.65	0.58	0.1802	0.6467	5	2.81	1.1978	0.7201	197.689***	184.468***
ADL0273	3	0.20	0.19	0.0901	0.1994	3	1.25	0.4160	0.5462	92.153***	48.941***
MCW0044	1	0.47	0.36	0.0631	0.4747	2	1.90	0.6655	0.8666	84.315***	96.788***
MCW0069	6	0.67	0.61	0.4324	0.6677	4	2.98	1.2180	0.3494	73.378***	97.483***
MCW0103	3	0.59	0.50	0.2432	0.5883	3	2.41	0.9552	0.5847	76.022***	88.635***
MCW0110	10	0.77	0.72	0.6036	0.7658	5	4.21	1.5176	0.2082	66.468***	79.640***
MCW0145	3	0.61	0.22	0.3423	0.6095	3	2.54	0.9943	0.4358	54.603***	65.439***
MCW0258	10	0.72	0.68	0.5315	0.7236	5	3.58	1.3921	0.2621	161.043***	176.833***
Mean ±SE		0.59	0.50	0.3036	0.5930	4.00	2.71	1.0654	0.5126		
		±0.05	±0.06	±0.0625	±0.0505	±0.37	±0.26	±0.1046	±0.0757		

H, Nei's heterozygosity; PIC, polymorphic information content; H_o, observed heterozygosity; H_e, expected heterozygosity; df, degrees of freedom; N_a, observed number of alleles; N_e, effective number of alleles; I, Shannon's index; F_{IS}, Wright's fixation index; ***$P \leq 0.001$.

genetic architecture of population analyzed or may be probably due to loss and or fixation of some of the alleles after long-term selection**.**

Population genetic analysis of microsatellite data: The mean±SE of observed and effective number of alleles, Shannon's index and Wright's fixation index were 4.00±0.37, 2.71±0.26, 1.0654±0.1046 and 0.5126±0.0757, respectively. Effective number of alleles ranged from 1.25 (ADL0273) to 4.21 (MCW0110) (Table 3).

Previously, Keambou *et al*. (2014) reported observed, effective number of allele, Shannon's index and Wright's fixation index as 10, 2.91,1.22 and 0.02 at MCW0069 and 2,1.71,0.59 and –0.01, respectively, at MCW0103 locus in Cameron indigenous chicken. Das *et al*. (2015a) reported the observed and effective number of alleles, Shannon's index and Wright's fixation index as 4, 2.4828, 1.1187 and –0.1163 at ADL0158 and 3, 2.3810, 0.9433 and –0.3846 at ADL0176, and 4, 3.6000, 1.3297 and –0.3846 at MCW0044 locus, respectively, in selected strain of RIR chicken. El-Sayed *et al.* (2011) reported effective number of alleles as 2.7397 at ADL0273, 2.4691 at MCW0145 and 6.6667 at MCW0258 locus in Egyptian chicken breed. Chatterjee *et al.* (2010) reported comparable observed, effective number of alleles as well as Shannon's index and Wright's fixation index as 5, 4.76, 1.58 and –0.17 at ADL0023; 5, 3.49, 1.39 and –0.37 at ADL0158; 6, 4.98, 1.65 and –0.22 at ADL0176; 2, 1.97, 0.69 and 0.30 at MCW0044 and 5, 3.73, 1.45 and –0.11 at MCW0110 locus in Kadaknath and Aseel breed and three different chicken lines. Pandey *et al*. (2005) reported high number of effective alleles as well as Shannon's index in Ankaleshwar chicken at ADL0023 and ADL0176 and low for ADL0158 locus and low Wright's fixation index for ADL0023, ADL0158 and ADL0176 in comparison to the present investigation. There was prevalence of heterozygosity in the studied population as the effective numbers of alleles were lesser than the observed number of alleles.

Hardy-Weinberg equilibrium: The mean±SE of observed and expected heterozygosities were 0.3036±0.0625 and 0.5930±0.0505, respectively (Table 3). The population is considered to be in Hardy-Weinberg equilibrium when $H_o \approx H_e$. However, in this study, the mean H_e was more than the mean H_o, which indicated that population was in Hardy-Weinberg disequilibrium, which might be due to influences of some forces like selection for some economic traits that might be associated with studied microsatellite loci. The results of Chi-square test and G-square tests also revealed significant differences between H_o and H_e frequencies demonstrating that the population was not in Hardy-Weinberg equilibrium for all loci, which might be due to influence of external forces. Since, the studied population was continuously being selected for 40-week part-period egg production and also smaller in size, this might have been the reason for it being in Hardy-Weinberg disequilibrium. Deshmukh *et al.* (2015) estimated high observed and expected heterozygosity in RIR chicken for ADL0176 (0.571 and 0.561), and MCW0069 (0.154 and 0.492). Suh *et al.* (2014) reported the observed and expected heterozygosity as 0.295 and 0.739 at ADL0176, 0.278 and 0.305 at MCW0103, and 0.68 and 0.811 at MCW0145, respectively. Das (2013) reported the observed and expected heterozygosity as 0.6667 and 0.6232 for ADL0158, 0.2000 and 0.6105 at ADL0176, and 1.0000 and 0.7536 at MCW0044, respectively, in selected strain of RIR chicken. El-sayed *et al*. (2011) reported higher expected heterozygosity as 0.71 and 0.87 at ADL0273 and MCW0258 locus, respectively. Chatterjee *et al.* (2010) reported higher observed and expected heterozygosity at ADL0023 (0.91 and 0.79), ADL0158 (0.92 and 0.72), ADL0176 (0.90 and 0.80), MCW0044 (0.27 and 0.49), MCW0014 (0.72 and 0.67) and MCW0110 (0.73 and 0.73) in Kadaknath and Aseel breed and three different chicken lines. The studied population was under Hardy-Weinberg disequilibrium since the mean expected heterozygosity was more than mean observed Heterozygosity and Chi-square and G-square estimates were significant.

In view of the findings that the 10 studied egg production-associated microsatellite loci revealed polymorphism in selected strain of RIR chicken and most of them showed PIC values of more than 0.5, it may be concluded that the study is suggestive of usefulness of the studied microsatellites as genetic markers to unravel genetic variability. Furthermore, study revealed that the population was under Hardy-Weinberg disequilibrium, which suggested that the ongoing selection for 40-week part-period egg production might have been associated with the studied microsatellite loci. Further exploration of these studied microsatellites and their association with layer economic traits may unravel critical information for their use in marker assisted selection programme in chicken.

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