



Association of microsatellite genotypes with layer economic traits in a selected strain of Rhode Island Red chicken

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Received: 19 May 2023; Accepted: 25 September 2023

ABSTRACT

Randomly selected 76 pullets of RIR selected strain were screened for microsatellite polymorphs and investigated for their association with layer economic traits. Microsatellite alleles were separated from genomic DNA samples through 3.4% MetaPhor® agarose gel electrophoresis. Data were recorded on age at first egg (AFE), egg weights and egg production (EP) at certain age. Microsatellite analysis revealed nine polymorphic loci (EW) and genotypes at ADL0020 locus were significantly associated with EP at 40 and 64 weeks of age, CC (110 bp/110 bp) genotype had the highest EP, whereas, AD (126 bp/102 bp) and BD (118 bp/102 bp) genotypes recorded the lowest EP. Genotypes at ADL0023 locus were associated with AFE, EP at 40 weeks of age, CC (176 bp/176 bp) genotype followed by AB (202bp/188bp) genotype had earlier AFE, whereas, AC (202bp/176bp) genotype followed by BB (188bp/188bp) genotype demonstrated late AFE. ADL0210 locus had significant impact on AFE, egg weight at 28/40/64 weeks of age; BB (124bp/124bp) genotype followed by AA (132 bp/132 bp) genotype had earlier AFE than AB (132 bp/124 bp) genotype. Both AB and AA genotypes revealed significantly higher egg weight at 28/40/64 weeks of age than BB genotype. The BB (176 bp/176 bp) genotype followed by AB (192 bp/176 bp) genotype at MCW0014 locus revealed higher egg weight at 28 weeks of age in comparison to AA (192bp/192bp) genotype. Allele C (110 bp) at ADL0020 and A (132 bp) at ADL0210 either in homozygous or heterozygous condition were linked to the traits of egg weight and egg production performance, respectively. The results might impact on genomic selection aided poultry breeding programme.

Keywords: Age at first egg, Egg production, Egg weights, Microsatellite genotypes, RIR chicken

Layer economic traits are genetically complex, controlled by numerous genes, and show a wide variation between low and high-performing birds (Chatterjee *et al.* 2008). The primary goals of poultry breeders are to produce birds that could reach an early age at sexual maturity, have a high rate of egg production and produce an optimal size of eggs with good quality (Thiruvankadan *et al.* 2010). Genetic improvement through traditional selection program based on phenotype is a very slow process. Breeders find it challenging to choose the best birds based solely on phenotype because of polygenic inheritance and higher environmental influence on majority of the production traits. Due to continuous selection pressure

predominantly with high rate of egg production, many strains of chicken exhibit selection limit due to exhaustion of additive genetic variance. In spite of selection ceiling, genetic variability still exists for the primary economic traits due to small molecular changes in genes. Selection changes the genetic background of population, and new sets of genes evolve to play as a source of variation stoking to discover novel genetic variation at gene level having substantial association with the desired traits for improvement through marker assisted selection (Dekkers 2004, Bahmanimehr 2012). Use of molecular makers can enhance selection accuracy and the genetic gain of the breeding programs. Various molecular markers are used for this purpose; among that microsatellites are the marker of choice (Debnath *et al.* 2023) because of being randomly distributed throughout the genome, highly polymorphic and ideal for deciphering genetic variability. Chicken genetic linkage maps contain more than 1900 loci and 800 of which are highly polymorphic microsatellite markers (Groenen *et al.* 2000). A pure selected strain of Rhode Island Red (RIR) chicken maintained at the layer farm of ICAR-Central Avian Research Institute, Izatnagar has undergone 30 generations of genetic selection based

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on 40th week part-period egg production record along with some independent culling levels practiced for egg weight (individual basis) (Das *et al.* 2020). The present study was therefore aimed to assess impact of microsatellite genotypes on the performance of layer economic traits in a population of RIR-selected strain chicken.

MATERIALS AND METHODS

Experimental birds: The study was conducted on randomly selected 76 pullets from a chicken flock of RIR selected strain maintained at the experimental layer farm of ICAR-Central Avian Research Institute, Izatnagar (India). All the experimental pullets were housed in individual laying cages according to standard practices adopted for layer birds at the institute and raised under similar feeding and other management conditions (Rahim *et al.* 2016).

Screening of birds for microsatellite genotypes: Approximately 0.5-1.0 ml venous blood was collected from each pullet and genomic DNA was isolated by phenol-chloroform extraction method (Kagami *et al.* 1990). The quality of DNA was assessed through 0.7% horizontal agarose gel electrophoresis. The purity and concentration of DNA were determined through NanoDrop® spectrophotometer. DNA of good quality having intact band without smearing and satisfactory purity were used for further analysis. A panel of 10 microsatellite loci (Table 1) was selected based on earlier reports having relationships with egg production traits (Chatterjee *et al.* 2008, Chatterjee *et al.* 2010, Das *et al.* 2016) and their primers were synthesized from M/s Xcelris Genomics Labs Ltd., Ahmedabad (India). The PCR conditions were optimized (Rahim *et al.* 2017) for amplification of all these

microsatellite loci (Table 1).

Each 25 µl PCR reaction mix for each DNA sample and microsatellite locus was prepared with 5 µl of 5X GoTaq® Flexi buffer (Promega, Madison, USA), optimized quantity of 25 mM MgCl₂ (Promega), 0.5 µl of 10 mM dNTPs (Thermo Fisher Scientific Inc., USA), 1 µl (10 pM) of each forward and reverse primer, 0.15 µl (5U/µl) GoTaq® DNA polymerase (Promega) and 50 ng genomic DNA as a template in nuclease free water. Amplification was carried out in programmable thermal cycler (PTC-200, MJ Research, USA) using PCR program consisting of initial denaturation 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 45 s and; (iii) extension at 72°C for 45 sec, followed by a final extension at 72°C for 5 min and 4°C forever. Amplified PCR products were checked on 1.5% horizontal submarine agarose gel electrophoresis and their final resolution was confirmed running with molecular size marker (50 bp DNA ladder) on 3.4% MetaPhor® agarose gel electrophoresis following Asif *et al.* (2008) to resolve microsatellite alleles and documented for further genotyping. Molecular sizes of various alleles at different microsatellite loci were determined using Quantity One® software on GelDoc-2000 (Bio-Rad Laboratories Inc., USA). Each individual pullet was genotyped with respect to each polymorphic locus using allelic data.

Data recording on layer economic traits: Data on layer economic traits such as age at first egg (AFE), egg weights at 28 (EW28), 40 (EW40) and 64 (EW64) weeks of age, egg production upto 40 (EP40) and 64 (EP64) weeks of age were recorded and used in this study.

Statistical analysis: Data on layer economic traits

Table 1. Panel of chicken microsatellite loci with their primers and optimized annealing temperatures (T_a)

Microsatellite loci	Chromosomal location*	Forward (F) and Reverse (R) primer sequences	T _a (°C)
ADL0020	1	F 5'-GCACTCAAAAAGAAAACAA T-3' R 5'-TAGATAAAAATCCTTCCCTT-3'	55
ADL0023	5	F 5'-CTTCTATCCTGGGCTTCTGA-3' R 5'-CCTGGCTGTGTATGTGTTGC-3'	61
ADL0102	30	F 5'-TTCCACCTTTCTTTTTTATT-3' R 5'-GCTCCACTCCCTTCTAACCC-3'	48
ADL0176	2	F 5'-TTGTGGATTCTGGTGGTAGC-3' R 5'-TTCTCCCGTAACACTCGTCA-3'	55
ADL0210	E30	F 5'-ACAGGAGGATAGTCACACAT-3' R 5'-GCCAAAAAGATGAATGAGTA-3'	52
MCW0007	1	F 5'-AGCAAAGAAGTGTCTCTGTTCAT-3' R 5'-ACCCTGCAAAGTGAAGGGTCTCA-3'	62
MCW0014	6	F 5'-AAAATATTGGCTCTAGGAAGTGC-3' R 5'-ACCGGAAATGAAGGTAAGACTAGGC-3'	60
MCW0041	2	F 5'-CCCATGTGCTTGAATAACTTGGG-3' R 5'-CCAGATTCTCAATAACAATGGCAG-3'	57
MCW0069	26	F 5'-GCACTCGAGAAAACCTTCTGCG-3' R 5'-ATTGCTTCAGCAAGCATGGGAGGA-3'	55
MCW0103	3	F 5'-AACTGCGTTGAGAGTGAATGC-3' R 5'-TTTCTAACTGGATGCTTCTG-3'	55

*Rahim *et al.* (2017); T_a denotes optimized annealing temperatures in °C.

recorded in the experimental birds were analysed by using JMP 9.0.0 statistical program package (SAS 2010) incorporating microsatellite genotypes at each polymorphic locus (independent) as fixed effect in the model.

$$Y_{jk} = \mu + M_j + e_{jk}$$

where Y_{jk} , an observation at j^{th} microsatellite genotypes of k^{th} bird; μ , a constant representing the overall mean; M_j , a constant representing the fixed effect of j^{th} microsatellite genotypes at an individual polymorphic locus; and e_{jk} , a random error assumed to be normally distributed with mean zero and variance σ^2 .

The critical differences among the least squares means of the performance of each trait under different genotypes at each microsatellite locus were assessed by Least Significant Difference (LSD) test at 5% probability level of significance.

RESULTS AND DISCUSSION

The present investigation documented some probable genotypes at each microsatellite locus in each experimental bird. The least squares analysis of variance revealed that four microsatellites (ADL0020, ADL0023, ADL0210, and MCW0014) out of nine polymorphic loci had significant ($P \leq 0.05$) effect on performance of layer economic traits (Table 2). The least squares means of the performance of each trait under different genotypes at each microsatellite locus are presented in Table 3. In the line of present findings, different microsatellites were earlier reported for significant association with different layer performance traits. Chatterjee *et al.* (2010) reported that MCW0041, ADL0210, and MCW0110 microsatellites were significantly ($P \leq 0.05$) associated with egg production traits in six genetic groups of White Leghorn chicken. Kim *et al.* (2008) reported that ADL0101 and ADL0238 had significant differences in allelic patterns for egg production traits in Korean native chicken. Singh *et al.* (2014) studied Z-chromosome-linked microsatellite loci in three strains of chicken and reported that HUR0412 locus was associated with egg production traits in White Leghorn and Punjab Red chicken.

Microsatellite genotypes associated with age at first egg: The analysis revealed that microsatellite genotypes at ADL0023 ($P \leq 0.01$) and ADL0210 ($P \leq 0.05$) loci had a significant influence on age at first egg (AFE) of the birds. The pullets with CC (176 bp/ 176 bp) genotype at ADL0023 locus had the earliest AFE (132.72±1.25 days) which was statistically not different from those pullets having AB (202 bp/ 188 bp) genotype (139.05±2.74 days) or BB (188 bp/ 188 bp) genotype (141.44±3.90 days). Birds with AC (202 bp/ 176 bp) genotype revealed late AFE (155.09±6.29 days) statistically not different from the pullets with BB (188 bp/ 188 bp) genotype. Again the pullets with BB (124 bp/ 124 bp) genotype at ADL0210 demonstrated the earliest AFE (134.80±1.04 days) followed by AA (132 bp/ 132 bp) genotype (135.80±6.02 days) and then AB (132 bp/ 124 bp) genotype (159.55±9.54 days), where BB and AA genotype did not differ significantly. In conformity, a non-significant ($P > 0.05$) association of AFE with ADL0102 microsatellite as observed in the present study was reported by Chatterjee *et al.* (2008) in a literature where AFE was significantly associated with ADL0176 microsatellite, while Das *et al.* (2016) recorded their non-significant associations in RIR chicken. Chatterjee *et al.* (2008) also reported significant association of ADL0023 microsatellite genotypes with age at sexual maturity in White Leghorn chicken. Birds' age at first egg trait was also reported earlier to be associated with different microsatellite genotypes in different chicken breeds, for instance, with MCW0075 in RIR chicken (Das *et al.* 2016), ADL0273, MCW0241, MCW0246 in other chickens (Roushdy *et al.* 2008). Variation in different studies might be affected by genetic architecture and most importantly by sample size.

Microsatellite genotypes associated with egg weight: The analysis revealed that microsatellite genotypes at ADL00210 ($P \leq 0.01$) and MCW0014 ($P \leq 0.05$) loci had a significant association with egg weights at different weeks of birds' age. The pullets with AA (132 bp/ 132 bp) and AB (132 bp/ 124 bp) genotypes at ADL0210 locus demonstrated significantly ($P \leq 0.05$) higher egg weight at 28, 40, and 64 weeks of age as compared to BB (124 bp/ 124 bp) genotype. It is evident from the results

Table 2. The least squares analysis of variance of performance of layer economic traits under different microsatellite loci in a selected strain of RIR chicken

Source of variation	df	P-value					
		AFE	EW28	EW40	EW64	EP40	EP64
ADL0020	5	0.3802	0.1482	0.3599	0.0742	0.0005***	0.0045**
ADL0023	3	0.0018**	0.4079	0.5268	0.3760	0.0207*	0.6817
ADL0102	5	0.1190	0.2346	0.7665	0.3743	0.9803	0.8349
ADL0176	7	0.4771	0.9026	0.9331	0.9701	0.8764	0.8399
ADL0210	2	0.0433*	0.0020**	0.0037**	0.0005***	0.6869	0.5958
MCW0007	4	0.1685	0.1391	0.4731	0.5311	0.4548	0.5337
MCW0014	2	0.3883	0.0464*	0.7849	0.1569	0.9211	0.1909
MCW0069	9	0.5840	0.7451	0.8198	0.6886	0.6755	0.3953
MCW0103	1	0.8285	0.1906	0.9420	0.3374	0.9111	0.4214

*df, degrees of freedom; AFE, EW28/40/64 and EP40/64 denote age at first egg (days), egg weight (grams) at 28/40/64 weeks of age and egg production (in numbers) up to 40/64 weeks of age, respectively; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Table 3. Estimated least squares means of layer economic traits under different genotypes at each microsatellite locus in a selected strain of RIR chicken

Microsatellite genotype	Genotype code	Least squares means± standard errors					
		AFE (days)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
<i>ADL0020 Microsatellite locus</i>							
126/110	AC	132.43± 9.76	41.86±3.78	44.94±4.96	51.98±5.79	111.9 ^{ab} ±18.41	197.77 ^a ±53.14
126/102	AD	128.87±10.56	52.08±4.50	46.32±3.88	67.06±6.26	61.29 ^b ±19.92	136.18 ^{ab} ±57.50
118/110	BC	150.50±6.92	46.47±2.69	49.32±3.45	54.3±3.78	100.4 ^{ab} ±13.05	186.67 ^a ±37.68
118/102	BD	130.64±5.94	50.22±3.28	52.20±2.97	49.83±5.28	62.40 ^b ±11.21	70.58 ^b ±32.36
110/110	CC	136.03±1.67	44.03±0.73	47.50±0.84	51.04±1.09	122.70 ^a ±3.14	214.17 ^a ±9.07
102/102	DD	128.37±8.08	44.06±3.65	46.30±4.03	50.57±5.09	80.29 ^b ±15.24	167.18 ^a ±44.00
<i>ADL0023 Microsatellite locus</i>							
202/188	AB	139.05 ^a ±2.74	45.86±1.20	49.79±1.55	53.47±2.06	116.92 ^a ±6.21	192.51±18.35
202/176	AC	155.09 ^b ±6.29	41.01±2.75	46.47±3.49	45.41±4.33	72.20 ^b ±14.25	167.60±42.14
188/188	BB	141.44 ^{ab} ±3.90	44.41±1.70	47.32±2.42	50.40±2.80	105.21 ^a ±8.83	179.66±26.11
176/176	CC	132.72 ^a ±1.25	44.23±0.55	47.21±0.70	51.41±0.93	115.57 ^a ±2.82	204.68±8.35
<i>ADL0102 Microsatellite locus</i>							
120/120	AA	141.37±4.45	46.41±1.79	49.31±2.38	55.59±2.82	112.57±10.30	212.21±28.18
120/112	AB	130.06±2.28	44.75±0.92	48.40±1.21	51.44±1.57	111.48±5.29	189.08±14.48
120/102	AC	138.88±4.39	41.56±1.77	44.94±2.47	44.26±3.79	113.69±10.18	209.94±27.85
112/112	BB	138.85±2.61	45.97±1.05	48.47±1.51	52.52±1.81	115.66±6.06	213.69±16.56
112/102	BC	138.45±9.48	39.16±3.81	42.66±4.99	51.24±5.87	101.61±21.96	165.61±60.07
102/102	DD	133.33±2.56	42.93±1.03	46.69±1.39	50.22±1.89	115.02±5.93	189.87±16.22
<i>ADL0176 Microsatellite locus</i>							
220/214	AB	138.63±7.37	44.75±3.01	48.83±3.83	50.29±4.64	110.78±16.45	207.03±45.36
220/206	AC	140.36±4.12	43.03±1.68	47.26±2.35	49.55±3.51	105.56±9.19	181.84±25.35
214/214	BB	140.94±4.39	44.92±1.79	47.98±2.30	49.73±3.40	103.68±9.80	179.41±27.02
214/206	BC	132.57±4.34	43.99±1.77	47.18±2.28	50.77±2.91	110.75±9.69	178.91±26.71
214/202	BD	137.82±3.89	43.72±1.59	46.78±2.06	50.84±2.52	113.75±8.68	222.26±23.94
206/206	CC	134.73±2.28	45.40±0.94	49.23±1.24	52.47±1.54	111.89±5.10	185.74±14.05
206/202	CD	130.46±8.22	44.87±3.36	46.52±4.27	53.96±6.42	125.98±18.36	215.05±50.61
202/202	DD	128.89±4.91	43.13±2.01	44.90±2.56	50.50±3.17	128.80±10.97	243.25±30.24
<i>ADL0210 Microsatellite locus</i>							
132/132	AA	135.80 ^a ±6.02	49.23 ^a ±2.26	56.38 ^a ±3.33	67.56 ^a ±4.73	113.47±13.65	165.25±37.67
132/124	AB	159.55 ^b ±9.54	54.88 ^a ±3.59	57.71 ^a ±4.55	62.82 ^a ±4.81	94.97±21.64	175.91±59.61
124/124	BB	134.80 ^a ±1.04	44.11 ^b ±0.39	47.32 ^b ±0.51	50.87 ^b ±0.63	113.97±2.36	200.91±6.52
<i>MCW0007 Microsatellite locus</i>							
302/302	AA	136.70±9.42	43.35±3.71	43.96±3.03	44.04±5.61	86.02±20.96	159.31±58.25
302/275	AB	135.24±3.26	42.96±1.28	46.31±1.67	48.66±2.35	103.06±7.25	177.47±20.14
302/262	AC	112.55±10.15	53.96±4.00	52.35±5.21	59.70±6.12	134.84±22.59	295.94±62.78
275/275	BB	137.88±1.97	43.84±0.78	47.51±1.03	50.76±1.41	109.60±4.38	195.84±12.18
275/262	BC	137.34±3.75	43.55±1.48	45.93±1.98	51.97±3.09	119.63±8.35	194.65±23.21
262/262	CC	132.15±2.63	45.83±1.04	49.01±1.36	53.15±1.95	122.45±5.86	213.59±16.28
<i>MCW0014 Microsatellite locus</i>							
192/192	AA	128.94±5.10	40.51 ^b ±1.97	45.80±2.84	43.58±4.00	112.96±11.21	157.57±30.24
192/176	AB	137.19±2.69	44.07 ^a ±1.04	47.60±1.36	51.94±1.69	115.85±5.91	220.25±15.94
176/176	BB	135.31±1.27	44.88 ^a ±0.49	47.83±0.65	51.58±0.86	113.14±2.79	197.96±7.22
<i>MCW0069 Microsatellite locus</i>							
232/194	AD	131.51±9.80	46.11±3.93	50.65±5.01	55.02±5.95	121.76±21.42	211.84±57.82
232/184	AE	126.43±7.15	40.61±2.87	42.72±3.83	51.70±4.40	125.51±15.63	203.46±42.19
221/194	BD	137.10±5.09	46.33±2.04	50.86±2.96	50.59±4.07	113.36±11.12	216.74±30.02
221/184	BE	140.25±6.93	42.36±2.78	46.61±3.56	49.48±4.18	118.50±15.13	233.49±40.85
210/184	CE	133.99±1.99	44.58±0.80	48.00±1.04	51.73±1.47	109.19±4.34	195.64±11.71
210/172	CF	127.95±10.17	42.21±4.08	47.96±5.20	NA	75.36±22.22	67.55±59.97
194/194	DD	141.10±9.46	43.37±3.79	46.17±5.03	42.43±6.47	133.19±20.66	289.57±55.78

Table 3 continued ...

Table 3. *Concluded*

Microsatellite genotype	Genotype code	Least squares means± standard errors					
		AFE (days)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
194/172	DF	131.96±4.20	43.34±1.69	46.12±2.16	49.71±2.90	113.06±9.17	180.53±24.75
184/184	EE	136.09±3.21	45.30±1.30	47.69±1.77	54.76±2.37	119.64±7.02	216.86±18.96
184/172	EF	140.86±3.86	43.86±1.55	47.27±2.00	51.19±2.88	114.42±8.42	171.51±22.74
<i>MCW0103 Microsatellite locus</i>							
298/276	AA	135.00±1.56	43.83±0.61	47.72±0.81	50.55±1.07	113.36±3.38	193.99±9.30
276/276	AB	135.50±1.58	45.04±0.62	47.63±0.81	52.03±1.05	113.93±3.44	205.20±9.46

*Means with different superscripts in a column (each trait) under a particular microsatellite locus differ significantly ($P \leq 0.05$).

that A (132 bp) allele at ADL0210 locus either in homozygous or heterozygous condition is linked to the trait of egg weight performance. Again, BB (176 bp/ 176 bp) and AB (192 bp/ 176 bp) genotypes at MCW0014 locus revealed significantly ($P \leq 0.05$) higher EW28 (44.88±0.49 g and 44.07±1.04 g, respectively; statistically not different in comparison to the pullets with AA (192 bp/ 192 bp) genotype (40.51±1.97 g). In conformity, Das *et al.* (2016) reported that 28th week's egg weight in RIR chicken was significantly associated with MCW0014 and MCW0005 microsatellites. The egg weight performance trait was also reported to be linked with other microsatellites such as ADL0273, MCW0241, MCW0246 and MCW0258 located on poultry Z-chromosome (Roushdy *et al.* 2008). The present investigation indicates that map location (E30) of ADL0210 microsatellite (Rahim *et al.* 2017) becomes hotspot for studying the QTL for egg weight performance trait in chicken (Sasaki *et al.* 2004).

Microsatellite genotypes associated with egg production: The analysis also revealed that microsatellites genotypes at ADL0020 ($P \leq 0.01$) and ADL0023 ($P \leq 0.05$) loci had significant effect on part-period egg productions. The genotypes of ADL0020 were found to be significantly associated with egg production upto 40 (EP40) and 64 (EP64) weeks of age. The pullets with CC (110 bp/ 110 bp) genotype at ADL0020 recorded the highest EP40 (122.70±3.14) and EP64 (214.17±9.07) followed by AC (126 bp/ 110 bp), BC (118 bp/ 110 bp), DD (102 bp/ 102 bp), and BD (118 bp/ 102 bp) genotypes (EP40) or AD (126 bp/ 102 bp) genotype (EP64). The CC, AC and BC genotypes did not differ significantly among egg production performances. Similarly, statistically non-difference performance was observed under AD and BD genotypes. It is evident from the results that C (110 bp) allele at ADL0020 locus either in homozygous or heterozygous condition is linked to the trait of egg production performance. Again, AB (202 bp/ 188 bp) > CC (176 bp/ 176 bp) > BB (188 bp/ 188 bp) genotypes at ADL0023 locus had higher ($P \leq 0.05$) EP40 than AC (202 bp/ 176 bp) genotype. In accordance with the present findings, significant ($P \leq 0.05$) association of ADL0023 microsatellite genotypes with egg production up to 64 and 72 weeks of ages in White Leghorn chicken was reported earlier (Chatterjee *et al.* 2008). Das *et al.* (2016) reported that genotypes at ADL0102, ADL0158 and MCW0044 microsatellites had a significant ($P \leq 0.05$) effect on EP40 in RIR chicken. Roushdy *et al.* (2008)

reported that four microsatellites (MCW0241, ADL0273, MCW0246 and MCW0258) located on Z-chromosome were significantly linked to the traits of egg number and egg weight. There is a trend noticed in the present results that C (110 bp) allele at ADL0020 locus either in homozygous or heterozygous condition produced more number of eggs in comparison to other genotypes and could be indicative of better egg production up to 40 and 64 weeks of age in RIR chicken. Microsatellites located on chromosome one, two and five in chicken carry some QTLs for egg number along with other QTLs (Abasht *et al.* 2006 a,b). The present study indicates that chromosome one and five housing ADL0020 and ADL0023 microsatellites, respectively (Rahim *et al.* 2017) become hotspot zone for studying the QTLs for egg production traits in chicken (Sasaki *et al.* 2004).

It could be concluded that different microsatellite genotypes at ADL0020, ADL0023, ADL0210 and MCW0014 loci are associated with layer economic traits and the birds with specific microsatellite genotypes that attain an early sexual maturity have produced more number of eggs at a certain age. C (110 bp) allele at ADL0020 locus and A (132 bp) allele at ADL0210 locus either in homozygous or heterozygous condition are linked to the traits of egg weight and egg production performance, respectively. Such information may have impact on poultry breeding program for rapid genetic improvement in layer performance. However, this may be evaluated and validated in different chicken populations investigating a quite large number of samples to reach a definite conclusion for further exploitation.

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