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Association of melatonin receptors type C (*MTNR1C*) gene polymorphism with layer economic traits in Rhode Island Red

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ABSTRACT

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The aim of this study was to assess allelic polymorphism of melatonin receptors type C *(MTNR1C)* gene and their association with layer economic traits in RIR chicken. Jugular vein was used to collect blood samples from 120 pullets belonging to two hatches. Data were recorded on age at sexual maturity (ASM), body weight at 20 and 40 week of age (BW20 & BW40), egg weight at 28 and 40 weeks of age (EW28 & EW40) and egg production at 40 week of age (EP40). Amplified product was digested with *MobI* restriction enzyme i.e. reveal the polymorphic pattern. Allele frequencies were observed as 0.29 (A) and 0.71 (B) and genotypic frequencies of AA, BB and AB genotype were 0.04, 0.46 and 0.5, respectively. A significant association was found of *MTNR1C-RFLP* genotype with ASM (P≤0.10). The overall least squares means along with standard error of ASM, EW28, EW40 and EP40 were observed as 186.37±1.67 days, 41.69±00.29g, 48.87±00.21g and 51.06±1.33g respectively. The birds with AB genotype achieved earliest age at sexual maturity (171.42±5.34 days). This study suggested that *MTNR1C* locus AB RFLP genotype can be used as a potential marker to enhance age at sexual maturity in RIR birds.

Keyword: Chicken, Layer economic traits, *MTNR1gene,* Rhode Island Red

INTRODUCTION

Poultry is the most expeditious growing segment of agricultural sector in India. According to the 20th livestock census (2020), the total poultry population of our country is 851.81 million, which has revealed a 16.81 increase over the previous census. India has the 3rd rank for egg production and 6th for poultry meat production in world. The availability of egg and meat is 95 eggs/year and 6.82 Kg/annum per person, respectively against ICMR Recommendations of 182 eggs/year and 11.5 Kg/annum (BAHS, 2021-22). This huge gap may be filled by genetic improvement in the productivity of birds through selection. RIR has a good egg-producing capacity compared to other different imported breeds. RIR has been maintained at CARI, Izatnagar since 1979. The selection programme based on 40-week part-period egg production was initiated and the selected strain of RIR has undergone 37th generation of selection. Molecular markers offer new possibilities to accelerate the selection of commonly measured traits or to select for new traits which are costly or hard to record in farm animals. Various molecular markers are applied for DNA polymorphism evaluation such as AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism), Microsatellites and SNP (Single Nucleotide Polymorphism), etc. PCR-RFLP based analysis is a simple, powerful gel-based technique for recognition and differentiation of species, extensively employed to assess DNA polymorphism and used for selection of superior genotype. Studies have been conducted to investigate the impact of candidate gene as *MTNR1C* gene on growth and reproductive traits in chickens, as reported by Tenzin *et al*., 2020. Melatonin is a hormone that regulates various physiological functions in birds, including neuroendocrine functions, circadian rhythms, feeding patterns and hibernation. According to Li *et al.* (2013) the presence of ovarian follicular fluid indicates the existence of an ovarian function. Present investigation was designed to determine the association of *MTNR1C-RFLP* genotype with layer economic traits in RIR chicken.

MATERIALS AND METHODS

One hundred twenty RIR birds (60 selected from high egg production and 60 from low production group) maintained at experimental layer farm of CARI, Izatnagar were selected on basis of their 40-weeks egg production for candidate gene polymorphism studies using PCR-RFLP. Blood was collected with a 24 gauge needle in autoclaved heparinized (50 IU/ml) 1.5 ml microfuge tubes from each experimental bird. Genomic DNA was extracted through phenol: chloroform extraction method by using blood sample. Purity and concentration of genomic DNA was evaluated by using a nanodrop spectrophotometer and the purity of samples was assessed by absorbance ratio (260nm/280nm). The quality of extracted genomic DNA was evaluated on 0.7% horizontal submarine agarose gel electrophoresis (AGE) and samples with intact band were considered as acceptable for further analysis.

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Primer sequence set for *MTNR1C* candidate gene was reported by Li *et al.*, 2013. Primer sequences, restriction enzymes, expected products of the amplicon of *MTNR1C* candidate genes are presented in table 1. PCR reactions were prepared in 0.2 ml clear, thin-walled sterile PCR tubes with clear flat caps and amplification of genomic DNA was done in programmable thermal cycler after optimization.

Table 1: Primer sequences, restriction enzymes, expected products of the amplicon of *MTNR1C* candidate genes

Gene	Forward/ Reverse Sequence	Annealing temp (T_a) (°C)	Predicted Amplicon size (bp)	Predicted fragment size	RE	Reference
MTNR1C	F: 5'GGTGTATCCGTATCCTCTA A R: 5'GACAGTGGGACAATGAAGT	49	372	333/ 39	*Mbo I*	Li *et al.*, 2013

PCR reaction was conducted in 0.2 ml nuclease-free PCR tubes with 25 ìl volume by genetle mixing of 12.5 ìl master mix, 2 ìl of each primer and 1 ng template into nuclease free water and all steps was done in ice box. To get reactant at bottom, mixing and spinning for 5-10 seconds at 3000 rpm was used. The PCR amplification programme was optimized for *MNTR 1C* candidate loci: initial denaturing at 95°C for 5min, followed by 30 cycles of (i) denaturation at 94°C for 1 minute, (ii) Annealing at T_a°C (T_a = optimized annealing temperature) for 45 seconds (iii) Extension at 72°C for 90 seconds followed by final extension at 72°C for 5 minutes. Genotypes of candidate gene were determined by PCR-RFLP technique after amplification of DNA. The PCR product was digested with *MobI* restriction enzyme under water bath at 37°C for overnight and checked through 2% agarose gel under gel documentation with fast low range DNA ladder. Genotypes were assigned based on the digested band patterns. The observed alleles in each sample for *MTNR1C/MboI* loci and its probable genotypes were recorded. Locus specific alleles were identified according to their molecular sizes. Allele and genotype frequencies were calculated with the help of following this formula:

Genotype frequency = number of specific genotype/ total number of genotypes in population

Gene frequency = 2D + H / 2N

Where, D = Frequency of homozygote, H = frequency of heterozygote, N = number of birds

Statistical analysis

Data recorded on layer economic traits were analyzed for association with genotypes of *MTNR1C* via *MboI–RFLP.* Association between egg production traits and genotypes at candidate gene loci were determined via least-square analysis (Harvey, 1990) using following model:

$$Y_{ijkl} = ì + S_i + H_j + G_k + e_{ijk}$$

where, Y_{ijkl} = value of traits measured on ijkth individual, ì = overall mean, S_i = random effect of ith sire, H_j = fixed effect of jth hatch, G_k = effect of kth genotype of particular candidate gene associated candidate gene in layer of individual Y_{ijkl}, e_{ijk} = Random error associated with each observed Y_{ijkl} and assumed to be NID (0, σ^2)

RESULTS AND DISCUSSION

Allelic profile of *MTNR1C* gene with 372 bp amplicon size was digested via restriction enzyme *MboI*. Allelic patterns observed in RIR breed are depicted in the figure 1. Two different alleles, B (333/39 bp) and A (372 bp) have been resolved after digestion and three type of genotypes were observed as BB, AA and AB, respectively in RIR chicken. The restricted digested fragments showed that *MTNR1C* gene was polymorphic with *MboI*. The number of alleles, genotype and their frequencies for polymorphic region was depicted in Table 2. In RIR, allele frequencies of A and B were observed as 0.29 and 0.71, respectively and genotypic frequencies of AA, BB and AB genotype were 0.04, 0.46 and 0.5, respectively. Chethan *et al*. (2018) studied *MTNR1C* gene polymorphism by *MboI* restriction enzymes in White Leghorn which revealed only two types of genotypes in White Leghorn chicken i.e. AA and AB genotype with two (333 bp and 39 bp) and three (333 bp, 39 bp and 372 bp) size of fragments, respectively. These studies were not reported in RIR birds, so it was not possible to compare the results from this study. Therefore, future research on other chicken breeds can use the current study *of MTNR1C/MboI* RFLP as baseline information.

Table 2: Number of alleles, number of genotypes and their frequencies at regions of *MTNR1C* candidate gene in RIR chicken

Gene	Genotypes	Genotypes	Alleles	Gene frequency
MTNR1C	AA (333 bp and 39 bp)	0.04	A (333 bp and 39 bp)	0.29
	BB (372 bp)	0.46	B (372 bp)	0.71
	AB (333 bp, 39 bp and 372 bp)	0.5		

Therefore, future research on other chicken breeds can use the current study of *MTNR1C/MboI* RFLP as baseline information. Chi-square test was reported that RIR population in Hardy-Weinberg equilibrium for a *MTNR1C* even under selection.

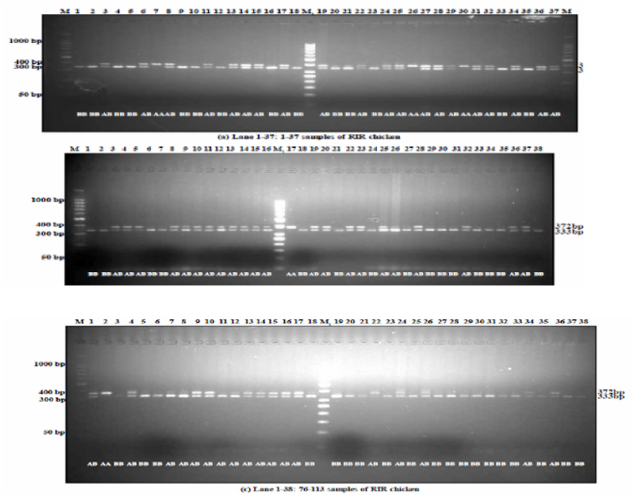

Fig. 1: Mbo PCR-RFLP pattern of MTNR1C gene in RIR chicken. (a-c) M : 100 bp DNA ladder; M1 : 50 bp DNA ladder; UC : uncut PCR product. Various genotypes have been indicated below the lanes, Genotypes with allele size have been shown on the right side; size of marker on the left side and the genotypes below the amplicons in each lane

Association of MTNR1C/MboI genotype with layer economic traits

In RIR breed, the least squares mean of layer economic traits for different *MTNR1C* RFLP genotypes was depicted in table 3 and 4. Resultant genotypes significantly affected ASM traits at 10% level of significance (P≤0.10). The birds with AB genotype achieved earliest ASM (171.42±5.34 days) and birds which possessing AB genotypes for ASM trait were statistically lesser compare to birds which possess BB genotyped as (182.97±5.56 days) and AA (179.34±11.15 days). Chethan *et al*. (2018) studied *MTNR1C* gene polymorphism by *MboI* restriction enzymes in white leghorn which found the effect of genotypes was significant ASM (P≤0.12). *MTNR1C* gene polymorphism showed that Pullets with AA genotype had lower ASM as 138.44±1.35 days than those pullets with AB genotypes (141.11±2.73 days). Pandey (2022) also reported significant effect on ASM in Aseel bird. Birds with AA genotype achieved earlier age at sexual maturity (185.23±15.52 days).

According to finding, significant association of MTNR1C was found with age at first egg. A similar association was not found in native chickens and in Thai Pradu Hang Dam reported by Tenzin, Chankitisakul & Boonkum (2020).

Table 3: Least squares ANOVA for *MTNR1C (Mbo1-RFLP)* genotypes on various layer economic traits in Rhode Island Red

Source	DF	P value			
		ASM	EW28	EW40	EP40
Sire		0.0083(43)	0.5791(38)	0.7710(43)	0.6241(43)
Hatch	1	0.1670	0.3504	0.8131	0.6282
MTNR1C (Mbo1) Genotype	2	0.0890	0.5017	0.5590	0.2553

Figure in parenthesis is the degree of freedom

Table 4: Least squares means and standard errors of various growth and layer economic traits for different genotypes at *MTNR1C (Mbo1*-RFLP*)* in Rhode Island Red (RIR)

Genotypes	N	ASM	EW28	EW40	EP40
	120	177.91±5.18	42.12±0.65(71)	48.87±0.63(118)	72.515±3.94
AA	5	179.34±11.15^{b}	41.93±1.96(2)	48.18±1.91(5)	80.98±11.87
BB	56	182.97±5.56^{c}	42.97±0.96(28)	49.62±0.74(56)	64.12±4.56
AB	59	171.42±5.34^{a}	41.45±0.72(40)	48.62±0.69(57)	72.43±4.22

Figure in parenthesis is the number of observations; Means with different supercripts in a column differ significantly

CONCLUSION

In conclusion, the association between age at sexual maturity and *MboI-RFLP* genotypes was determined. Present findings suggest that *MTNR1C* locus AB RFLP genotype demonstrated a potential marker to enhance age at sexual maturity and this loci can be used for further studies on association of *MboI-RFLP* genotype with layer economic traits in RIR chicken. Furthermore, the association with this trait may be advantageous for elaboration of chicken breeding programme in molecular marker-assisted selection (MAS).

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