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## Effect of melatonin gene on growth and production traits in Kadaknath and Jabalpur colour birds

**Pratibha Padwar and MS Thakur**

### Abstract

The present study was undertaken to explore polymorphism of Melatonin (MTNR1C) gene and its association with quantitative traits such as age at first egg (AFE), body weight at sexual maturity (BWSM), body weight at 20 (BW20), 30 (BW30) and 40 (BW40) weeks of age, egg production at 40 weeks of age (EP40), egg weight at 40 (EW40) weeks of age in Jabalpur colour and Kadaknath birds. Total of 30 birds of each Kadaknath and Jabalpur colour were included in the study. The RFLP pattern for MTNR1C/*MboI* yielded monomorphic fragment of 372 bp (i.e. AA genotype) in all the birds of Kadaknath and Jabalpur colour. The results of analysis of variance showed highly significant differences among breeds, whereas the effect of genotypes and breed x genotype was found to be non significant. The means for AFE, BWSM, BW20, BW30, EW40 and EP40 were significantly ( $p < 0.05$ ) higher in Jabalpur colour as compared to Kadaknath birds.

**Keywords:** MTNR1C, Kadaknath, Jabalpur colour, growth, production

### Introduction

The indigenous poultry breeds play an important role in rural economy and socio-cultural life of the rural community. The chicken and its by-products is a good source of high quality animal protein which provides cash income to the farmers. The native chicken can survive in the harsh environmental condition and poor husbandry practices without much loss in production. The Jabalpur colour breed of chicken is an improved breed of chicken developed by the University under AICRP on poultry which is utilized as parental line to produce commercial colour chicks suitable for rural and tribal areas poultry farming in the state. Kadaknath is a local breed of chicken found in tribal dominated Jhabua, Dhar and Alirajur districts of Madhya Pradesh. In poultry industry, quantitative traits like egg production and growth are considered to be the most economically valued traits. These traits are affected by the various endocrine factors, environment factors and different feeding allowances (Li *et al.*, 2013) [5]. Melatonin (N-acetyl-5-methoxytryptamine) is an indole hormone, which is primarily secreted by the pineal gland. It has three receptor subtypes (i.e. MTNR1A, MTNR1B and MTNR1C) that regulate the various biological functions in vertebrates (Li *et al.*, 2013) [5]. In birds, melatonin (MTNR1C) regulates circadian rhythm, hibernation, feeding pattern, thermoregulation, neuroendocrine functions and also affects the various ovarian functions (Courtilot *et al.*, 2010) [2]. It is thought that many of the variations in growth and production traits such as body weight, egg production rate and egg quality are directly or indirectly influenced by some candidate genes such as melatonin (MTNR1C) genes in a variety of poultry species (Xu *et al.*, 2010) [8].

### Materials and Methods

**Experimental Material:** Total number of 30 birds of each Kadaknath and Jabalpur colour were randomly selected in the study from the flocks of two genetic groups maintained at All India Coordinated Research Project (AICRP) on Poultry Breeding, Department of Poultry Science, Adhartal, N.D.V.S.U., Jabalpur. The different quantitative traits were recorded from each bird of Kadaknath and Jabalpur colour *viz.*, Age at first egg (AFE), Body weight at sexual maturity (BWSM), Body weight at 20/30/40 weeks (BW20/BW30/BW40, respectively), Egg production up to 40 weeks of age (EP40), Egg weight at 40 weeks of age (EW40) for association study.

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**Blood sample collection:** About 1-2 ml blood sample was collected in ethylene diamine tetra-acetate (EDTA) containing vacutainer aseptically from via wing vein of each experimental bird of Kadaknath and Jabalpur colour by using disposable syringe. The blood samples were brought immediately in icebox to the Molecular Genetics Laboratory, Department of Animal Genetics and Breeding, College of Veterinary Science & A.H., Jabalpur. The samples were stored at 4°C till the completion of work.

**Extraction of genomic DNA:** Genomic DNA was extracted from blood samples using the method as described by John *et al.* (1991) [3] with slight modifications. DNA quality was assessed through 0.8% horizontal submarine agarose gel electrophoresis.

**DNA concentration and purity check:** The purity of DNA and concentration was checked via using Nanodrop spectrophotometer (ND-1000 USA). Optical density (OD) value at 260 nm and 280 nm were measured. DNA samples with an OD<sub>260/280</sub> ratio of 1.7 to 1.9 were considered pure and used for further analysis. The DNA concentration was determined and sample was diluted for obtaining a final concentration of 30ng/µl in miliQ water for further use.

**PCR amplification of MTNR1C gene:** Gene specific primer (Forward: 5- GGTGTATCCGTATCCTCTAA-3; Reverse: 5- GACAGTGGGACAATGAAGT-3) was used to amplify the region of interest in the MTRN1C gene of 372 bp (Li *et al.*, 2013) [5]. All the reactions were carried out in 0.2 ml thin wall PCR tubes. Each PCR tube contains 2X PCR Master mix (Fermentas) 12.5 µl, forward and reverse primers 1.25 µl each, genomic DNA 3.0 µl and DNA ase free water 7.0 µl to make the final volume of 25 µl. The PCR tubes were placed in PCR thermal cycler (Eppendorf, Germany), programmed for 36 cycles. The reaction consists of initial denaturation at 95°C for 5 min followed by 36 cycles of denaturation at 94°C for 60 sec then annealing at 49.1°C for 45 sec and extension at 72°C for 50 sec and final extension at 72°C for 5 min. The PCR product was analyzed on 2.0 % agarose gel. 2% w/v agarose gel was prepared in 0.5 X TBE buffer in a similar way as used for checking the quality of DNA. The electrophoresis was conducted at constant voltage of 70 volt for 90 min at 37°C using 10 X TBE buffer. The amplified products in the gel were visualized by UV trans illuminator and photographed using Gel documentation system (Gel-Doc, Bio-Rad, USA).

**PCR-RFLP of MTNR1C gene fragment:** The amplified PCR product (10µl) of each sample was digested with 1µl *MboI*, 10x fast digest buffer RE 2 µl, DNAase free water 17 µl in manufacturers recommended assay buffers in a final reaction volume of 30 µl. The reaction mixture was incubated at 37°C for overnight digestion in water bath. After digestion, the digested products were electrophoresed on 2% agarose gel (Sambrook and Russel, 2001) [6] and visualized by UV trans illuminator and photographed using Gel documentation system (Gel-Doc, Bio-Rad, USA) to detect the banding pattern/ genotype of MTNR1C gene of each sample.

**Statistical analysis:** Genotype frequencies, gene frequencies and genetic equilibrium at different loci were estimated using software POPGENE 32 version, the user-friendly software for Population Genetic Analysis (Yeh *et al.*, 1999) [9]. The data on

various quantitative traits was analysed using analysis of variance using SPSS (1996) computer package.

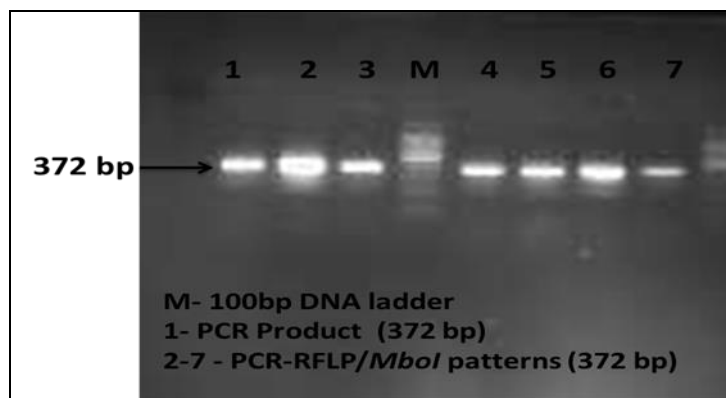
**Results and Discussion**

The amplified PCR product of size 372 bp was obtained for MTNR1C gene in Jabalpur colour and Kadaknath birds. The RFLP pattern for MTNR1C/*MboI* yielded monomorphic fragment of 372 bp of AA genotype in all the birds of Kadaknath and Jabalpur colour (Plate 1 and Plate 2). The allelic frequency for allele A was 1.00 and for allele B was 0.00 in all birds of both the breeds. The Non significant chi-square value in Jabalpur colour and Kadaknath showed that populations were in Hardy-Weinberg equilibrium (HWE) at this locus (Table 1).

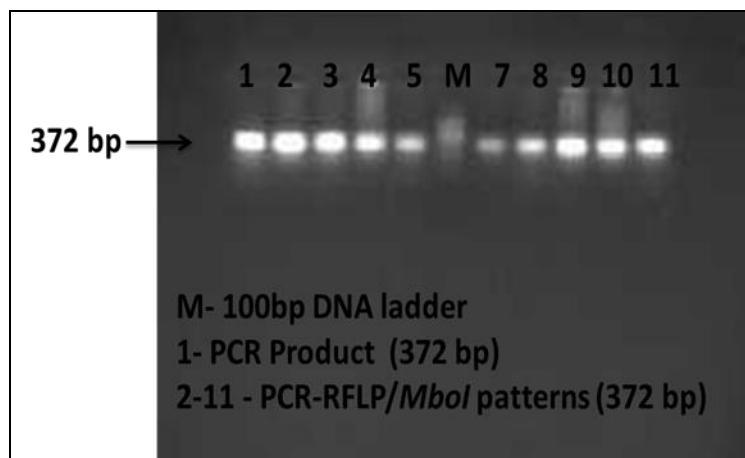
**Table 1:** Frequencies of genotypes and alleles at MTNR1C gene locus

Genotype	Breed	
	Jabalpur colour	Kadaknath
AA	1.00 (30)	1.00 (30)
AB	0.00 (0)	0.00 (0)
BB	0.00 (0)	0.00 (0)
Allele		
A	1.00	1.00
B	0.00	0.00
Chi-square value	0.00 <sup>NS</sup>	0.00 <sup>NS</sup>

NS- Non significant, Figure in parenthesis denotes number of birds



**Plate 1:** PCR-RFLP/*MboI* digested products in Kadaknath birds



**Plate 2:** PCR-RFLP/*MboI* digested products in Jabalpur colour birds

**Association of MTNR1C gene polymorphic variants with quantitative traits in chicken**

The RFLP pattern for MTNR1C/*MboI* yielded monomorphic fragment of 372 bp in all the birds of Kadaknath and Jabalpur

colour so association of gene with quantitative traits could not be studied. The comparative means for various economic traits of both the breeds has been presented in table 2. The effect of breed was found significant ( $p < 0.05$ ) for all the economic traits. The results of least square analysis of variance showed highly significant differences among breeds ( $p < 0.01$ ), whereas the effect of genotypes and breed x genotype interaction were found to be non-significant. The means for age at first egg was significantly higher in Kadaknath ( $153.47 \pm 0.48$ ) as compared to Jabalpur colour ( $163.43 \pm 0.64$ ). The averages for body weight at sexual maturity, adult body weight at 20, 30 and 40 weeks of age, egg weight at 40 weeks and egg production at 40 weeks of age were significantly higher in Jabalpur colour as compared to Kadaknath.

The RFLP fragments and patterns of genotypes obtained in the present study are in agreement with RFLP fragment and patterns obtained by Chau and Nguyen (2016) [1] in Noi chicken. However, Li *et al.* (2013) [5] reported three genotypes (i.e., AA, AB and BB) in Erlang Mountain Chicken. They reported significantly lower age at first egg and higher total egg production for AB genotype as compared to AA and BB genotypes in Erlang Mountain Chicken. The population of

Jabalpur colour and Kadaknath chicken was found in Hardy Weinberg equilibrium. The results reported in the present study are not in agreement with the results reported by Li *et al.* (2013) [5] in Erlang Mountain Chickens and Chau and Nguyen (2016) [1] in Noi chicken. Similar results of HWE were also reported by Thakur *et al.* (2009) [7] in Kadaknath breed of poultry for chicken growth hormone gene.

The mean body weight at sexual maturity was significantly ( $p < 0.01$ ) higher in Jabalpur colour and Kadaknath chicken. The results reported in the present study are in agreement with the results reported by Li *et al.* (2013) [5] in Erlang Mountain chickens. However, the results are not in agreement as reported by Chau and Nguyen (2016) [1] for body weight at sexual maturity in Noi native chicken.

The overall least square means for adult body weight at 20, 30 and 40 weeks were found to be significantly higher for Jabalpur colour birds. Li *et al.* (2015) [4] reported higher adult weight for different genotypes in Chinese local Erlang mountain chickens. The mean egg weight at 40 weeks (g) was significantly high. The results reported in the present study for Jabalpur colour birds are in close agreement with the results reported by Li *et al.* (2013) [5] in Erlang Mountain chickens and Chau and Nguyen (2016) [1] for in Noi chicken

**Table 2:** Means for various economics traits at MTNR1C gene locus in Kadaknath and Jabalpur colour chicken

Traits	Kadaknath	Jabalpur colour
Age at first egg (days)	153.47 <sup>b</sup> ±0.48	163.43 <sup>a</sup> ±0.64
Body weight at Sexual maturity (g)	1659.67 <sup>a</sup> ±18.95	1177.67 <sup>b</sup> ±9.02
Adult body weight at (20 weeks) (g)	1619.67 <sup>a</sup> ±18.42	1107.67 <sup>b</sup> ±8.60
Adult body weight at (30 weeks) (g)	1829.33 <sup>a</sup> ±17.69	1318.67 <sup>b</sup> ±9.14
Adult body weight at (40 weeks) (g)	2037.00 <sup>a</sup> ±18.07	1542.00 <sup>b</sup> ±10.04
Egg weight at 40 weeks (g)	58.35 <sup>a</sup> ±0.07	47.29 <sup>b</sup> ±0.09
Egg production at 40 weeks (Nos)	90.57 <sup>a</sup> ±0.56	59.57 <sup>b</sup> ±0.68

<sup>a,b</sup>Values with different superscript in row differ significantly ( $p < 0.05$ ).

## Conclusions

All the screened birds of Jabalpur colour and Kadaknath was found to be monomorphic at this gene locus. The differences between breeds were significant and Jabalpur colour birds were found comparatively superior than Kadaknath birds for all the quantitative traits.

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