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Research Article
Association of *Clock* gene (*turClock*) Polymorphism with Growth and Reproductive Traits in Turkeys, *Meleagris gallopavo*

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Abstract

Background and Objective: A study was conducted to test the hypothesis that differences in DNA sequence variations of turkey C*lock* gene may be associated with growth and reproductive traits. The *turClock* gene for DNA sequence variations was screened and evaluated the relationships among its haplotypes (based on haplogroups) with growth and reproductive traits of the turkey. **Methodology:** A total of 290 birds including hybrid turkeys and seven different varieties of heritage turkeys were used. DNA sequences of *turClock* gene (24.6 kb) were screened by re-sequencing of individual amplicons. Haplogroups were determined based on the output from Visual Haplotypes and data were analyzed to find out the associations between genotype and phenotypic traits. **Results:** Twelve SNPs, including five, four and three in introns 6, 9 and 16 respectively, were identified in the *turClock* gene. Reported SNPs were not in the Hardy-Weinberg Equilibrium (HWE) ($p \leq 0.05$). Linkage disequilibrium (D') among SNPs ranged from 0.05-1.00. Pairwise fixation index (F_{ST}) ranged from 0.03-0.90. Five haplogroups were developed from 12 SNPs. Haplogroups frequencies ranged from 0.03-0.93, were significantly associated with body weight (BW) at 309 days of age, feed conversion ratio (FCR) for the periods of 34-68 and 69-159 days, egg production and average egg weight ($p \leq 0.05$). **Conclusion:** The haplogroups of the *turClock* gene are associated with growth and reproductive traits. DNA sequence variations of *Clock* genes at the nucleotide and haplotype levels are associated with differences in performance traits. The genomic information described in the present study would be valuable for future association studies between *Clock* gene and other economically important traits in the turkey using a candidate gene approach.

Key words: *turClock* gene, single nucleotide polymorphism, growth and reproductive traits, turkey, body weight, egg production, semen quality

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A wide variety of activities including behavioral, physiological and biochemical rhythms of the circadian cycle are controlled by a biological clock. Molecular components of the biological clock have been identified in different animal species[1]. Molecular mechanism of the circadian clock is controlled by several genes including Clock, Bmal1, Period1 (Per1), Period2 (Per2), Period3 (Per3), Cryptochrome1 (Cry1) and Cryptochrome2 (Cry2)[2]. The *Clock*, one of the core genes involved in generating endogenous rhythms, encodes the transcription factor CLOCK which dimerizes with BMAL1 protein to activate the transcription of target genes. The CLOCK and BMAL1 produce rhythmic transcription activation that serves as a basic driving force for the circadian clock[3].

In birds, *Clock* gene shares high sequence identities with mammals. These genes have been cloned and their expression localized to the retina, pineal gland and central and peripheral nervous tissues in different varieties of avian species including chicken, Japanese quails, pigeon, house sparrow, garden warbler and barn owl[4-7]. Some studies have shown that *Clock* gene does not exhibit pronounced rhythmicity in chicken and quails[7-8] while others show rhythmic expression of *Clock* gene with peak values at different time points[4-5]. The *Clock* gene is expressed at higher levels during light in the pineal gland, eyes of quails and brain of the house sparrow[5-6]. It seems that expression of *Clock* gene has been inconsistent in different species of avian suggesting that expression of *Clock* gene is species specific[7].

Even though many studies on polymorphisms of *Clock* gene and its association have been carried out in wild birds, association studies of circadian *Clock* gene are limited in poultry species. Therefore, turkey birds, *Meleagris gallopavo,* were used for the present study. It is believed that turkey genome sequence and genomic resources provide the tools that are required to improve growth and reproductive traits in the turkey industry. The objectives of the current study, therefore, were to screen the *turClock* gene for DNA sequence variations and to evaluate the relationships among its haplotypes (based on haplogroups) with growth and reproductive traits of turkeys.

MATERIALS AND METHODS

Genotyping and screening the turkey population: A total of 290 birds including hybrid turkeys (CC) and seven different varieties of heritage turkeys; NA: Narragansett, RP: Royal Palm, BS: Blue Slate, SB: Spanish Black, MW: Midget White, WH: White Holland and BR: Bourbon Red were used. The management and measurement of growth and reproductive traits were described elsewhere[9]. Genomic DNA from 290 turkey birds was isolated using a standard salting out procedure[10]. The DNA sequences of the *turClock* gene were used to design primers using Primer 3 software[11]. The information for the primers including the sequences, annealing temperature and expected sizes of the Polymerase Chain Reaction (PCR) amplicon are presented in Table 1. Amplification was in a final volume of 25 uL consisting of standard reagents including Taq DNA polymerase (Takara Bio, Inc., Japan), 200 uM dNTPs and 2 mM $MgCl_2$. The PCR reaction was performed for a total of 30 cycles in a GeneAmp, PCR System 9700 (Applied Bio-system, CA,). Following PCR, each amplicon was purified using Diffinity RapidTips (Diffinity Genomics, Inc., West Henrietta, NY) and sequenced (VBI, Blacksburg, VA) using the BigDye Terminator, Version 3.1, Sequencing kit (Applied Biosystems, Carlsbad, CA). The sequences were analyzed for SNPs using Phred, Phrap, Polyphred and Consed as previously described by Guan *et al.*[10].

Data analyses: Allele, genotype and haplotype frequencies were determined by standard counting. The computer program, Arlequin ver3.5[12] was used to estimate the pairwise linkage disequilibrium (LD) among SNP loci, to test genotype frequencies for Hardy-Weinberg Equilibrium (HWE) and to estimate the fixation index (F_{ST}) among turkeys. Haplogroups were determined based on the output from Visual Haplotypes (VH1) software (http://gvs.gs.washington.edu/GVS/).

Data were analyzed with the PROC GLIMMIX of SAS 9.3 (SAS Inst. Inc., Cary, NC). The following statistical model was used for the analysis of associations between the genotype and phenotypic traits.

$$Y = \mu+L+S+G+(L\times G)+(G\times S)+e_i$$

where, Y is the trait measured and estimated on turkeys, μ is the overall population mean, L is the fixed effect of the turkey variety, S is the fixed effect of sex, G is the fixed effect associated with the genotype, (L×G) is the interaction between the turkey variety and genotype, (G×S) is the interaction between the sex and genotype and it was excluded from the model if its effect was $p\geq0.05$ and e is the residual error. A separate ANOVA was run for BW at each measurement day, ADG and FCR for each period and each reproductive parameter. Multiple comparisons were analyzed using Tukey's test. The values were presented as least square Means±standard error. Results were considered significant at $p\leq0.05$.

RESULTS AND DISCUSSION

Gene variation of *turClock*: The amplicons produced by the five primer-pairs spanned a 24.6 kb region that included the *turClock* gene (Table 1). A total of 12 Single Nucleotide Polymorphisms (SNPs) were detected in the sequences scanned and validated. The SNPs, the sequence contexts, alleles and GenBank identification (dbSNP) are presented in Table 2. Of the 12 SNPs identified, five, four and three SNPs were detected in introns 6, 9 and 16, respectively. The putative SNPs discovered in the current study have not been published earlier in the dbSNP, NCBI and these SNPs represent novel nucleotide variants of the *turClock* gene.

As expected, most of the SNPs showed C-T/A-G transitions. Within the 290 birds screened, the minor alleles ranged from 0.05-0.49 with the observed heterozygosity of 0.09 and 0.49, respectively. The reported SNPs were not in the HWE ($p \leq 0.05$). Across all SNPs, D' ranged from 0.05-1.00. The correlation coefficient (r^2) for the SNPs, ranged from 0.003-0.81 (Table 3). The pairwise F_{ST} estimated for the eight turkey varieties ranged from 0.03-0.90. The highest F_{ST} (0.90) reported between BR and MW turkeys while lowest (0.03) between RP and NA turkeys. Most of the F_{ST} were significantly different ($p \leq 0.05$) (Table 4).

The haplotypes observed from the 12 SNPs were grouped into five haplogroups. The haplogroups ranged from 0.03-0.93 in the turkey varieties (Table 5). The most common haplogroup identified for BR, CC and SB turkeys was Hap1 with the frequencies of 0.91, 0.82 and 0.48, respectively. Hap2 was predominant for MW, NA and RP turkeys with the frequencies of 0.93, 0.52 and 0.46, respectively. Hap3 and Hap2 were the leading haplogroups for WH and BS turkeys with the frequencies of 0.46 and 0.42, respectively (Table 5).

The *turClock* gene is located over a 24.47 kb region of the chromosome 4 and contains 23 exons and 22 introns. Three transcripts of the *turClock* gene have been identified (www.ensembl.org). However, nucleotide variants of the *turClock* gene have not been published yet in the dbSNP, NCBI. Therefore, the reported SNPs in the present study, would be novel genetic variants of the *turClock* gene according to our knowledge. The genetic structure of *turClock* gene was compared with chicken and zebra finch genome. The genetic structure of the turkey *Clock* gene has 94 and 93% sequence similarity with chicken and zebra finch genome respectively, (http://blast.ncbi.nlm.nih.gov/) suggesting that most of the nucleotides of the *Clock* gene are conserved within these birds. In the present study, the genetic relatedness between CC and heritage turkeys using Fst was also investigated to measure the genetic differentiation between the turkeys. Small Fst values between RP and NA, CC and BR turkeys indicated that closer relatedness between those turkeys. As per the study conducted by Smith *et al.*[13], it showed that closer relatedness between RP and NA turkeys, which were also observed in the present study. In addition, we found that CC turkey was more closely related with BR turkey which was consistent with the previous reports[14]. The pairwise Fst estimates showed that BR turkey was genetically closely related to CC turkey while BS and SB turkeys were distantly related to CC turkey. The CC turkeys have been developed from heritage turkeys using different selection and mating procedures. However, further improvements with respect to disease resistant and reproductive performances are essential to meet the demand of the turkey meat. Some of the genetic information in the present study would be useful to genetically improve the CC turkey population by introgression using heritage turkeys which show the good reproductive performances and are believed high resistance for many diseases.

Associations of *turClock* haplogroups with growth and reproductive traits: The statistical analysis showed that there was a significant association ($p \leq 0.05$) of haplogroups with BW at 309 days of age (Table 6). The results showed that Hap4 had

Table 1: Primer sequences, the expected sizes of amplicons and PCR characteristics for *turClock* gene

Primer ID	Primers[1]	Sequences	Tm[2] (°C)	Amplicon length[3] (bp)
Clk_1	For(47784885)	5'-CAGATTATAATGGTAATGGAGACTAGATTAGAG-3	63.4	4900
	Rev(47784917)	5'-GTACTTCCAGAAAAGTAGAAATAACATACCTC-3'	63.4	
Clk_2	For(47789520)	5'-ATGCTAGAGTAGTGAAGAATTGATAAATGAG-3'	62.0	5300
	Rev(47794850)	5'-AGGTGTAGCTAATCTAACTGTGGCTATAAA-3'	63.3	
Clk_3	For(47794450)	5'-GACAGTTAAATAATAGTCCATAGGAAAAGTG-3'	60.5	4800
	Rev(47799257)	5'-ATAACTAGAAGAGAATGGACTTCATACTTGAC-3'	63.4	
Clk_4	For(47798812)	5'-AATCTTAGTAACGTGAGAAAATACAATGCT-3'	62.0	4800
	Rev(47803674)	5'-GTAACACTACTGATTATGATAAGCCTATGAAC-3'	63.4	
Clk_5	For(47803208)	5'-GGTAATTACAATGAGGAAGATGATTGTAGTAG-3'	63.4	4800
	Rev(47808012)	5'-AATAATTTAACCTCGAGAAGTCAACTAACC-3'	61.9	

[1]For: Forward primer, Rev: reverse primer. Primer-binding sites in the turkey genome (GenBank accession No: LOC100008576) are presented in parentheses. [2]The optimized annealing temperature at which a single amplicon of the expected size was obtained. [3]Length in base pairs (bp) of the expected amplicon based on the binding sites of the forward and reverse primers

Table 2: Characteristics of single nucleotide polymorphisms identified in the *turClock* gene in eight divergent turkey varieties

SNP	Location[1]	Nucleotide position	Sequence context[2]	dbSNP identification[3]	Genotype	Genotype frequency (%)	MAF[4]	HWE[5]
Clk-1	Intron 6	47789799	AAGTA(G/C)AATAT	rs271791595	C/C	68.3	0.29	0.00*
					C/G	5.5		
					G/G	26.2		
Clk-2	Intron 6	47789821	GAGCT(G/C)TGAGT	rs271791596	C/C	47.6	0.39	0.00*
					C/G	26.9		
					G/G	25.5		
Clk-3	Intron 6	47789868	CATTT(G/T)TATAT	rs271791597	T/T	54.1	0.36	0.01*
					T/G	19.7		
					G/G	26.2		
Clk-4	Intron 6	47789923	TTCTA(T/C)GTACA	rs271791598	C/C	47.2	0.44	0.00*
					C/T	17.6		
					T/T	35.2		
Clk-5	Intron 6	47790105	CAGAA(T/G)TGTGT	rs271791599	G/G	56.5	0.43	0.00*
					G/T	1.4		
					T/T	42.1		
Clk-6	Intron 9	47794113	TGATT(G/A)TAGTA	rs271791600	A/A	50.7	0.49	0.00*
					A/G	0.7		
					G/G	48.6		
Clk-7	Intron 9	47794196	AGTTA(G/C)CTGTC	rs271791601	C/C	53.8	0.46	0.00*
					C/G	0.0		
					G/G	46.2		
Clk-8	Intron 9	47794209	ATTTG(A/G)AGTTT	rs271791602	G/G	56.6	0.44	0.00*
					G/A	0.0		
					A/A	43.4		
Clk-9	Intron 9	47794694	GATGC(A/G)GCATG	rs271791603	G/G	60.0	0.32	0.00*
					G/A	0.0		
					A/A	40.0		
Clk-10	Intron 16	47798932	TGCTA(G/A)TATGC	rs271791604	A/A	95.2	0.05	0.00*
					A/G	0.0		
					A/A	4.8		
Clk-11	Intron 16	47798940	ATGCT(A/G)TGTTT	rs271791605	G/G	51.4	0.42	0.00*
					G/A	12.8		
					A/A	35.8		
Clk 2-12	Intron 16	47798954	GTTTA(G/A)AAACA	rs271791606	A/A	65.9	0.33	0.00*
					A/G	2.1		
					G/G	32.0		

[1]Position of the SNP in Ensembl on the forward strand of chromosome 4 of the *Meleagris gallopavo* genome sequence, [2]Within each sequence context, alleles at the SNP locus appear in parentheses. The minor allele is italicized in the parentheses, [3]rs prefix indicates novel SNPs detected in the present study and available in dbSNP, NCBI. [4]MAF: Minor allele frequency of 12 SNPs markers, [5]Significance of deviation from HWE for the 12 SNPs. *Refers to significant at $p<0.05$

Table 3: Linkage disequilibrium as measured by D′ and r^2 between the 12 segregating SNPs in the *turClock* gene

SNPs[1]	Clk-1	Clk-2	Clk-3	Clk-4	Clk-5	Clk-6	Clk-7	Clk-8	Clk-9	Clk-10	Clk-11	Clk-12
Clk-1		0.93	0.85	0.89	0.89	0.82	0.87	0.74	0.68	NS	0.74	0.56
Clk-2	0.56		0.95	0.53	0.69	0.70	0.66	0.55	0.58	NS	0.51	0.55
Clk-3	0.52	0.81		0.54	0.69	0.72	0.70	0.59	0.59	0.33	0.56	0.53
Clk-4	0.41	0.22	0.21		0.66	0.55	0.52	0.50	0.86	0.38	0.62	0.54
Clk-5	0.43	0.40	0.36	0.43		0.57	0.60	0.54	0.75	NS	0.55	0.57
Clk-6	0.29	0.32	0.31	0.25	0.25		0.76	0.73	0.80	0.69	0.75	0.77
Clk-7	0.36	0.32	0.33	0.25	0.31	0.52		0.81	0.76	0.42	0.68	0.70
Clk-8	0.28	0.25	0.25	0.25	0.28	0.45	0.62		0.74	NS	0.68	0.68
Clk-9	0.42	0.24	0.28	0.43	0.34	0.61	0.31	0.32		NS	0.83	0.47
Clk-10	NS	NS	0.01	0.01	NS	0.02	0.01	NS	NS		0.93	1.00
Clk-11	0.31	0.22	0.25	0.37	0.29	0.43	0.40	0.44	0.42	0.05		0.87
Clk-12	0.26	0.25	0.25	0.19	0.21	0.31	0.28	0.29	0.20	0.09	0.51	

[1]SNP identification (clk5-12), NS: Non significant ($p\geq0.05$) D′ and r^2 values, D′ values are listed in upper right section and r^2 values are listed in lower left section

the highest BW at day one and 68 days of age while Hap5 had the highest values for BW at 34, 159, 231 and 309 days of age. The BW of Hap5 was significantly greater compared to Hap3 at 309 days of age ($p\leq0.05$) (Table 6). There was no significant association ($p\geq0.05$) of haplogroups with ADG. However, Hap5 numerically appeared to be advantageous haplogroup for all

Table 4: The pairwise fixation index (Fst) estimated among turkey varieties using *turClock* SNPs

Turkey varieties[1]	BR	BS	NA	MW	SB	WH	RP	CC
BR	0.00							
BS	0.16	0.00						
NA	0.56	0.38	0.00					
MW	0.90	0.74	0.30	0.00				
SB	0.16	0.10	0.37	0.74	0.00			
WH	0.68	0.51	0.19	0.26	0.48	0.00		
RP	0.51	0.34	0.03	0.25	0.34	0.12	0.00	
CC	0.04	0.18	0.62	0.91	0.25	0.74	0.58	0.0

[1]Seven different varieties of heritage turkeys including BR: Bourbon Red, BS: Blue Slate, NA: Narragansett, RP: Royal Palm, SB: Spanish Black, WH: White Holland and MW: Midget White and CC: Commercial turkeys were used for the present study

Table 5: Haplogroup frequencies of *turClock* gene in eight turkey populations

	Haplogroups					
Turkey varieties[1]	N	Hap1	Hap2	Hap3	Hap4	Hap5
BR	23	0.91	0.00	0.09	0.00	0.00
BS	36	0.39	0.00	0.08	0.11	0.42
CC	50	0.82	0.00	0.00	0.18	0.00
MW	31	0.00	0.93	0.00	0.07	0.00
NA	33	0.09	0.52	0.24	0.03	0.12
RP	39	0.08	0.46	0.23	0.10	0.13
SB	37	0.48	0.03	0.14	0.27	0.08
WH	37	0.00	0.32	0.46	0.22	0.00

[1]Seven different varieties of heritage turkeys including BR: Bourbon Red, BS: Blue Slate, NA: Narragansett, RP: Royal Palm, SB: Spanish Black, WH: White Holland and MW: Midget White and CC: Commercial turkeys were used for the present study. N: No. of birds from each variety used for calculation of haplogroup frequencies

Table 6: Associations between haplogroups of *turClock* gene and the body weight (BW) at different ages of turkeys

	BW[1] (kg)					
Haplogroups	1 days	34 days	68 days	159 days	231 days	309 days*
Hap1	0.046±0.001[a]	0.69±0.02[a]	1.97±0.05[a]	6.61±0.13[a]	8.76±0.16[a]	9.09±0.16[a,b]
Hap2	0.046±0.001[a]	0.70±0.02[a]	2.06±0.06[a]	6.50±0.15[a]	8.75±0.17[a]	8.86±0.17[a,b]
Hap3	0.046±0.001[a]	0.70±0.02[a]	2.05±0.06[a]	6.42±0.16[a]	8.46±0.19[a]	8.73±0.19[a]
Hap4	0.047±0.001[a]	0.72±0.02[a]	2.09±0.06[a]	6.64±0.16[a]	8.68±0.20[a]	9.05±0.20[a,b]
Hap5	0.045±0.001[a]	0.73±0.03[a]	2.07±0.08[a]	6.77±0.21[a]	8.88±0.24[a]	9.35±0.24[b]

[a,b]Means within columns with different superscripts are significantly different ($p \leq 0.05$). [1]BW: Body weight (kg) was measured at 1, 34, 68, 159, 231 and 309 days (d). Least square Means±SE. *$p \leq 0.05$. Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

Table 7: Associations between haplogroups of *turClock* gene and the average daily gain (ADG) by period of age for turkeys

	ADG[1] (kg)					
Haplogroups	1-34 days	35-68 days	69-159 days	160-231 days	232-309 days	1-309 days
Hap1	0.019±0.001[a]	0.038±0.001[a]	0.051±0.001[a]	0.030±0.002[a]	0.004±0.002[a]	0.029±0.001[a]
Hap2	0.020±0.001[a]	0.040±0.001[a]	0.049±0.002[a]	0.031±0.002[a]	0.001±0.002[a]	0.029±0.001[a]
Hap3	0.020±0.001[a]	0.040±0.001[a]	0.048±0.002[a]	0.028±0.002[a]	0.003±0.002[a]	0.028±0.001[a]
Hap4	0.020±0.001[a]	0.040±0.001[a]	0.050±0.002[a]	0.027±0.002[a]	0.004±0.002[a]	0.029±0.001[a]
Hap5	0.021±0.001[a]	0.041±0.002[a]	0.052±0.002[a]	0.029±0.003[a]	0.006±0.003[a]	0.030±0.001[a]

[a]Means within columns with different superscripts are significantly different ($p \leq 0.05$). [1]ADG: Average daily gain (kg) was estimated at different periods of the age. Least square Means±SE. Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

the periods of age except 160-231 days where Hap2 had the highest (Table 7). In general, Hap5 appeared to be the most advantageous haplogroup for BW and ADG.

Haplogroups were significantly ($p \leq 0.05$) associated with FCR during the periods of 34-68 and 69-159 days. During the period of 34-68 days, Hap2 had significantly lower FCR compared to Hap1. Hap1 had significantly lower FCR than that of Hap3 during the period of 69-159 days. Hap1 reported the lowest FCR for the other two periods though not significant ($p \geq 0.05$) among haplogroups (Table 8). The FCR numerically increased with the age of turkeys in all the haplogroups. Generally, Hap1 numerically appeared to be the most efficient feed conversion haplogroup with compared to others within the different periods of age for the turkeys.

As shown in Table 9, haplogroups were not significantly ($p \geq 0.05$) associated with semen quality traits. However, Hap1

Table 8: Associations between haplogroups of *turClock* gene and the feed conversion ratio (FCR) by periods of age for turkeys

	FCR[1]			
Haplogroups	34-68 days*	69-159 days*	160-231 days	34-231 days
Hap1	3.19±0.14[a]	4.33±0.12[a]	11.32±0.58[a]	7.11±0.18[a]
Hap2	2.49±0.15[b]	4.69±0.13[a,b]	11.72±0.64[a]	7.31±0.20[a]
Hap3	2.73±0.16[b]	4.78±0.14[b]	12.88±0.71[a]	7.61±0.22[a]
Hap4	2.85±0.17[a,b]	4.52±0.14[a,b]	12.32±0.73[a]	7.57±0.23[a]
Hap5	2.78±0.21[a,b]	4.39±0.18[a,b]	12.13±0.89[a]	7.28±0.28[a]

[a,b]Means within columns with different superscripts are significantly different ($p \leq 0.05$). [1]FCR: Feed conversion ratio was estimated at different periods of the age. Least square Means±SE. *$p \leq 0.05$. Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

Table 9: Associations between haplogroups of *turClock* gene and semen quality traits for turkeys

	Semen quality traits[1]			
Haplogroups	Ejaculate volume (mL)	Sperm concentration ($\times 10^9$ mL^{-1})	Total number of sperm ($\times 10^8$/ejaculate)	Sperm viability (%)
Hap1	0.11±0.01[a]	2.32±0.16[a]	2.63±0.38[a]	83.65±0.83[a]
Hap2	0.11±0.01[a]	2.31±0.17[a]	2.72±0.39[a]	83.17±0.85[a]
Hap3	0.12±0.01[a]	2.29±0.19[a]	2.80±0.44[a]	83.83±0.97[a]
Hap4	0.10±0.01[a]	2.23±0.20[a]	2.37±0.45[a]	84.13±0.98[a]
Hap5	0.11±0.01[a]	2.15±0.20[a]	2.58±0.45[a]	82.32±0.99[a]

[a]Means within columns with different superscripts are significantly different ($p \leq 0.05$). 1Least square Means±SE. Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

Table 10: Associations between haplogroups of *turClock* gene and the egg production traits for turkeys

	Egg production traits[1]				
		Egg production[2]		Average egg weight[3] (g)	
Haplogroups	AFE[4] (days)	6 weeks*	10 weeks*	6 weeks*	Total wt
Hap1	231.56±6.34[a]	14.82±1.80[b]	22.26±2.61[a]	79.28±0.61[a]	78.87±0.56[a]
Hap2	228.63±6.82[a]	12.55±2.10[a,b]	19.48±3.05[a,b]	77.78±0.69[a,b]	77.58±0.64[a]
Hap3	227.62±7.03[a]	12.69±2.20[a,b]	20.35±3.19[a,b]	76.80±0.82[b]	76.96±0.71[a]
Hap4	229.03±8.74[a]	9.17±2.26[a]	14.89±3.28[b]	76.20±0.83[b]	77.47±0.76[a]
Hap5	253.09±14.15[a]	6.86±3.46[a]	10.30±5.01[b]	77.84±1.48[a,b]	79.18±1.42[a]

[a,b]Means within columns with different superscripts are significantly different ($p \leq 0.05$). 1Least square Means±SE. *$p \leq 0.05$, [2]Egg production was individually recorded for a period of 10 weeks starting from 30-40 weeks of age. The total egg production for each hen was estimated for a period of 6 and 10 weeks. The individual egg production of 6 weeks was calculated excluding the first and last two weeks egg production from the period of 10 weeks of egg production. [3]The average egg weight (g) was calculated for the period of 6 and 10 weeks separately for each hen. [4]AFE: Age at first egg was recorded and given in days (d). Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups

had the highest values for sperm concentration and sperm viability while Hap3 reported the highest ejaculate volume and total sperm count per ejaculate compared to others. For the reproductive traits including AFE and average egg weight, haplogroups were significantly ($p \leq 0.05$) associated with egg production for both 6 and 10 weeks periods and the average egg weight for 6 weeks period. The egg production of Hap1 for the periods of 6 and 10 weeks was significantly greater than that of Hap4 and Hap5. The average egg weight of the Hap1 for the period of 6 weeks was significantly greater compared to Hap3 and Hap4. For AFE, Hap3 had the lowest value compared to Hap5 though not significant ($p \geq 0.05$) (Table 10). In general, Hap1 appeared to be the most advantageous haplogroup for egg production parameters.

Most of the reported SNPs in the *Clock* genes did not follow the HWE suggesting that gene and genotype frequencies of these variants change from one generation to another due to operational of some evolutionary forces like selection, migration, mutation and random mating. A deviation from HWE may be due to natural selection, population admixture, inbreeding, experimental and duplication[15]. Most of the D′ values among SNPs were significantly different suggesting that these SNPs are in linkage disequilibrium though they are apart which shows the SNPs tend to be inherited together more often than expected by chance[16]. The strong genetic differentiation between two populations is confirmed by the large Fst values[17]. The candidate gene approach is considered as one of the powerful methods to investigate associations of gene polymorphisms with economically important traits in farm animals. Non-synonymous SNPs translate into amino-acid polymorphisms in the proteins they encode. Regulatory SNPs can also affect the expression, tissue-specificity or function of relevant proteins[18]. The association studies have been used to find any

association between the genetic variants and phenotype of the animals. With the discovery of SNPs, it has been increasing interests in genetic associations with closely linked SNPs[19]. Haplotype-based association analysis has several advantages compared to association analysis of single SNP. Haplotypes can reduce the number of comparisons to be made during the analysis. Thus, haplotypes are naturally interpreted as genetic polymorphisms of SNP alleles on the same chromosome and tend to be conserved by evolutionary processes[20].

CONCLUSION

The novel SNPs found in the present study could be useful in biochemical and molecular studies of economically important traits in turkeys and future genetic studies. It also provides a foundation to evaluate the important of those variants in the molecular mechanism of the circadian clock in the turkey. The haplogroups developed for each gene were used to analyze the association study with the growth and reproductive parameters of the turkey. The association study reveals that haplogroups of the different genes are associated with some of the growth and reproductive traits. DNA sequence variations of the *turClock* genes may have some regulatory role in the molecular mechanism of the circadian clock which may affect the overall mechanism of the circadian clock. However, further association studies are required to show the significance of genetic data in the present study for the genotype : phenotype correlations in the turkey.

SIGNIFICANCE STATEMENT

This study discovers 12 novel SNPs which were detected in introns 6, 9 and 16 as five, four and three SNPs, respectively. The reported SNPs represent novel nucleotide variants in the turkey genome. These novel information could be beneficial for future genotype : phenotype studies. The information gathered from genetic and association analyses of *turClock* gene would be useful for the breeder to select the best birds at the early stage of their life. This study will be helpful for researchers to uncover the correlation of genotype : phenotype studies between other clock genes and traits in the turkey.

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