DNA Sequence and Haplotype Variation Analyses of Circadian Clock Genes and Their Effects on Phenotypes in the Turkey, *Meleagris gallopavo*

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ABSTRACT

Present study was planned to compare the phenotypic variation of performances traits among commercial (CC) and heritage varieties of turkeys. Information about heritage turkey varieties continues to be limited. In addition, the emerging turkey genome sequence provides a unique opportunity to understand the DNA sequence variation and its associations with performance traits. The *turClock, turPer3, turCry1 and turCry2* genes were screened and evaluated for its association with their performance traits. As expected, CC turkeys were superior to heritage birds in performance for most of the traits evaluated. However, heritage turkeys showed better reproductive performances compared to CC turkeys. A total of 41 SNPs were identified from the genes that screened. The haplogroups in the *turClock* gene were significantly associated with body weight (BW) at 309 d of age, feed conversion ratio (FCR) for 34 - 68 d and 69 - 159 d, egg production and average egg weight ($P \leq 0.05$). The haplogroups developed from *turPeriod-3* gene were significantly associated with BW at 231 d of age, average daily gain (ADG) for 160 - 231 d, FCR for 69 - 159 d and 160 - 231 d, egg production traits, semen quality traits and plasma melatonin concentration ($P \leq 0.05$). In the *turCry1* gene, haplogroups were significantly associated with ADG for 35 - 68 d, FCR for 160 - 231 d and 34 - 231 d, egg production and ejaculate volume ($P \leq 0.05$). The haplogroups identified from *turCry2* gene were significantly associated with BW at 34, 68 and 231 d of age, ADG for 160 - 231 d, FCR for 34 - 68 d, average egg weight ($P \leq 0.05$). These findings reveal that phenotypic variation observed in

growth and reproductive parameters among turkeys could be used for selecting birds for future breeding programs. DNA sequence variations at the nucleotide and haplotype levels are associated with some of growth, reproductive parameters and plasma melatonin of turkeys. Thus DNA sequence variations that identified of the circadian genes may have some regulatory role in the molecular mechanism of the circadian clock which may affect the circadian rhythm of the animal.

DEDICATION

I dedicated my dissertation to my family. I extend a special feeling of gratitude to my beloved parents, brother and sisters whose words of encouragement. I dedicated this work with special thanks to my loving wife Deepthi for being with me throughout the Ph.D. program. I also dedicated this work to my wonderful son Sanuka who has been my best cheer leader.

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CHAPTER 1

Introduction

1.1 Genotype and phenotype association

The genotype and phenotype have been defined in different ways. However, one of the accepted definitions for the genotype is the sum of the total of the genetic information contained in the chromosome of an organism. The phenotype is defined as observable properties of an organism (Rieger et al., 1991). The term genotype was first used by Danish geneticist, Wilhelm Johannsen in 1909 to describe the entire genetic constitution of an organism (Mahner and Kary, 1997). The phenotypes are based on the content of genes that comprise the genotype. The expression of those genes in observable forms of an organism is influenced by environmental factors. The relationship between genotype and phenotype is an area of interest of geneticists to predict the effect of genetic change on phenotypic processes. The genotype and phenotype represent the differences between genetic composition and expressed form. In addition, the genotype is a group of genetic markers that describe the variations (alleles) of gene of an individual. The individual's genotype includes various alleles which determine the range of traits of the organism. Therefore, the phenotype of the organism reflects the expression of the genotype (Gilbert, 2000).

The main aim of genetic association study is to identify the relationship between one or more genetic polymorphisms and a phenotypic trait. Compared to linkage studies, association analysis has greater power to detect small effects because it operates within a short distance in the genome, though many more markers have to be examined. The discovery of large number of

single nucleotide polymorphisms (SNPs) throughout the genome has increased the importance of genetic association studies especially because of the low genotyping cost for SNPs. SNPs are most widely used as a marker in association studies (Lewis and Knight, 2012). The association between a genetic polymorphism and a phenotypic trait could be due to several factors: polymorphism has a causal role, polymorphism has no causal role but linked to causal alleles or association may be due to the admixture of populations (Cordell and Clayton, 2005).

Three types of genetic associations have been described including direct, indirect, and confounded. Direct association involves putative causal genetic variants that lead the amino acid change in the candidate gene affecting the gene regulation and expression. The indirect association involves polymorphisms which are located close to causal variants. This usually needs to identify the surrounding markers with a high chance for the association. The third type of association between genotype and phenotype in a population involves confounding by stratification and admixture (Cordell and Clayton, 2005).

1.2 Biological clock

Most living organisms change their behavior and physiology rhythmically throughout the day. Environmental stimuli, including light:dark cycle, temperature are responsible for the changing behaviors. Others behaviors change even without environmental cues (Harmer et al., 2001). The behavioral rhythms are referred to as 'circadian' (*circa* – about and *dies* – day). A circadian rhythm is a biological rhythm that persists within the 24 h light:dark cycle (Chong et al., 2003). The first known experiment on biological rhythms was carried out in 1729 by the French biologist De Mairan. He observed that the leaves of the heliotrope plant, *Mimosa pudica* open during day and fold at the night and also continued to open and fold their leaves even at

constant darkness (Gardner et al., 2006). This biological phenomenon confirmed that these plants maintain the daily rhythm even in the absence of daylight as the environmental cue. In order to respond to external stimuli, there should be a mechanism in living organisms for their survivability. Developing an endogenous time keeping mechanism known as the circadian clock ensures that behavioral and physiological processes occur at the optimal time of the 24 h cycle (Froy, 2010).

Biological clocks are fundamental systems of most living organisms ranging from single cell to multicellular plants and animals (Bell-Pedersen et al., 2005). They provide the mechanism responsible for anticipating rhythmic changes in the environment and accordingly adjust the physiological state of the body (Pittendrigh, 1993). In addition, the biological clock regulates the rhythmic variations in physiology and behavior of the living organisms including the sleep-wake cycle, body temperature, hormone production, and locomotor activities (Okano and Fukada, 2003). Thus, the circadian clock provides adaptive advantage to living organisms (Ouyang et al., 1998).

Most circadian clock systems generally consist of three components including the input pathway (environmental timing cue), an oscillator (rhythm generator) and the output pathway (Harmer et al., 2001). The input pathway is upstream of the pacemaker that receives the diverse external cues and then entrains or resets the pacemaker. The oscillator or pacemaker is located at the center and drives the intrinsic rhythms in the presence or absence of external stimuli. The output pathway is downstream of the pacemaker which directly regulates the physiological and behavioral rhythms of the organisms (Kwon et al., 2011). In addition, the circadian clock has three basic properties: First, the circadian clock persists for a period of up to 24 hours in the

absence of external cues. Second, the clock can be entrained to an appropriate environmental stimulus (usually a light/dark or temperature cycle). The final characteristic is the period of the free-running rhythm within the physiological range (Edery, 2000).

1.3 Mammalian clock system

In mammals, the master circadian clock is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Takahashi et al., 2008). The SCN plays a vital role in coordinating the circadian rhythms associated with numerous physiological and behavioral functions in mammals. The SCN are small paired structures that contain approximately 20,000 neurons. The circadian rhythms are endogenously generated in these neurons with the influence of light-dark cycle (Antle and Silver, 2005). Damage of the mammalian SCN has been shown to abolish the circadian patterns of behavior, endocrine, cardiovascular rhythms and many biochemical processes in rats (Moore and Eichler, 1972; Stephan and Zucker, 1972; Turek, 1985). Transplantation of SCN tissues restores the circadian rhythmicity of behavioral activities of rats that previously had the SCN abolished (Ralph et al., 1990; Ralph and Lehman, 1991). Therefore, it is believed that the SCN contains all the features required for a circadian clock and is vital for generations of circadian rhythms throughout the organisms (Cassone and Stephan, 2002). The important characteristic of the SCN is that each neuron is capable of generating oscillations, which couple to each other to form the complete SCN pacemaker (Bell-Pedersen et al., 2005).

Light plays a major role as a time cue that synchronizes the circadian clock with the environmental light-dark cycle. Light is perceived by the retina of the eyes and then signals are sent via the retinohypothalamic tract (RHT) to the SCN (Lucas et al., 2001). Once the SCN collect the information, it processes and interprets the information received via RHT and

transmits it further to the peripheral oscillators via neuronal connections and humoral factors (Froy, 2007). Several humoral factors such as prokineticin and cardiotrophin-like cytokine have been identified that connect the SCN to the peripheral oscillators. Peripheral oscillators have been found in diverse organisms including the liver, heart, intestine and retina (Froy, 2010). The sub-paraventricular zone (SPZ) and the dorso-medial nucleus of the hypothalamus (DMH) are two important areas of the hypothalamus which receive most of the output from the SCN. DMH connects the intermedio-lateral column which is in the spinal cord and send the information to the pineal gland through the superior cervical ganglia. The pineal gland secrets melatonin to the blood stream based on light information. The plasma melatonin concentration is important for coordination of circadian rhythms associated with feeding, sleep-wake cycle, locomotor activity, body temperature and endocrine (Saper et al., 2005).

1.4 Avian circadian system

The avian clock system appears to be more complex than that of mammals. It consists a total of three autonomous and anatomically distinct pacemakers each of which is in the retina, the pineal gland, and the SCN (Bell-Pedersen et al., 2005). These three components interact to produce a stable circadian rhythm (Cassone and Westneat, 2012). A 'neuroendocrine loop model' has been proposed for the avian circadian system (Cassone and Menaker, 1984).

The pineal gland was the first avian oscillator discovered and it is considered the primary component of the avian circadian system (Cassone and Menaker, 1984; Gwinner, 1989). Evidence of research importance is based on observations that pinealectomy in some birds including house sparrow (*Passer domesticus*), European starling (*Sturnus vulgaris*), Japanese quail (*Coturnix coturnix japonica*) and Indian weaver (*Ploceus philippinus*) severely affects

circadian behavior including locomotor activity (Gaston and Menaker, 1968), feeding (Heigl and Gwinner, 1994), hormonal imbalance (Ubuka et al., 2005), and body temperature (Binkley et al., 1971). Restoration of rhythmicity occurs with transplantation of the pineal gland into the anterior chamber of the eye of the pinealectomized birds (Zimmerman and Menaker, 1979). These observations provide strong support for the pineal gland as a core stimulant in the avian circadian system.

In a study involving Japanese quail, the retina was determined to be another organ that involving in the avian circadian (Underwood et al., 1990; Underwood, 1994). Locomotor activity in Japanese quails and pigeon kept in either light-dark cycle or in constant darkness was shown to be rhythmicity (Underwood and Siopes, 1984; Oshima et al., 1989). The avian retina produces melatonin rhythmically. Surgical removal of pineal and eyes eliminates the plasma melatonin and behavioral rhythms (Gwinner and Brandstatter, 2001). The periodic application of melatonin restores the behavioral rhythmicity of sparrows (Heigl and Gwinner, 1994; Heigl and Gwinner, 1995).

The third avian pacemaker resides in the hypothalamus, described as a homolog of the mammalian SCN (Ebihara and Kawamura, 1981). Two distinct structures of the hypothalamus have been identified in birds: medial SCN (mSCN) and visual SCN (vSCN) (Cantwell and Cassone, 2006). The mSCN and the vSCN together form the functional unit, that is similar to the mammalian SCN (Kumar et al., 2007). The vSCN receives light information via retinohypothalamic tract (RHT) while mSCN receives extraocular photoreceptor input (EPR) located in the deep brain (Cassone et al., 2009). The vSCN is the primary retinorecipient nucleus in the avian hypothalamus (Kumar et al., 2004). Experimental evidence reveals that the avian

SCN also rhythmically regulates biological processes. Consistent with observations reported in mammals, lesions of the SCN in avian species including the House sparrow, Java sparrow, and Japanese quail, abolishes circadian rhythmic locomotor activity (Ebihara and Kawamura, 1981; Simpson and Follett, 1981; Takahashi and Menaker, 1982). As a whole, evidence clearly shows that the avian circadian system is a multi-oscillatory system that is comprised of oscillators in the pineal gland, retina, and the SCN (Cassone and Westneat, 2012).

1.5 Molecular mechanism of the circadian clock

A transcriptional and translational feedback loop underlies the basic molecular mechanism of the circadian clock (Helfer et al., 2006). This mechanism has been defined based on studies in the model systems like *Drosophila* and mouse. In the feedback loop system, there are two elements referred to as positive and negative. Positive elements dimerize to activate the transcription of the negative elements, which in turn are translated, dimerized, and inhibit their own transcription (Ripperger and Schibler, 2001). In mice, the negative regulatory elements include three *Period* homologs (Per1, Per2 and Per3) and two Cryptochrome homologs (Cry1 and Cry2). The positive regulatory elements include two BMAL homologs (BMAL1 and BMAL2) and CLOCK which bind to E-box *cis*-element in the promoters of *Per1* and *Per2* genes to activate its transcription (Gekakis et al., 1998; Lee et al., 2001). The PER1 and PER2 proteins are then translocated into the nucleus and form the negative complex with CRY1 and CRY2 proteins. Negative complexes suppress the transcription of *Per1* and *Per2* genes binding with the positive regulatory complexes (CLOCK/BMAL). Circadian rhythm is generated at the gene level and integration mechanism of rhythm starts from gene to system level of the organism. Finally,

circadian rhythm at the system level changes the behavior, peripheral neuronal activities, and hormonal secretion. (Okamura et al., 1999; Okamura, 2003).

Several avian clock genes including *Per2, Per3, Clock, Bmal1, Cry1* and *Cry2* with high sequence identities to mammals have been cloned and characterized in several species including the chicken, Japanese quail, Java sparrow, pigeon, house sparrow, garden warbler, and barn owl. Their expressions have been localized to the retina, pineal gland and other central nervous and peripheral tissues (Chong et al., 2000; Yoshimura et al., 2000; Doi et al., 2001; Yamamoto et al., 2001; Fu et al., 2002; Yasuo et al., 2002; Fidler and Gwinner, 2003; Mouritsen et al., 2004; Helfer et al., 2006).

1.6 Regulation of avian clock genes

Expression studies have shown that the *Clock* gene in Japanese quails is expressed throughout the day while *Per2* and *Per3* show robust circadian rhythms in the eye and the pineal gland. In addition, these genes expressed in various tissues of quails including the heart, kidney, liver, spleen, testis and ovary (Yoshimura et al., 2000). Chicken *Cry2* is expressed at high levels in the pineal gland and retina. *Cry2* is expressed in multiple tissues of the chicken including heart, intestine, hypothalamus and liver (Bailey et al., 2002). In the other found, *Per2* has been detected in the pineal gland and SCN of the chicken embryo under light dark cycle, but not in constant darkness, suggesting that light dark cycle is required for the expression of the *Per2* gene (Okabayashi et al., 2003).

The expression of *Clock* and *Bmal* genes has been shown to be inconsistent. Some studies show that the *Clock* gene does not exhibit pronounced rhythmicity in the chicken and quail (Chong et al., 2000; Yoshimura et al., 2001). However, rhythmic expression of *Clock* gene with

peak values at different time points have been shown in other birds (Yoshimura et al., 2000; Yasuo et al., 2003). *Bmal1* and *Bmal2* have both been identified in the chicken and shown to be expressed in the chicken pineal gland with the peak observed at the day-night transition (Chong et al., 2000; Okano et al., 2001; Chong et al., 2003). Avian *Per2* and *Per3* are also rhythmically expressed in the pineal gland, retina and hypothalamus in both light-dark conditions with the peak at early light phase (Helfer et al., 2006). *Cry1* and *Cry2* have also been identified and described to be expressed rhythmically in the chicken and house sparrow and quail. The expression of *Cry1* is high during the light phase while *Cry2* is shown to be expressed at high levels during late night (Yamamoto et al., 2001; Bailey et al., 2002; Fu et al., 2002; Helfer et al., 2006). Though circadian clock genes have shown to be important in diverse birds, very little is known about these important genes in the turkey.

1.7 Dissertation research hypothesis and objectives

The turkey, *Meleagris gallopavo,* is the second most important poultry meat species and fourth most economically important source of meat for consumers in the United States of America. The recently described turkey genome sequence and other genomic resources provide tools that may be useful in improving growth and reproduction in the turkeys. Therefore, these resources provide unique opportunities to define DNA sequence variations that may be associated with performance traits. Variability in performance traits associated with physiological and behavioral functions considered diurnally rhythmic is of particular interests in animal agriculture because those are regulated by photoperiod. We believe that sequence variations in the circadian clock genes may affect the performance traits associated with physiological and behavioral functions considered diurnally rhythmic. Specially because of the

importance of these genes in rhythmic behavior, I hypothesized that DNA sequence variations in these genes at the nucleotide and haplotype levels may be associated with differences in performance traits including growth and reproduction in the turkeys.

Overall hypothesis

H_0: there is no association between DNA sequence variations in circadian clock genes and performance traits.

H_1: there is association between DNA sequence variations in circadian clock genes and performance traits.

To test the hypothesis and generate data for the overall goal of determining the association between DNA sequence variations in circadian clock genes and performance traits, the following three specific aims were proposed for my dissertation research;

Specific Aims:

1. To evaluate differences among turkey varieties for growth, reproductive performances and plasma melatonin concentration between commercial (CC) and seven heritage varieties of turkeys.

   Heritage turkey is defined as the turkey which has ability to natural mating, long productive lifespan and slow growth rate. Heritage turkey includes Bourbon Red (BR): it is a large bird with rich chestnut-red plumage developed by crossing Buff, Bronze and White Holland in Pennsylvania and Kentucky. Blue Slate (BS): this is a beautiful bird with a blue base color due to unusual gene combination which can lead to blindness. Narragansett (NA):

developed in 1700s by crossing of domestic turkeys from Europe and wild turkey and a very old variety. Royal Palm (RP): lovely and beautiful bird developed for ornamental purpose in 1920s, Spanish Black (SB): developed in Europe and brought back to USA, the conservation status is critical. White Holland (WH): originated in Europe in 1800s and was imported to USA and used for commercial production after 1950s. Midget White (MW): fairly a new breed developed by Bob Smyth at the University of Massachusetts in late 1950s and a smallest variety of turkey with critical status.

2. To screen the circadian clock genes for DNA sequence variations based on SNPs and haplotypes using a panel of heritage and commercial turkeys and to estimate frequency and distribution of SNPs and haplotypes among them.
3. To evaluate the association, if any, of variation in the haplotypes (based on haplogroups) of each of the clock genes studied with performance traits including body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), age at first egg (AFE) , egg number, egg weight, semen quality traits and plasma melatonin concentration.

I have divided the dissertation into several chapters which describe different aspects as follows:

Chapter 2. As a proof of concept, we screened the *chLEAP-2* gene for DNA sequence variation and evaluated the relationships among its haplotypes variation (based on haplogroups), with resistance to coccidiosis.

Chapter 3. We compared the phenotypic variations of growth, reproductive performances and plasma melatonin concentration between commercial (CC) and seven heritage varieties of turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW).

Chapter 4. We screened the several clock genes including *turClock, turPeriod3, turCry1 and turCry2* for DNA sequence variations and evaluated the relationships among its haplotypes (based on haplogroups) with growth, reproductive traits and plasma melatonin of turkeys.

In chapter 2, original works were carried out by Samantha Casterlow (Casterlow et al., 2011) who was supervised by Eric A. Wong in the Department of Animal and Poultry Sciences, Virginia Tech. Based on their research findings, we were interested in looking at the DNA sequence variation of *chLEAP-2* gene and designed the present study to evaluate the relationships among its haplotypes variation (based on haplogroups), with resistance to coccidiosis.

CHAPTER 2

Haplotype Structure and DNA Sequence Variation of the Liver Expressed Antimicrobial Peptide-2 (*chLEAP-2*) Gene in *Eimeria maxima*-Challenged Chickens

2.1 Abstract

Coccidiosis, a common poultry enteric disease typically characterized by intestinal lesions and reduced performance, is caused by *Eimeria* species. Lesions induced by *Eimeria* parasites in coccidiosis-affected chickens have been associated with the expression of the chicken liver expressed antimicrobial peptide-2 (*chLEAP-2*) gene, a component of the innate immune system. Here, we screened the *chLEAP-2* gene for DNA sequence variation and evaluated the relationships among its haplotypes (based on haplogroups), with the gene expression levels, weight gain (WG) as well as lesion score (LS): a measure of susceptibility to *Eimeria,* in two chicken lines. A total DNA sequence of 4.6 kb including the *chLEAP-2* gene was screened by re-sequencing of individual amplicons. Sixteen single nucleotide polymorphisms (SNPs), including seven each in the promoter and introns, and two in exons, were identified. One of the exonic SNPs was non-synonymous, involving a cysteine to tyrosine codon change. About 25% of the SNPs were in Hardy Weinberg equilibrium. Linkage disequilibrium (D′) among the SNPs ranged from 0.02 to 1.00. The haplotypes observed from the 16 SNPs were assembled into 5 haplogroups. The estimated frequencies of the haplogroups ranged from 0.17 to 0.23 in the combined chicken lines. Although not significant ($P \geq 0.05$), the *chLEAP-2* gene expression difference varied among haplogroups. Differences among haplogroups for LS and WG were consistent, but not statistically significant ($P \geq 0.05$).

However, Hap4 numerically appeared to be the haplogroup least susceptible to coccidiosis. At a minimum, the data do not support an association between *chLEAP-2* DNA variations and susceptibility to coccidiosis. Therefore, earlier reports of differences between resistant and susceptible lines in *chLEAP-2* expression may be due to trans-factors. The genomic reagents reported here provide resources for testing the trans expression control theory and will be useful for future genotype:phenotype evaluation studies between *chLEAP-2* gene and other traits in the chicken.

2.2 Introduction

Coccidiosis is an intestinal disease that affects many animals, including birds. In poultry, it is a major cause of economic losses to the industry worldwide. Seven species of *Eimeria* are responsible for causing avian coccidiosis (McDougald, 2003). Infections by the parasites produce lesions that damage the intestinal epithelia leading to reduced bird performance. This damage can interfere with the food digestion and nutrient absorption, as well as causing dehydration and blood loss. The tissue damage can also expose the bird to bacterial infections. Infection of the mid intestine caused by *Eimeria maxima* is one of the most economically devastating to the poultry industry. Gross clinical signs include lesions with a salmon pink exudate, thickened intestinal wall, and haemorrhagic patches. Though its pathogenicity is considered medium, mortality can be as high as 20% (Schnitzler and Shirley, 1999). Reducing the damage and associated losses caused by *E. maxima* thus remains a major goal of the poultry industry.

Biological molecules like antimicrobial peptides (AMPs) are being explored as an option that may afford the host the capacity to resist *E. maxima* infection (Bao et al., 2006). Antimicrobial peptides are essential components of the host's innate defense system and are synthesized in response to invasion and infection by microbes. The AMPs disrupt the membrane integrity of invading microbes or inhibit metabolic processes of the pathogen (Satchell et al., 2003). Two AMPs identified in vertebrates that are involved in the host defense mechanism are liver-expressed antimicrobial peptide (LEAP)-1 and LEAP-2 (Schnitzler and Shirley, 1999). LEAP-1, the first discovered blood-derived AMP, is secreted from the liver and is involved in iron homeostasis (Pigeon et al., 2001). LEAP-2, discovered shortly thereafter, is expressed in a

number of tissues including the small intestine, lungs and kidney, with greatest expression in the liver (Krause et al., 2003).

The chicken *LEAP-2* gene (*chLEAP-2*) was originally described by Smith et al. (2004) in a genome-wide expression profiling as a member of chicken immune related genes. It is located on chicken chromosome 13 and has three exons that together encode a 76-amino acid peptide (Michailidis, 2010). The encoded peptide exhibits a classic pre/pro-peptide structure. The pro-LEAP-2 is secreted as a 53 amino acid pro-peptide, which is cleaved to a 40 amino acid mature peptide that contains two disulfide bonds (Townes et al., 2009). The chicken LEAP-2 has been shown to kill pathogens, though the mechanism remains unclear (Townes et al., 2004). Chicken LEAP-2 also functions in preventing pathogenic microbes from interacting with the epithelial surface, thereby impeding microbial invasion of tissues (Michailidis, 2010).

In an earlier study, Casterlow et al. (2011) reported that two genetically selected chicken lines (A and B) showed differential *chLEAP-2* expression in response to an *E. maxima* challenge with line A birds exhibiting higher resistance. In the genome-wide expression analysis of the lines using DNA microarrays, they showed that though *chLEAP-2* was down-regulated by 20-fold in line A, the down-regulation in line B was on average much higher and ranged from 11- to 71-fold. Here, we hypothesized that differences in the *chLEAP-2* gene expression in the selected lines may be associated with DNA sequence variation based on SNPs and haplotypes. The objectives of the current study, therefore, were to screen the *chLEAP-2* gene for DNA sequence variation and to evaluate the relationships among its haplotypes (based on haplogroups) with the gene expression levels, lesion score (LS) and weight gain (WG).

2.3 Materials and methods

Molecular Analyses

Blood samples of Aviagen's lines A and B chickens were used from Casterlow et al. (2011) study. The selection history and performance characteristics of the two lines was described elsewhere (Gilbert et al., 2007) with the two lines exhibiting differential responses to *Eimeria* infection (Casterlow et al., 2011). Genomic DNA from 82 birds (41 from Line A and 41 from Line B) were isolated using a standard salting out procedure (Guan et al., 2007). The DNA sequence of the *chLEAP-2* gene (GenBank accession No: LOC414338) was used to design primers using Primer 3 (Rozen and Skaletsky, 2000). The information for the primers including the sequences, annealing temperature, and expected sizes of the PCR amplicons is presented in Table 2. 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 $MgCl_2$. 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 (Guan et al., 2007).

Statistical Analyses

Allele, genotype and haplotype frequencies were determined by standard counting. The computer program Arlequin ver3.5 (Excoffier and Lischer, 2010) was used to estimate pairwise linkage disequilibrium (D´) among SNP loci, to test genotype frequencies for Hardy-Weinberg Equilibrium (HWE) and to estimate the fixation index (F_{ST}**)** between the two lines. The within lines F_{ST} for each locus was estimated using the following formula:

$$F_{ST} = (H_T - H_S)/H_T$$

where H_T = expected heterozygosity for line A and B birds, and H_S = expected average heterozygosity in line A or B.

Haplogroups were determined based on the output from Visual Haplotype (VH1) software (http://gvs.gs.washington.edu/GVS/). The following statistical model was used for the analysis of the relationship among gene expression, WG, LS and haplogroups.

$$Y = \mu + G + L + (G \times L) + e$$

where Y is the trait measured and estimated on chicken (*chLEAP-2* gene expression, WG and LS: data were taken from Casterlow et al. (2011) study), μ is the overall population mean, G is the fixed effect of genotype, L is the fixed effect of lines, $(G \times L)$ is the interaction between the genotype and lines and e is the residual error. Data were analyzed using PROC GLIMMIX of SAS (SAS Inst. Inc., Cary, NC). Significant effects were further evaluated using Tukey's test. The values were presented as least square means ± standard error. Results were considered significant at $P \leq 0.05$.

2.4 Results and discussion

The amplicons produced by the two primer-pairs spanned a 4.6 kb region that included the *chLEAP-2* gene (Table 2. 1). A total of 16 SNPs were detected in the sequences scanned including two in the exons, 7 in the introns and 7 in the promoter region. The complete list of the SNPs, their sequence contexts, alleles, and GenBank identification (*dbSNP*) numbers are presented in Table 2. 2. While 6 SNPs have previously been reported, ten SNPs represent novel nucleotide variants. One of the SNPs detected in the exons was non-synonymous involving an amino acid codon change from cysteine to tyrosine at 21 amino acid (aa) position of the chLEAP-2 peptide. As expected, most of the SNPs were C-T/ A-G transitions. Within the 82 birds screened, the minor alleles ranged in frequency from 0.01 to 0.47 with the observed heterozygosity of 0.02 and 0.50, respectively. Approximately 25% of the SNPs were in HWE ($P \geq 0.05$). F_{ST} estimated for the 16 loci ranged from 0.01 to 0.36 and from 0.01 to 1.00 for lines A and B, respectively (Table 2. 2). The pairwise F_{ST} value for the two genetic lines was 0.17 ($P = 0.03$) which was significantly different suggesting that two chicken lines are genetically different. The relatively low F_{ST} estimate suggests low genetic differentiation between the two genetic lines and probably a reflection of their common ancestry.

Across all SNPs, D´ ranged from 0.01 to 1.00. The correlation coefficient (r^2) for the SNPs ranged from 0.001 to 0.75 (Table 2. 3). The haplotypes observed from the 16 SNPs were grouped into five haplogroups (Figure 2. 1). The haplogroups ranged in frequency from 0.17 to 0.23 in the combined chicken lines (Table 2. 4). The most common haplogroups were Hap1 and Hap3 with frequencies of 0.22 and 0.23, respectively. The frequencies of Hap1, 4 and 5 were higher in line A, while those of Hap2 and 3 were higher in line B (Table 2. 4)

The average down-regulation of *chLEAP-2* expression of the haplogroups in lines A and B ranged from 3.89- to 27.70-fold and 9.68- to 108.29-fold, respectively (Table 2. 4). Hap3 and Hap5 showed the highest average down-regulation in line A (27.70-fold) and line B (108.29-fold), respectively. Hap4, on the other hand, showed the lowest average down-regulation in lines A and B of 3.89- and 9.68-fold, respectively (Table 2. 4). Though differences between lines for *chLEAP-2* expression appear large, the lack of statistical significance may be due to the large variation and standard errors observed.

The average LS of lines A and B for the haplogroups ranged from 0.85 to 1.91 and 1.53 to 3.77, respectively. In line A, Hap4 had the lowest LS (LS of 0.85) and Hap3, had the highest (LS of 1.91). In line B, Hap4 had the lowest LS (LS of 1.53) while Hap5 had the highest (LS of 3.77) (Table 2. 4). Differences among haplogroups for LS were not statistically significant ($P \geq 0.05$). The average WG (g) of lines A and B during the challenged period for the haplogroups ranged from 226.05 to 276.28 and 115.91 to 230.05, respectively. In line A, Hap3 had the lowest WG (226.05 g) and Hap4, had the highest WG (276.28 g). In line B, Hap5 had the lowest WG (115.91 g) while Hap4 had the highest WG (230.05 g) (Table 2. 4). However, differences among haplogroups for WG were not statistically significant ($P \geq 0.05$). The average *chLEAP-2* expression of the combined chicken lines for haplogroups ranged from 6.78- to 56.29-fold. The average LS and WG of the combined chicken lines for the haplogroups ranged from 1.19 to 2.64, and from 184.29 to 253.17 g respectively (Table 2. 5). These results suggest that the association among *chLEAP-2* expression levels, LS, WG, and haplogroups are inconsistent.

The *chLEAP-2* gene was selected as a marker for genetic resistance to coccidiosis based on the earlier study where Casterlow et al. (2011) reported that two genetically selected chicken lines (A and B) showed differential *chLEAP-2* expression in response to an *E. maxima*

challenge. In addition, they showed that *chLEAP-2* gene was more down-regulated in line B compared to line A. For an immunological trait, in our study, we selected LS which is one of the best methods to assess the coccidiosis in poultry (Johnson and Reid, 1970). Body WG was used as one of the economical traits where infection leads to reduced WG resulting severe economic losses for the poultry industry (Casterlow et al., 2011). In the present study, we described new genetic variants in the *chLEAP-2* gene and used them to identify haplotypes and haplogroups that span the *chLEAP-2* gene. Of the variants identified, we found one non-synonymous variant in exon 2 of the *chLEAP-2* gene which was involved an amino acid codon change from cysteine to tyrosine at 21 amino acid (aa) position of the chLEAP-2 peptide. The mature chLEAP-2 peptide is encoded in exon 2 of the chicken gene as a 40 aa peptide (amino acids 37 to 76) (Townes et al., 2004) suggesting that the genetic variant which we detected was not within the mature peptide encoded region of the chLEAP-2 and therefore it has no effect on the mature peptide. Haplotypes were constructed with the 16 SNPs and were used to develop the haplogroups. We analyzed the association between the haplogroups and lines for *chLEAP-2* gene expression, LS and WG. The results showed that differences among the haplogroups for gene expression, LS and WG were not significant. However, line A showed the lowest *chLEAP-2* gene expression, LS and the highest WG compared to line B across all haplogroups. The differences between line A and line B were consistent but not significant. The Hap4 numerically appeared to be the haplogroup least susceptible to coccidiosis (lowest average down-regulation of *chLEAP-2*, lowest mean LS, and highest mean WG) while Hap5 was most susceptible.

In this study, we only examined the role of cis-acting SNPs in *chLEAP-2* gene expression. However, further studies are needed to determine if trans-acting factors explain the differences previously reported in *chLEAP-2* expression in lines divergent for susceptibility to

coccidiosis. Earlier work by Casterlow et al. (2011) showed that line B birds had, on average, higher LS and a greater down-regulation of *chLEAP-2* than line A following *E. maxima* challenge. Sumners et al. (2011) also reported that expression of *chLEAP-2* in *E. praecox* infected birds was significantly decreased in the duodenum and jejunum. Interestingly, the expression of *chLEAP-2* in the small intestine and liver increased significantly in 5 day-old chicks infected with *Salmonella enterica* (Townes et al., 2004). Michailidis (2010) also reported that *chLEAP-2* is developmentally regulated during chicken embryonic development, it is constitutively expressed in the chicken epididymis, and is induced in the chicken gonads in response to *Salmonella enteritidis* infection.

In summary, our results do not support the hypothesis that differences in the *chLEAP-2* gene expression in the selected lines may be associated with DNA sequence variation based on SNPs and haplotypes. We believed that chicks from selected lines susceptible to *E. maxima* would possess a common *chLEAP-2* haplogroup. However, our study showed that *chLEAP-2* haplogroups were not associated with *chLEAP-2* expression levels, LS, WG. But, Hap4 numerically appeared to be the haplogroup least susceptible to coccidiosis in the present study. The identification of particular haplogroups that are less affected by *E. maxima* is vital for management and breeding purposes in poultry. The DNA sequence variations of *chLEAP-2* gene reported in the present study would be useful for future genotype: phenotype evaluation studies between *chLEAP-2* gene and other traits in the chicken using a candidate gene approach.

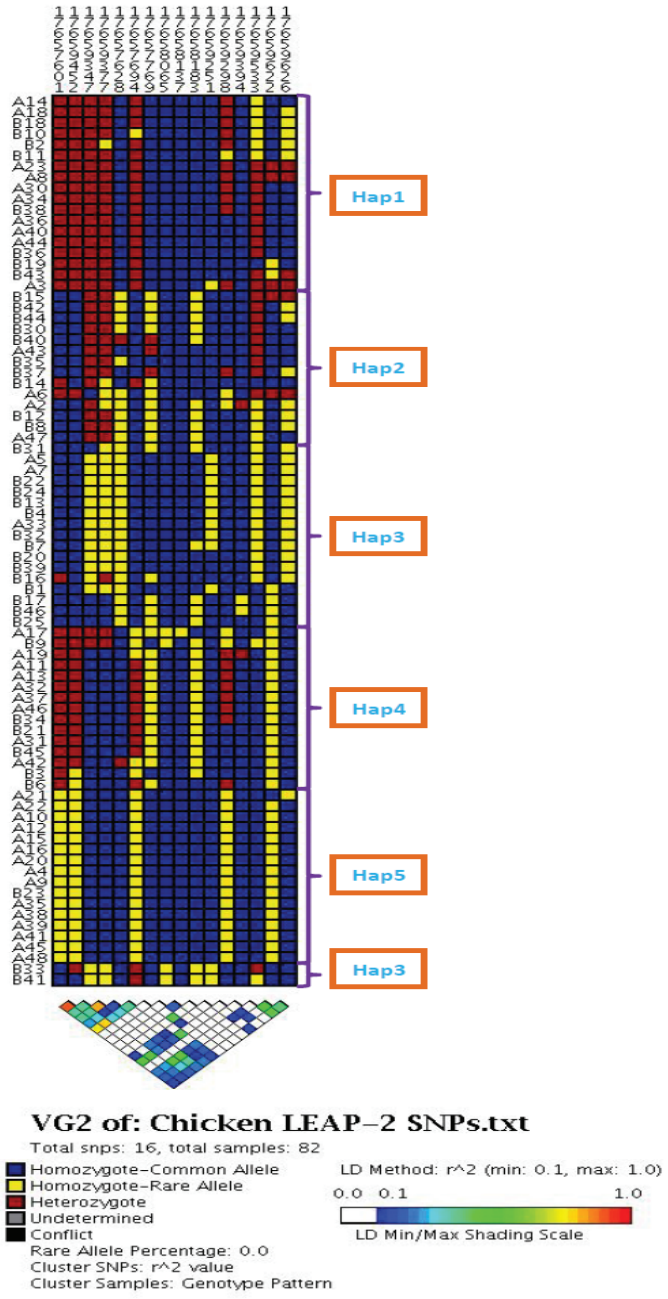

Figure 2. 1. Visualization of haplogroups using Visual Haplotype (VH1) software. (http://gvs.gs.washington.edu/GVS/).

Table 2. 1. Primer sequences, expected sizes of amplicons and PCR characteristics for *chLEAP-2* gene.

Primer ID	Primers[1]	Sequences	Tm[2] (^{0}C)	Amplicon length[3] (bp)
LP_1	For(17657521)	5'-CAATGTAGCTAAAGCACAATTATTTCAT-3'	63.5	2180
	Rev(17659663)	5'-CACAGAATTAATCCCATATCTTATTTGA-3'		
LP_2	For(17659324)	5'-TCTATGACTCCTTCACTTAAAAGTGTTT-3'	63.5	2470
	Rev(17661775)	5'-TAGGATTTCTAAGTCAGTATGTGCATTT-3'		

[1]*For, forward primer; Rev, reverse primer.* Primer-binding sites in the chicken genome (GenBank accession No: LOC414338) 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. 2. Characteristics of single nucleotide polymorphisms (SNPs) identified in the *chLEAP-2* gene in two divergent commercial chicken lines.

SNP	Location	Nucleotide position[1]	Sequence context[2]	*dbSNP* Identification[3]	Genotype	Genotype Frequency (%)	MAF[4]	HWE[5]	F_{ST}[6]	
									Line A	Line B
cL2-1	Promoter	17657601	GAACC(*C*/T)GACAC	*ss*472336150	C/C	20.7	0.41	NS	0.07	0.17
					C/T	42.7				
					T/T	36.6				
cL2-2	Promoter	17657628	GTTAC(*A*/T)CTGCA	*ss*472336151	A/A	32.9	0.34	0.00*	0.36	0.04
					A/T	2.5				
					T/T	64.6				
cL2-3	Promoter	17657694	GGATG(*T*/C)ATAAA	*ss*472336152	C/C	36.6	0.45	0.01*	0.09	0.16
					C/T	37.8				
					T/T	35.6				
cL2-4	Promoter	17657769	CACAC(*C*/T)ACTCC	*rs*15710013	C/C	34.2	0.36	0.00*	0.09	0.05
					C/T	3.6				

					T/T	62.2				
cL2-5	Promoter	17658065	ATGGA(*A*/G)CAACC	*ss*472336153	A/A	4.9	0.05	0.00*	0.08	0.04
					A/G	0.0				
					G/G	95.1				
cL2-6	Promoter	17658137	ACTGC(*A*/G)CATGA	*rs*13505627	A/A	1.2	0.01	0.00*	0.01	1.00
					A/G	0.0				
					G/G	98.8				
cL2-7	Promoter	17658183	AAGTA(*A*/T)TAACG	*ss*472336154	A/A	35.4	0.35	0.00*	0.26	0.09
					A/T	0.0				
					T/T	64.6				
cL2-8	Intron 1	17659251	CCTGG(*G*/T)CTGCT	*ss*472336155	G/G	15.9	0.16	0.00*	0.26	0.13
					G/T	0.0				
					T/T	84.1				
cL2-9	Intron 1	17659298	GATGA(*G*/T)CATTG	*rs*14065141	G/G	23.2	0.38	0.00*	0.19	0.38
					G/T	26.8				
					T/T	50.0				

cL2-10	Intron 1	17659347	CTCCT(*T*/C)CACTT	*rs*14065142	C/C	43.9	0.38	NS	0.20	0.04
					C/T	38.8				
					T/T	18.3				
cL2-11	Intron 1	17659377	GACTC(*G*/A)AGCAT	*rs*14065143	A/A	39.0	0.42	NS	0.12	0.01
					A/G	36.6				
					G/G	24.4				
cL2-12	Intron 1	17659394	ATGAC(*C*/T)CATGC	*rs*14065144	C/C	3.5	0.04	0.01*	0.29	0.08
					C/T	2.6				
					T/T	93.9				
cL2-13[7]	Exon 2	17659452	GGTGT(*A*/G)CTGTG	*ss*472336156	A/A	23.2	0.42	NS	0.07	0.14
					A/G	37.8				
					G/G	39.0				
cL2-14	Exon 2	17659533	AGACC(*C*/T)GTTGG	*ss*472336157	C/C	30.5	0.43	0.00*	0.13	0.03
					C/T	25.6				
					T/T	43.9				
cL2-15	Intron 2	17659622	ATGCT(*C*/T)GATGC	*ss*472336158	C/C	50.0	0.47	0.00*	0.05	0.04

					C/T	7.3				
					T/T	42.7				
cL2-16	Intron 2	17659626	TTGAT(*A*/G)CTGTG	*ss*472336159	A/A	29.3	0.34	0.00*	0.16	0.04
					A/G	8.5				
					G/G	62.2				

[1]Position of the SNP in Ensembl on the forward strand of chromosome 13 of the *Gallus gallus* 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 the SNP that has been previously reported; *ss* prefix indicates novel SNPs detected here and submitted for *rs* assignment in *dbSNP*, NCBI.

[4]Minor allele frequency (MAF) of 16 SNPs markers.

[5]Significance of deviation from HWE for the 16 SNPs. NS indicates non-significant ($P \geq 0.05$) while * refers to significant at $P \leq 0.05$.

[6]Fixation index (F_{ST}) estimates for each of the 16 SNPs.

[7]Non synonymous variant which changes the codon from TGC to TAC (cysteine to tyrosine).

Table 2. 3. Linkage disequilibrium as measured by *D´* and r^2 between the 16 segregating SNPs in the *chLEAP-2* gene.

SNPs[1]	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***
1		0.91	0.21	0.47	NS	NS	0.45	0.56	0.29	0.84	NS	NS	0.17	0.17	0.38	0.39
2	0.29		1.00	0.35	1.00	NS	0.19	0.34	0.81	0.42	0.29	0.75	0.96	0.21	0.47	0.56
3	0.04	0.43		0.44	NS	NS	0.27	0.52	0.82	NS	0.64	NS	0.92	0.37	0.44	0.55
4	0.09	0.11	0.09		NS	NS	0.75	0.71	0.42	0.31	NS	1.00	0.48	NS	NS	NS
5	NS	0.03	NS	NS		1.00	0.61	0.39	NS	NS	0.57	NS	NS	NS	NS	1.00
6	NS	NS	NS	NS	0.24		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
7	0.08	0.04	0.03	0.54	0.04	NS		NS	0.49	0.28	NS	1.00	0.38	NS	NS	NS
8	0.04	0.05	0.05	0.06	0.04	NS	NS		0.62	0.65	0.82	NS	0.49	0.49	0.77	0.36
9	0.07	0.21	0.50	0.06	NS	NS	0.08	0.05		0.32	0.61	NS	0.77	0.32	0.45	0.34
10	0.31	0.15	NS	0.03	NS	NS	0.03	0.14	0.04		NS	1.00	NS	NS	0.46	0.32
11	NS	0.07	0.24	NS	0.02	NS	NS	0.19	0.16	NS		NS	0.72	0.61	0.81	0.36
12	NS	0.04	NS	0.06	NS	NS	0.07	NS	NS	NS	NS		1.00	NS	NS	NS
13	0.03	0.43	0.75	0.09	NS	NS	0.06	0.04	0.51	NS	0.27	0.03		0.43	0.48	0.55
14	NS	0.03	0.09	NS	NS	NS	NS	0.07	0.05	NS	0.35	NS	0.10		0.67	0.23
15	0.11	0.10	0.18	NS	NS	NS	NS	0.11	0.14	0.12	0.41	NS	0.19	0.30		0.57
16	0.07	0.25	0.16	NS	0.03	NS	NS	0.04	0.04	0.10	0.12	NS	0.14	0.04	0.18	

[1]SNP identification (*cL2-1 – cL2-16*) (see Table 2. 2).

NS in the table indicates non-significant ($P \geq 0.05$) *D´* and r^2 values.

D´ values are listed in upper right section, and r^2 values are listed in lower left section.

Table 2. 4. Haplogroups frequency, gene expression, lesion score and weight gain.

Haplogroup ID[1]	N[2]	Frequency		Expression ± SE[3**]		Lesion Score ± SE[4**]		Weight Gain (g) ± SE[5**]	
		Line A	Line B	Line A	Line B	Line A	Line B	Line A	Line B
Hap1	18	0.12	0.10	6.63 ± 12.63	16.19 ± 13.66	1.72 ± 0.51	2.48 ± 0.55	268.91 ± 21.11	208.43 ± 22.84
Hap2	14	0.04	0.13	11.83 ± 19.85	14.89 ± 12.85	1.71 ± 0.81	2.17 ± 0.52	232.25 ± 33.17	209.60 ± 21.48
Hap3	19	0.04	0.19	27.70 ± 24.08	40.46 ± 9.83	1.91 ± 0.98	2.25 ± 0.40	226.05 ± 40.23	222.95 ± 16.42
Hap4	15	0.11	0.07	3.89 ± 13.15	9.68 ± 15.92	0.85 ± 0.53	1.53 ± 0.65	276.28 ± 21.98	230.05 ± 26.61
Hap5	16	0.18	0.02	4.30 ± 9.85	108.29 ± 39.92	1.52 ± 0.40	3.77 ± 1.60	252.66 ± 16.46	115.91 ± 65.71

[1]Hap1 -C-T-T*-T-G-G-T-T-G*-C-A*-T-G-C*-C*-A*-

Hap2 -T-A*-C-C*-G-G-A*-T-T-T-G-T-G-C*-C*-A*-

Hap3 -T-A-C-T*-G-G-T*-G*-T-T*-G*-T-G-C*-C*-A*-

Hap4 -C-T-T*-C*-G*-G*-A*-T-T*-C-A-T-A*-T*-T-G-

Hap5 -C-T-T-T-G-G-T-T-G-C-A-T-A-T-T*-G*-

The variant nucleotide within each haplogroup is represented by *(Alternate alleles are shown in Table 2. 2).

[2]Number of birds (N = 82) within each haplogroup.

[3]Values are expressed as LSmeans of *chLEAP-2* gene expression ± SE of birds within each haplogroup. Down-regulation of intestinal *chLEAP-2* was determined by real-time PCR using the $2^{-\Delta\Delta Ct}$ method and is indicated as negative fold change values (Casterlow et al., 2011).

[4]LSmeans of LS ± SE of birds within each haplogroup. LS ranged from 0 to 4.

[5]LSmeans of WG ± SE of birds within each haplogroup. Body weight was measured at the age of day 14 and day 20 to estimate the weight gain.

[**]Gene expression, LS and WG between both lines, within each haplogroup are not significantly different ($P \geq 0.05$).

Table 2. 5. Associations of haplogroups with gene expression, lesion score and weight gain[1]

Haplogroups	Gene Expression*	LS*	Weight gain (g)*
Hap1	11.41 ± 9.05	2.10 ± 0.37	238.67 ± 15.13
Hap2	13.36 ± 12.35	1.94 ± 0.50	220.92 ± 20.64
Hap3	34.08 ± 13.46	2.08 ± 0.55	224.50 ± 22.49
Hap4	**6.78 ± 10.03**	**1.19 ± 0.41**	**253.17 ± 16.76**
Hap5	56.29 ± 20.35	2.64 ± 0.83	184.29 ± 34.00

Bold values represent the least susceptible haplogroup.

Underline values represent the most susceptible haplogroup.

[1]Least square means ± standard error

*$P \geq 0.05$

CHAPTER 3

Phenotypic Variation of Growth, Reproductive Performances and Plasma Melatonin Concentration among the Turkeys, *Meleagris gallopavo.*

3.1 Abstract

Turkey production in the United States has increased about 110 percent since 1970. The growth is due to increased consumption of turkey meat and meat products. Turkey consumption has also increased by 102 percent since 1970 due to its taste and nutritional value. The turkey is the second most important poultry meat species in the U.S. However, information about heritage turkey varieties, an emerging resource for turkey improvement, continues to be limited. Thus, the present study was planned to compare the phenotypic variation of growth, reproductive performances and plasma melatonin among commercial (CC) and seven heritage varieties of turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW). A total of 40 one day-old poults from each heritage variety and 50 CC one day-old poults were raised for a period of 309 d. Differences among varieties for body weight (BW) were significant ($P \leq 0.05$). In all varieties, the mean average daily gain (ADG) gradually increased up to 159 d of age and then decreased thereafter. As expected, males had higher ADG compared to females. The NA turkeys had higher ADG among heritage turkeys. The CC turkey had consistently better feed conversion ratio (FCR). Males also showed better FCR. In all varieties, the FCR for heritage varieties was higher than that for CC turkeys. Differences among varieties for plasma melatonin concentration were significant ($P \leq 0.05$). The SB and RP turkeys had the highest and the lowest plasma melatonin concentrations, respectively. The NA turkeys were characterized by the largest average semen

volume and highest sperm count while BR turkeys had the lowest. The WH turkeys had the highest sperm concentration while CC turkeys had the highest sperm viability. Age at first egg (AFE) was also different among the varieties. The CC turkeys reached sexual maturity earlier than heritage turkeys which were generally characterized by late sexual maturity. The MW turkeys laid more eggs than other heritage birds. As expected, CC turkeys were superior to heritage birds in performance for all the traits evaluated. Within the heritage turkeys, the observed differences in performances could be used for selecting birds for future breeding programs.

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3.2 Introduction

The turkey, *Meleagris gallopavo*, which originated from North America, is raised throughout the temperate parts of the world (Aldrich, 1967). The modern domesticated turkey descends from one of the six subspecies of wild turkeys which were domesticated in Mexico between 200 B.C. and 700 A.D. In early 1500s, domesticated turkeys were taken to Europe from U.S. and developed into many distinct varieties. Later, in 1607, those developed varieties were re-introduced to Jamestown, Virginia (Hogan, 2008). The turkey belongs to the order *Galliformes* and the family *Meleagrididae.* The present domesticated turkey has been developed by crossbreeding and line breeding programs and are characterized as a single breed with 8 distinct varieties based on the plumage color (Kennamer et al., 1992). The *American Standard of Perfection* (American Poultry Association, 2001) has recognized 8 distinct varieties of *Meleagris gallopavo* as Black, Bronze, Narragansett, Slate, Beltsville Small White, Bourbon Red, Royal Palm and White Holland (WH). The American Livestock Breeds Conservancy also recognizes other naturally mating domesticated turkey varieties such as Midget White and Jersey Buff that have not been accepted into the American Poultry Science standards including the American Poultry Association.

These varieties have been called heritage turkeys because of historic, range-based production system in which the birds are normally reared. To qualify as a heritage turkey, the certain criteria including ability of natural mating, long productive lifespan and slow growth rate must be met. The Broad Breasted Bronze (BBB) variety was developed by selecting for large size and greater breast width from 1920 to 1950. The BBB carcasses were unattractive due to dark pin feathers. This led to develop Broad Breasted White turkeys in the 1960s (American

Livestock Breeds Conservancy, 2008). The most widely raised turkey at present is known as the commercial or hybrid turkeys, which were developed mainly for meat from WH and Bronze varieties using different mating and selection procedures (Austic and Nesheim, 1990). Commercial turkeys have a relatively higher rate of disease susceptibility, mainly because they have been highly selected for increased body weight and growth rate (Huff et al., 2005). High disease susceptibility may be due to narrow genetic background (Kamara et al., 2007).

The turkey is important as wild bird and as the second most important poultry meat species in the USA (Kamara et al., 2007). The number of turkey birds raised during 2011 was estimated to be 248, a 2% increase from 2010 (USDA, 2011). The United States of America is the leader in turkey consumption followed by Canada (http://www.uspoultry.org/ economic_ data/). The USA turkey consumption has increased by 102% since 1970 due to its taste and nutritional value. The U.S. per capita consumption of turkey was 16.4 pounds in 2010, which was double that in 1970 (National Turkey Federation – Statistics, 2010). Six states in the US accounted for nearly two thirds of the turkey production in 2011. Minnesota produced the most followed by North Carolina, Arkansas, Missouri, Virginia and Indiana (USDA, 2011). In 2010, U.S. exported about 583 million pounds of turkey or about 10% of the total produced (National Turkey Federation – Statistics, 2010).

The plumage coloration of the turkey has been used as the primary criteria for identifying different turkey varieties (Aldrich, 1967). Though turkey varieties are considered a single breed, research evidence shows that significant differences exist among the populations and the heritage turkey and commercial birds (Hartman et al., 2006). Several attempts have been made to examine the differences among turkey varieties at the phenotypic, molecular, and biochemical

levels. Gyenai (2005) in his unpublished thesis work reported that the turkey varieties differed in the incidence and severity of susceptibility effects of toxic levels of furazolidone, which induces dilated cardiomyopathy. In addition, significant differences among heritage varieties have also been observed for plasma uric acid, a biomarker of oxidative stress (Hartman et al., 2006).

Differences among turkey varieties at the molecular level have also been investigated. Smith et al. (2005) distinguished the relatedness of five heritage turkey varieties using three molecular marker systems including randomly amplified polymorphic DNA (RAPD), microsatellite and SNPs. They reported that the Royal Palm is distinct from other varieties investigated. Kamara et al. (2007) compared the genetic relatedness of five turkey varieties using microsatellite markers. They also reported that Spanish Black was closely related to Bourbon Red which is similar to the findings observed by Smith et al. (2005). The study did not support the Smith et al. (2005) study that showed a closer relationship between Royal Palm and Narragansett.

In birds, several studies have shown that there is a relationship between plasma melatonin and seasonal processes including sexual development, egg production, gonadal activity, endocrine activity of the adrenal gland, and thermogenesis. The melatonin rhythm is mainly influenced by the photoperiod. These physiological processes are also dependent on photoperiod changes. Further, it is assumed that regulatory mechanisms are involved for adjusting the physiology and behavior for annual changes. Therefore, melatonin may play an important role by providing photoperiodic signals to influence physiological processes in birds (Zawilska et al., 2007).

Further improvement of domesticated turkeys to meet the human demand is dependent on within and between variations among turkeys. Such variations among individuals or varieties of turkeys provide ample opportunities to select the best animals for breeding purposes. However, limited work pertaining to phenotypic differences among turkeys has been carried out. Thus, the primary objective of the current study was to compare the growth, reproductive performance and plasma melatonin concentration among commercial (CC) and seven heritage varieties of turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW).

3.3 Materials and methods

Turkey populations

A total of 40 - 50 one-day-old poults from each of the heritage varieties and a commercial strain (CC) were obtained from two commercial breeders (Welp's Hatchery, Bancroft, IA and AG Forte LLC, Harrisonburg, VA). The heritage birds comprised of seven different varieties including Narragansett (NA), Royal Palm (RP), Blue Slate (BS), Spanish Black (SB), Midget White (MW), White Holland (WH) and Bourbon Red (BR).

Management and phenotypes

Standards animal care procedures were carried out as described in the protocol approved by the Virginia Tech Institutional Animal Care and Use Committee (11-028-APSC). On receipt, the poults were weighed and wind-banded. The birds were raised at the Turkey Farm of the Department of Animal and Poultry Sciences, Virginia Tech according to standard management protocol in floor pens for a period of 309 days. They were fed with a commercial diet (Big Spring Mill, Inc., Elliston, VA), which met the recommendations of National Research Council (Nutrition and Council, 1994). Birds were randomly allocated to pens according to variety and sex. The normal commercial diets fed were Pre-starter (T-1) from 1-3 wks of the age, Starter (T-2) from 3-6 wks of the age, Grower (T-3) from 6-9 wks of the age, Finisher (T-4) from 9-18 wks of the age, Pre-breeder (TPB-1) from 18-24 wks of the age and Breeder (TB-2) from 24 wks of the age. Feed and water were provided *ad libitum*. Body weight (BW) was measured in kilograms at 1 (1 d), 34 (34 d), 68 (68 d), 159 (159 d), 23 (231 d) and 309 (309 d) days of age. Feed intake was recorded for each strain starting from 34 to 231 d. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated using standard arithmetic.

Semen collection and evaluation

Semen was collected at three different times from each tom at weekly intervals starting from 35 wks of age using the abdominal massage technique (Burrows and Quinn, 1937). The volume (V) of the semen was determined using a syringe. Sperm concentration (C) was estimated using a hemocytometer after dilution at a ratio of 1:500 (Bakst and Cecil, 1997). The total number of sperm/ejaculate (T) was calculated for each tom using the formula T = C x V.

Sperm viability was examined microscopically (400x) using eosin, nigrosin stains (Blom, 1950) and hancock stain and semen were gently mixed. The strain and semen smears were air dried and examined directly on a microscope. The proportions of live (eosin-impermeable) and dead (eosin–permeable) sperm cells in a sample were assessed on the basis of 100. The sperm cells that were white (unstained) were classified as live and those with pink or red coloration were classified as dead.

Egg production parameters

Turkey hens were wing-tagged for easy identification during the period of laying. Trap nests were used from 20 wks of age. Age at first egg (AFE), number of eggs and egg weight were recorded for each hen. Number of egg parameters was based on egg laid for a period of 10 wks starting from 30 wks of age. Eggs were weighed daily on the same day they were laid. Total egg production for each hen was estimated for two periods including 6 and 10 wks. The individual egg production for 6 wks was calculated excluding the first and last two weeks of 10 wks of egg production**.** The average egg weight (g) was also calculated for 6 and 10 wks separately for each hen. The egg weight was obtained using an electronic weighing balance with a sensitivity of 0.01g.

Blood and plasma collection, and biochemical assays

Blood was collected twice from each bird at 10 and 32 wks of age by brachial venipuncture. The whole blood was collected into tubes containing EDTA as anticoagulant. Plasma was obtained by centrifuging the blood samples at approximately 10,000 x g for 15 minutes at 4 ^{0}C. All the samples were stored at – 20 ^{0}C until ready for use. Plasma melatonin concentration was measured using an enzyme-linked immunosorbent assay (Cheung et al., 2003) following the manufacturer's guidelines (ELISA) (IBL, Hamburg, Germany). The assay has a sensitivity limit of 1.6 pg/mL. To standardize the measurements, a calibration curve was constructed using samples provided as standards.

Statistical analyses

Data were analyzed using the GLIMMIX procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The following statistical model was used for the analysis of the phenotypic traits described above:

$$Y = \mu + L + S + (L \times S) + e$$

where Y is BW, ADG, FCR, AFE, semen quality traits and egg production parameters for turkeys, μ is the overall varietal mean, L is the fixed effect of the turkey variety, S is the fixed effect of sex, $(L \times S)$ is the interaction between the sex and variety and e is the residual error. Multiple comparisons were analyzed using Tukey's test. The values were presented as least square means ± standard error. Results were considered significant at $P \leq 0.05$.

3.4 Results and discussion

Body weight (BW)

The BW means of the different varieties are presented in Table 3. 1. The sex x variety interaction on BW was significant ($P \leq 0.05$). The BW of males and females at 1, 34, 68, 159, 231, and 309 d of ages was significantly different ($P \leq 0.05$) among different varieties of turkeys. The mean BW of males and females at 1 d ranged from 0.042 ± 0.001 to 0.056 ± 0.001 kg and from 0.039 ± 0.001 to 0.055 ± 0.001 kg, respectively. As expected, CC turkeys had significantly higher average BW for males and females at all ages. The SB and MW males had the highest and lowest BW respectively while for females; both BR and MW turkeys had the lowest at 1 d. The mean BW of males and females at 34 d of ranged from 0.612 ± 0.03 to 1.350 ± 0.03 kg and from 0.522 ± 0.04 to 1.293 ± 0.02 kg respectively. NA turkeys for both males and females had the highest BW. Birds with the lowest BW in males and females were RP and BR turkeys, respectively. The mean BW of males and females at 68 d of age ranged from 1.727 ± 0.09 to 5.009 ± 0.10 kg and from 1.386 ± 0.10 to 4.094 ± 0.07 kg, respectively. The NA and BS toms had the highest and lowest BW among heritage varieties. For females, highest and lowest BW was reported for NA and RP turkeys respectively. The mean BW of males and females at 159 d of age ranged from 5.192 ± 0.29 to 22.017 ± 0.28 kg and from 3.461 ± 0.18 to 12.546 ± 0.19 kg respectively. NA and WH turkeys had the highest BW for both males and females respectively while RP turkeys had the lowest. The mean BW of males and females at 231 d of age ranged from 8.149 ± 0.34 to 26.849 ± 0.32 kg and from 4.321 ± 0.23 to 14.086 ± 0.24 kg respectively. NA turkeys had the highest BW for both males and females while RP had the lowest. The mean BW of males and females at 309 d of age ranged from 8.127 ± 0.36 to 26.979 ± 0.36 kg and from

4.507 ± 0.23 to 14.843 ± 0.19 kg respectively. NA turkeys had the highest BW for both males and females while RP had the lowest for both sexes.

In our study, the comparison of males and females CC turkeys with their counterparts of heritage varieties showed that CC turkeys had significantly higher BW than that of heritage turkeys at all the ages. The CC turkeys have been developed by crossing a sire line and a dam line. Sire lines are normally selected for the better growth related traits while dam lines are selected for both growth and reproductive traits (Huff et al., 2005; Kamara et al., 2007). The expectation is that the male turkey should grow to 40 lb in 140 d compared to 20 lb in 140 d of age 25 years ago (Heffernan, 2000). The changes of BW for heritage varieties have a similar pattern compared to CC turkeys. In general, RP had smaller BW compared to other heritage varieties. Heritage turkeys have been characterized as slow growth (American Livestock Breeds Conservancy, 2008). Several studies have shown changes of BW with age in different lines of turkeys. Ilori et al. (2010) compared the growth performances of pure and crossbred turkeys at different ages up to 140 d of age. The average BW of exotic turkeys on 1, 7, 28, 56, 84, 112 and 140 d of age were higher than that of the crossbred. The values reported by Ilori et al. (2010) were lower than their for CC turkeys in our study and those were closer to these of the heritage varieties. Havenstein et al. (2007) compared the change of BW of 1966 vs 2003 type turkeys at different ages starting from one day old to 196 d of age. BWs of CC turkeys of the current study were similar to 2003 type turkeys. The body weights of heritage varieties were lower than these of 1966 vs 2003 type turkeys. Laudadio et al. (2009) reported the BW of Nicholas Large White female turkeys at different ages starting at 30 d up to 114 d of age. The BW reported was similar to CC turkeys of our study, but as expected higher than of the heritage turkeys.

In the present study, we measured BW up to 309 d of age, while in earlier investigations, BW was measured up to 140 d of age . The BWs of both male and female CC turkeys were approximately three times than that of heritage birds at 159, 231 and 309 d of age. At 34, 68, 159, 231 and 309 of ages, NA males and females were heaviest while RP birds were lightest among heritage birds. Differences in BW may be due to different varieties of turkeys used for the studies. The BW is dependent on the varieties of turkeys suggesting that genotype accounts for the BW differences.

Average daily gain (ADG)

The ADG for the different varieties of turkeys at different ages is presented in Table 3. 2. The ADG was estimated for the five different periods at which BW measurements were made between day one and 309 d of age. The sex x variety interaction for ADG was also significant ($P \leq 0.05$) at the different ages except from 232 - 309 d. Differences among varieties for ADG at different periods were significant ($P \leq 0.05$) except between 232 and 309 d of age. The mean ADG of males and females between 1 and 34 d of age ranged from 0.017 ± 0.001 to 0.039 ± 0.001 kg and from 0.014 ± 0.001 to 0.37 ± 0.001 kg, respectively. As expected, CC turkeys had significantly higher ADG for males and females. In heritage birds, NA turkeys had the highest ADG for males and females. The lowest ADG for males was reported in RP and BS turkeys while BR had the lowest for females. The mean ADG of males and females between 35 d and 68 d of age ranged from 0.032 ± 0.002 to 0.108 ± 0.002 kg and from 0.024 ± 0.002 to 0.082 ± 0.001 kg respectively. NA and BS turkeys had the highest and lowest ADG for males. For females, NA and BS turkeys had the highest ADG. The mean ADG of males and females between 69 and 159 d of age ranged from 0.037 ± 0.003 to 0.187 ± 0.003 kg and from 0.022 ± 0.002 to 0.093 ± 0.003

kg respectively. NA and WH turkeys had the highest ADG for both males and females respectively while RP had the lowest for both sexes. The mean ADG of males and females between 160 and 231 d of age ranged from 0.035 ± 0.003 to 0.066 ± 0.003 kg and from 0.010 ± 0.002 to 0.021 ± 0.002 kg respectively. BR and NA turkeys had the highest ADG for males and females respectively while WH and BS turkeys had the lowest ADG for males and females. The mean ADG of males and females between 1 and 309 d of age ranged from 0.026 ± 0.001 to 0.087 ± 0.001 kg and from 0.014 ± 0.001 to 0.048 ± 0.001 kg respectively. NA males had the highest ADG while RP turkeys had the lowest for both sexes.

As expected, the mean ADG increased up to 159 d of age and then decreased thereafter in all the different varieties. The CC turkeys differed in the pattern of ADG and had significantly higher ADG when compared to heritage turkeys. The heritage turkeys showed similar pattern of ADG throughout the study. Both males and females reached a maximum ADG between the 69 and 159 d of age. As discussed earlier, CC turkeys have been highly selected for increased body weight and growth rate (Huff et al., 2005; Kamara et al., 2007). Case et al. (2012) reported that the ADG of breeder turkey sire was 0.230 kg from 105 to 133 d of age. The ADG of Nicholas Large White females was estimated as 0.077 kg between 31 and 114 d of age (Laudadio et al., 2009). Torres-Rodriguez et al. (2007) used CC turkey hens to estimate the ADG up to 92 days of age and reported that ADG of those hens was 0.075 kg. Brenoe and Kolstad (2000) compared the ADG between BUT-9 and Nicholas turkeys and reported that males showed a higher ADG compared with females. Nicholas showed the lower ADG compared with BUT-9. Both turkeys had a maximum ADG (0.130 kg for BUT-9 and 0.120 kg for Nicholas) at 56 to 77 d of age followed by a slight decrease thereafter. The ADG for CC turkeys studied here were higher than those from previously reported studies except that of Case et al. (2012) study, where they used

breeder turkey sires. The ADG for heritage varieties was lower than these described in published reports. This is a good indication of heritage turkeys characterized for slow growth. Overall, NA turkeys had higher ADG among heritage birds while RP turkeys had least.

Feed conversion ratio (FCR)

The average FCR for different varieties of turkeys at different ages is presented in Table 3. 3. The sex x variety interaction on FCR was significant ($P \leq 0.05$) at each age. Differences among varieties for FCR at different periods of age were also significant ($P \leq 0.05$). The mean FCR of males and females between 34 and 68 d of age ranged from 1.38 ± 0.28 to 3.21 ± 0.26 and 1.99 ± 0.20 to 3.91 ± 0.28 respectively. As expected, CC turkey had significantly lower FCR for both males and females at all ages except between 160 - 231 d. In heritage birds, the lowest FCR for both males and females were reported for RP and NA turkeys respectively. The mean FCR of males and females between 69 and 159 d of age ranged from 2.86 ± 0.21 to 5.45 ± 0.25 and 4.08 ± 0.17 to 6.15 ± 0.28 respectively. The lowest FCR for both males and females was reported for WH turkeys. The mean FCR of males and females between 160 and 231 d of age ranged from 7.29 ± 1.25 to 12.88 ± 1.19 and from 12.88 ± 1.02 to 18.73 ± 1.20 respectively. Among heritage birds, BR and NA turkeys had significantly lower FCR for both males and females respectively. The mean FCR of males and females between 34 and 231 d of age ranged from 5.19 ± 0.38 to 6.72 ± 0.33 and 6.97 ± 0.28 to 10.62 ± 0.38 respectively. The CC turkey had significantly lower FCR for males and females. In heritage birds, BS and WH turkeys had lowest FCR for males and females respectively.

Feed efficiency is often assessed as either FCR or residual feed intake (RFI). In our study, we used FCR which indicates each variety's ability to convert feed to body weight as a

measure of feed efficiency. In general, males showed the better FCR compared to females. The FCR for males and females of all turkey varieties increased gradually with age. The FCR for heritage varieties was higher than these in the CC turkeys except during the period between 160 and 231 d. WH and BS turkeys had the better FCR among the heritage varieties for the period of age between 34 and 231 d. According to Case et al. (2012), the FCR estimated for the breeder turkey sires was 2.96 between 105 and 133 d of age. In another study, FCR estimated for Nicholas Large White females was 2.98 for the period between 31 d and 114 d of age and also reported that the FCR gradually increased with the age (Laudadio et al., 2009). According to Havenstein et al. (2007), the FCR increased with age, which is consistent with present work. Heritage turkeys are characterized as having slow growth. It is therefore not surprising that FCR of heritage turkeys is higher than that for CC birds. However, WH and BS turkeys among the heritage turkeys have shown fairly a better FCR. The direct measures of FCR can be used to study the performance traits that affect the overall efficiency. Therefore, FCR information can be incorporated in breeding programs as one of the efficiency related parameters. In general, low FCR reduces the cost of production for feeds and improves the profit margin of the turkey industry.

Plasma melatonin concentration

The plasma melatonin concentration for the different varieties of turkeys is presented in Table 3. 4. The sex x variety interaction and varietal differences in melatonin concentration was significant ($P \leq 0.05$). The mean plasma melatonin concentration for the varieties ranged from 12.23 ± 6.59 to 48.49 ± 6.27 pg/ml. SB and RP turkeys had the highest and lowest plasma melatonin concentration, respectively. For males and females, it ranged from 15.57 ± 10.59 to

59.14 ± 8.83 pg/ml and 8.88 ± 7.13 to 48.14 ± 8.16 pg/ml respectively. For males, SB turkey had significantly higher melatonin concentration ($P \leq 0.05$) while RP turkey had the lowest. For females, WH had significantly higher melatonin concentration and RP had the lowest.

Light is the dominant environmental factor that controls melatonin biosynthesis in avian species. Light suppresses the melatonin synthesis at day time while dark induces the melatonin synthesis at night. Zawilska et al. (2006) showed that plasma melatonin concentrations exhibited a diurnal rhythm in CC turkeys. The melatonin levels were low during the light phase and increased in the first half of the dark phase. They further reported that the mean day-time plasma melatonin concentration was 35 ± 3 pg/ml. The effect of photoperiod on plasma melatonin concentrations in CC turkeys was assessed (Zawilska et al., 2007). Turkey birds were subjected to three different photoperiods including short, long and regular periods and observed the change of plasma melatonin concentrations. During short photo period, the amplitude of the plasma melatonin rhythm was significantly lower than the long and regular photoperiods. Based on the results, the plasma melatonin concentrations exhibited a diurnal rhythm in turkey. The photo-period induced changes the plasma melatonin levels in the turkey. In the present study, we individually measured day-time plasma melatonin levels of turkeys at 32 wks of age considering that they reached to mature body weight. Among the turkey varieties, the SB and RP turkeys had the highest and the lowest plasma melatonin concentrations. The plasma melatonin concentrations of other turkey varieties are consistent with the previously reported values (Zawilska et al., 2006). The differences in plasma melatonin concentration may be due to variety specific rhythm. As discussed earlier in the introduction, photoperiod changes melatonin signal which may play a role in controlling the reproduction of the turkey (Zawilska et al., 2007).

Semen quality characteristics

The comparison of the mean values of ejaculate volume, sperm concentration, total number of sperm per ejaculate and sperm viability among the different varieties of turkeys is reported in Table 3. 5. The ejaculate volume, total number of sperm per ejaculate and sperm viability were significantly different ($P \leq 0.05$) among varieties. The mean ejaculate volume for ranged from 0.049 ± 0.02 to 0.179 ± 0.01 mL across different varieties of turkeys. The largest ejaculate volume was collected from NA birds while BR birds produced smallest average volume. The mean sperm concentration of varieties of turkeys was not significant ($P \geq 0.05$). The mean sperm concentration ranged from 1.754 ± 0.24 and 2.598 ± 0.19 ($x10^9$/mL). The WH turkey had the highest sperm concentration while BR birds had the lowest. The mean total number of sperm per ejaculate ranged from 0.970 ± 0.55 to 4.124 ± 0.43 ($x10^8$/ejaculate). NA turkeys had significantly higher number of sperm per ejaculate while BR had the lowest. The mean sperm viability ranged from 79.689 ± 0.96 to 86.315 ± 1.27% for the different varieties. CC turkeys had significantly higher sperm viability while BS turkeys had the lowest.

In the present study, NA turkeys were characterized by the largest average semen volume and highest total number of sperm. BR turkeys had the smallest volume of semen, lowest sperm concentration and lowest total number of sperm. WH turkeys had the highest sperm concentration. When compared the sperm viability, CC turkeys were characterized by the highest sperm viability while BS turkeys had the lowest. In general, these results indicated that heritage turkey had better semen quality parameters compared to CC turkeys. The differences in semen quality parameters in relation to turkey varieties indicated in the present study were also reported by Kotlowska et al. (2005). The data that have been published recently provide the following

information for average semen volume, average sperm concentration and total sperm count per ejaculate. Kotlowska et al. (2005) reported the sperm characteristics of three strains of turkey including Big-6 (0.53 mL, 6.90 x 10^9/mL, 3.64 x10^9), Hybrid Large White (0.44 mL, 6.3 x10^9/mL, 2.83 x10^9) and Nicholas (0.36 mL, 7.02 x10^9/mL, 2.59 x10^9). In addition, several studies reported the semen volume and concentration of Big-6 strain: 0.37 mL and 5.94 x 10^9/mL (Kozłowski et al., 2004), 0.35 mL and 6.54 x 10^9/mL (Jankowski et al., 2002). The semen volume and sperm concentration depend on the strains, age and frequency of semen collection (Bakst and Cecil, 1992; Noirault and Brillard, 1999; Kotlowska et al., 2005; Słowińska et al., 2011). The ejaculate volume, sperm concentration and total number of sperm of the present study were lower than that of previously published data (Noirault and Brillard, 1999; Kotlowska et al., 2005; Słowińska et al., 2011). This may be due to strain differences used for different studies. By analyzing present data, it was obvious that semen volume was affected by the variety of turkeys which is consistent with previously published data (Kotlowska et al., 2005). However, we did not see any significant difference for sperm concentration among the turkey varieties in the present study. Semen viability was also consistent with data published by Noirault and Brillard (1999).

Egg production performance

The comparison of the mean AFE, egg production for 6 and 10 wks, average egg weight for 6 and 10 wks among the different varieties of turkeys is presented in Table 3.6. The AFE, egg production for 6 and 10 wks, average egg weight for 6 and 10 wks was significant ($P \leq 0.05$) among varieties. The mean AFE ranged from 184.62 ± 8.59 to 258.10 ± 7.91 d across the different varieties. CC turkeys had significantly lower AFE. In heritage birds, MW had the

lowest AFE (228.20 ± 9.93 d). The mean egg production for the periods of 6 and 10 wks among varieties was significant ($P \leq 0.05$). The mean egg production for the periods of 6 and 10 wks ranged from 1.96 ± 3.79 to 24.36 ± 3.28 and from 6.85 ± 5.49 to 33.95 ± 4.76, respectively. In the both periods, BR turkeys had significantly lower egg production ($P \leq 0.05$) while MW turkeys had significantly higher egg production. The mean average egg weight for the periods of 6 and 10 wks among varieties was significant ($P \leq 0.05$). The mean average egg weight for the periods of 6 and 10 wks ranged from 73.39 ± 0.96 to 90.73 ± 0.84 g and from 74.21 ± 0.92 to 91.31 ± 0.80 g respectively where CC turkeys had significantly higher average egg weight ($P \leq 0.05$) while MW turkeys had the lowest for both periods.

One of the major problems faced by turkey industry today is low production of eggs. Therefore, increased selection pressure for egg numbers, modifications in management and lighting programs have been using as a solution to improve the low egg production. Photo-stimulation is generally used for CC turkey industry to increase the egg production. In the lighting program, turkey hens are exposed to a low lighting schedule (8 h light: 16 h dark) at the beginning and then photo-stimulated (>14 h light) between 29 and 31 wks of age. However, photo-stimulation at young ages will have negative effects of delayed sexual maturity, low egg production, low fertility and low hatchability. The non-commercial turkey strains achieve optimal reproductive body weight at approximately 30 wks of age (Applegate and Lilburn, 1998). But, if photo-stimulation is carried out at correct stage, present day commercial turkey can reach their reproductive body weight well before 30 wks of age. Turkey hens reached sexual maturity and laid eggs as early as 21 to 22 wks of age with the proper photo-stimulation for egg production (Siopes, 2010). According to present study, mean AFE for CC turkeys was reported as 184.62 d which was higher than that of the published reported. The mean AFE of heritage

varieties ranged between 228.20 - 258.10 d which was also higher than that of published data. It appeared that AFE depends on the variety of turkeys. Heritage turkey hens are generally characterized by late sexual maturity. Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The cumulative egg production for each hen was estimated for a period of 6 wks and 10 wks. The cumulative egg production for the period of 6 wks for a hen was calculated excluding first and last two weeks egg production from the period of 10 wks of egg production. Egg production gradually increased over the 10 wks production period. When compared the egg production among different varieties of turkeys, MW turkeys showed the much better egg laying performances compared to BR turkeys. There is a negative correlation between egg production and body weight which is mediated mainly as a decrease in egg production (Nestor et al., 2000). CC turkeys have been selected for increased growth and body weight. We did not observe high egg production from CC hens though they reached sexual maturity earlier. The average egg weight increased from 6 to 10 wks except RP and SB turkeys. CC turkeys had significantly higher average egg weight compared to heritage turkeys. The average egg weight among heritage varieties did not significantly differ ($P \geq 0.05$) between the production periods of 6 wks and 10 wks. Nestor et al. (2000) compared the egg production performance of two turkey lines which included the random bred control line (RBC) and subline of RBC (F) line selected for increased body weight. The mature body weight of the RBC was 21 lb compared to 38 lb for the F line. The 180 day egg production for the RBC line was 92.6 eggs per hen compared to 68.7 eggs per hen for F line. Egg weight increased from 89 to 98 g for the RBC vs the F line. This suggests that when turkeys are selected for increased growth and body weight, the body weight is negatively associated with egg production but positively associated with egg weight.

In summary, the phenotypic variation of growth, reproductive performances and plasma melatonin concentrations between commercial (CC) and seven heritage varieties of turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) were compared. Since CC turkeys have been highly selected for increased body weight and growth rate, the highest growth parameters were reported for CC turkeys. As expected, CC turkeys were superior to heritage birds in performance for the traits evaluated. NA males and females were heaviest while RP birds were lightest among heritage birds. When compared the male and female reproductive performances of turkeys, heritage turkeys showed better in performances compared to CC turkeys in general though heritage turkey varieties are characterized for slow growth as defined. The MW turkeys laid more eggs than other heritage birds. In conclusion, the phenotypic variation among different varieties of turkeys used in the present study account for observed differences in the growth, reproductive parameters and plasma melatonin concentration suggesting that these differences could be used for selecting birds for future breeding programs.

Table 3. 1. The changes of body weight (BW) in heritage and commercial turkeys from day old to 309 days of age.

BW (kg)[1]	Turkey varieties[2]							
	BR	BS	CC	MW	NA	RP	SB	WH
1 d								
Male	0.043 ± 0.001^{b,c}	0.047 ± 0.001^{b}	0.056 ± 0.001^{a}	0.042 ± 0.001^{c}	0.044± 0.001^{b,c}	0.046 ± 0.001^{b,c}	0.048 ± 0.001^{b}	0.044 ± 0.001^{b,c}
Female	0.039 ± 0.001^{d}	0.047 ± 0.001^{b,c}	0.055 ± 0.001^{a}	0.039 ± 0.001^{d}	0.047± 0.001^{b}	0.044 ± 0.001^{b,c}	0.045 ±0.001^{b,c}	0.043 ± 0.011^{c,d}
34 d								
Male	0.703 ± 0.04^{b}	0.619 ± 0.03^{b}	1.350 ± 0.03^{a}	0.663 ± 0.03^{b}	0.725 ± 0.03^{b}	0.612 ± 0.03^{b}	0.707 ± 0.03^{b}	0.663 ± 0.03^{b}
Female	0.522 ± 0.04^{b}	0.575 ± 0.03^{b}	1.293 ± 0.02^{a}	0.554 ± 0.03^{b}	0.602 ± 0.03^{b}	0.538 ± 0.02^{b}	0.593 ± 0.03^{b}	0.583 ± 0.03^{b}
68 d								
Male	1.984 ± 0.10^{b}	1.727 ± 0.09^{b}	5.009 ± 0.10^{a}	1.744 ± 0.10^{b}	2.092 ± 0.09^{b}	1.783 ± 0.11^{b}	1.896 ± 0.09^{b}	1.910 ± 0.09^{b}
Female	1.539 ± 0.12^{b}	1.567 ± 0.08^{b}	4.094 ± 0.07^{a}	1.386 ± 0.10^{b}	1.608 ± 0.08^{b}	1.422 ± 0.07^{b}	1.526 ± 0.08^{b}	1.489 ± 0.08^{b}
159 d								
Male	6.447 ± 0.29^{b,c}	6.153 ± 0.25^{b,c}	22.017 ± 0.28^{a}	5.776 ± 0.27^{c}	7.294 ± 0.25^{b}	5.192 ± 0.29^{c}	6.112 ± 0.24^{b,c}	6.602 ± 0.25^{b,c}
Female	3.987 ± 0.32^{b,c}	3.895 ± 0.22^{b,c}	12.546 ± 0.19^{a}	3.794 ± 0.28^{b,c}	4.111 ± 0.24^{b}	3.461 ± 0.18^{c}	3.797 ± 0.22^{b,c}	4.197 ± 0.22^{b}
231 d								
Male	10.124±0.34^{b}	9.496±0.30^{b,c}	26.849±0.32^{a}	8.272±0.32^{c}	10.150±0.29^{b}	8.149±0.34^{c}	9.140±0.28^{b,c}	9.216±0.30^{b,c}

Female	4.865±0.37^{b,c}	4.658±0.26^{b,c}	14.086±0.24^{a}	4.523±0.33^{b,c}	5.379±0.27^{b}	4.321±0.23^{c}	5.047±0.26^{b}	5.039±0.26^{b,c}
309 d								
Male	10.357±0.33^{b}	9.765±0.29^{b,c}	26.979±0.36^{a}	8.853±0.32^{c,d}	10.743±0.28^{b}	8.127±0.36^{d}	9.781±0.28^{b,c}	9.567±0.29^{b,c,d}
Female	5.202±0.37^{b}	4.739±0.25^{b}	14.843±0.19^{a}	4.733±0.32^{b,c}	5.381±0.27^{b}	4.507±0.23^{c}	5.281±0.25^{b}	5.354±0.26^{b}

^{a,b,c,d}Means within rows with different superscripts are significantly different ($P \leq 0.05$).

[1]BW (kg) of male and female birds was measured at 1, 34, 68, 159, 231 and 309 days of age. Values are expressed as LSmeans of BW ± SE.

[2]Seven different varieties of heritage turkeys including Bourbon Red (BR: M=13, F=9), Blue Slate (BS: M=15, F=21), Narragansett (NA: M=16, F=17), Royal Palm (RP: M=11, F=25), Spanish Black (SB: M=16, F=20), White Holland (WH: M=15, F=21) and Midget White (MW: M=15, F=15) and Commercial strain (CC: M=11, F=30) turkeys were used for the present study. The number of males (M) and females (F) from each variety is given within the parenthesis.

Table 3. 2. The changes of average daily gain (ADG) for heritage and commercial turkeys by different periods of age.

ADG (kg)[1]	Turkey varieties[2]							
	BR	BS	CC	MW	NA	RP	SB	WH
$ADG_{1 - 34 d}$								
Male	0.020 ± 0.001^{b}	0.017 ± 0.001^{b}	0.039 ±0.001^{a}	0.019 ± 0.001^{b}	0.021 ± 0.001^{b}	0.017 ± 0.001^{b}	0.020 ± 0.001^{b}	0.018 ± 0.001^{b}
Female	0.014 ± 0.001^{b}	0.016 ± 0.001^{b}	0.037 ±0.001^{a}	0.016 ± 0.001^{b}	0.017 ± 0.001^{b}	0.015 ± 0.001^{b}	0.016 ± 0.001^{b}	0.016 ± 0.001^{b}
ADG_{35-68d}								
Male	0.038 ± 0.002^{b}	0.033 ± 0.002^{b}	0.108 ±0.002^{a}	0.032 ± 0.002^{b}	0.040 ± 0.002^{b}	0.034 ± 0.002^{b}	0.035 ± 0.002^{b}	0.037 ± 0.002^{b}
Female	0.030 ± 0.003^{b}	0.029 ± 0.001^{b}	0.082 ±0.001^{a}	0.024 ± 0.002^{b}	0.029 ± 0.002^{b}	0.026 ± 0.002^{b}	0.027 ± 0.002^{b}	0.027 ± 0.001^{b}
$ADG_{69-159 d}$								
Male	0.049 ±0.003^{b,c}	0.049 ±0.002^{b,c}	0.187 ±0.003^{a}	0.044 ±0.003^{b,c}	0.057 ± 0.003^{b}	0.037 ± 0.003^{c}	0.046 ±0.003^{b,c}	0.051 ±0.002^{b,c}
Female	0.027 ±0.003^{b,c}	0.025 ±0.002^{b,c}	0.093 ±0.003^{a}	0.025 ±0.003^{b,c}	0.027 ±0.003^{b,c}	0.022 ± 0.002^{c}	0.025 ±0.002^{b,c}	0.029 ± 0.002^{b}
$ADG_{160 – 231d}$								
Male	0.051 ±0.003^{a,b}	0.046 ± 0.003^{b}	0.066 ±0.003^{a}	0.035 ± 0.003^{b}	0.039 ± 0.003^{b}	0.041± 0.003^{b}	0.042 ± 0.003^{b}	0.036 ± 0.003^{b}
Female	0.012 ±0.004^{a,b}	0.010 ± 0.003^{b}	0.021 ±0.002^{a}	0.010 ±0.002^{a,b}	0.018 ±0.003^{a,b}	0.012 ±0.002^{a,b}	0.017 ±0.003^{a,b}	0.012 ± 0.003^{b}
$ADG_{232 – 309 d}$								
Male	0.003 ± 0.003^{a}	0.003 ± 0.003^{a}	-0.001±0.004^{a}	0.007 ± 0.004^{a}	0.008 ± 0.003^{a}	-0.001± 0.003^{a}	0.008 ± 0.003^{a}	0.004 ± 0.003^{a}

Female	0.004 ±0.004^{a,b}	0.001 ± 0.002^{b}	0.010 ±0.002^{a}	0.002 ±0.003^{a,b}	0.001 ±0.003^{b}	0.002 ± 0.003^{b}	0.003 ±0.002^{a,b}	0.004 ± 0.003^{b}
$ADG_{1-309\ d}$								
Male	0.034 ± 0.001^{b}	0.031 ±0.001^{b,c}	0.087± 0.001^{a}	0.029 ±0.001^{c,d}	0.035 ± 0.001^{b}	0.026 ± 0.001^{d}	0.032 ±0.001^{b,c}	0.031±0.001^{c,b,d}
Female	0.017 ±0.001^{b,c}	0.015 ±0.001^{b,c}	0.048 ±0.001^{a}	0.015 ±0.001^{b,c}	0.017 ± 0.001^{b}	0.014 ± 0.001^{c}	0.017 ± 0.001^{b}	0.017 ± 0.001^{b,c}

^{a,b,c,d}Means within rows with different superscripts are significantly different ($P \leq 0.05$).

[1]ADG (kg) of male and female birds was estimated at different periods of the age. $ADG_{1-34\ d}$ – ADG between 1 d and 34 d, ADG_{35-68d} – ADG between 35 d and 68 d, $ADG_{69-159\ d}$ – ADG between 69 d and 159 d, $ADG_{160-231\ d}$ – ADG between 160 d and 231 d, $ADG_{232-309\ d}$ – ADG between 232 d and 309d and $ADG_{1-309\ d}$ – ADG between 1 d and 309d. Values are expressed as LSmeans of ADG ± SE.

[2]Seven different varieties of heritage turkeys including Bourbon Red (BR: M=13, F=9), Blue Slate (BS: M=15, F=21), Narragansett (NA: M=16, F=17), Royal Palm (RP: M=11, F=25), Spanish Black (SB: M=16, F=20), White Holland (WH: M=15, F=21) and Midget White (MW: M=15, F=15) and Commercial strain (CC: M=11, F=30) turkeys were used for the present study. The number of males (M) and females (F) from each variety is given within the parenthesis.

Table 3. 3. The changes of feed conversion ratio (FCR) for heritage and commercial turkeys by different periods of age.

FCR[1]	Turkey varieties[2]							
	BR	BS	CC	MW	NA	RP	SB	WH
$FCR_{34\text{-}68\ d}$								
Male	2.37 ± 0.30^{b}	3.21 ± 0.26^{b}	1.38 ± 0.28^{a}	3.15 ± 0.28^{b}	2.97 ± 0.25^{b}	2.27 ± 0.30^{b}	2.44 ± 0.25^{b}	2.45 ± 0.26^{b}
Female	3.11 ± 0.33^{a}	3.25 ± 0.22^{a}	1.99 ± 0.20^{c}	3.91 ± 0.28^{a}	2.90 ± 0.24^{b}	2.93 ± 0.19^{b}	3.27 ± 0.22^{a}	3.34 ± 0.22^{a,b}
$FCR_{69\text{-}159\ d}$								
Male	5.02 ± 0.26^{a,b}	3.96 ± 0.23^{b,c}	2.86 ± 0.21^{d}	3.68 ± 0.24^{b,c}	3.94 ± 0.22^{b,c}	5.45 ± 0.25^{a}	4.70 ± 0.21^{a,b}	3.38 ± 0.22^{c,d}
Female	6.15 ± 0.28^{a}	4.93 ± 0.19^{b}	4.08 ± 0.17^{c}	4.93 ± 0.24^{a,b}	5.36 ± 0.20^{a,b}	5.08 ± 0.16^{a,b}	4.79 ± 0.19^{b}	4.44 ± 0.19^{b}
$FCR_{160 - 231\ d}$								
Male	7.29 ± 1.25^{c}	8.03 ± 1.11^{b,c}	12.88 ± 1.19^{a}	8.91 ± 1.17^{b,c}	10.14±1.07^{a,b}	7.67 ± 1.25^{b,c}	9.19 ± 1.04^{b,c}	9.84±1.09^{a,b,c}
Female	14.08 ± 1.39^{a,b}	15.86 ± 0.94^{a,b}	15.37 ± 0.86^{a,b}	18.73 ± 1.20^{a}	12.88 ± 1.02^{b}	15.12 ± 0.86^{a,b}	12.88 ± 0.95^{b}	14.32 ± 0.95^{a,b}
$FCR_{34 - 231\ d}$								
Male	6.12 ± 0.40^{a,b}	5.89 ± 0.35^{a,b}	5.19 ± 0.38^{b}	6.13 ± 0.37^{a}	6.10 ± 0.34^{a}	6.67 ± 0.40^{a}	6.72 ± 0.33^{a}	5.92 ± 0.35^{a,b}
Female	9.54 ± 0.44^{a,b}	8.71 ± 0.30^{b}	6.97 ± 0.28^{c}	10.62 ± 0.38^{a}	8.13 ± 0.32^{b,c}	9.27 ± 0.27^{a,b}	8.25 ± 0.30^{b}	7.76 ± 0.30^{b,c}

^{a,b,c,d}Means within rows with different superscripts are significantly different ($P \leq 0.05$).

[1]FCR of male and female birds was calculated at different periods of the age. $FCR_{34\text{-}68d}$ – FCR between 34 d and 68 d, $FCR_{69\text{-}159\ d}$ – FCR between 69 d and 159 d, $FCR_{160-231\ d}$ – FCR between 160 d and 231 d and $FCR_{34-231\ d}$ – FCR between 34 d and 231d. Values are expressed as LSmeans of FCR ± SE.

[2]Seven different varieties of heritage turkeys including Bourbon Red (BR: M=13, F=9), Blue Slate (BS: M=15, F=21), Narragansett (NA: M=16, F=17), Royal Palm (RP: M=11, F=25), Spanish Black (SB: M=16, F=20), White Holland (WH: M=15, F=21) and Midget White (MW: M=15, F=15) and Commercial strain (CC: M=11, F=30) turkeys were used for the present study. The number of males (M) and females (F) from each variety is given within the parenthesis.

Table 3. 4. The plasma melatonin concentration in heritage and commercial turkeys at 32 wks of age.

Turkey varieties[1]	Plasma melatonin[2] (pg/ml)		
	All	Male	Female
BR (N=22)	42.13 ± 8.82[a]	41.37 ± 11.20[a]	42.89 ± 11.76[a]
BS (N=36)	32.54 ± 6.50[a]	31.73 ± 9.70[a]	33.34 ± 7.95[a]
CC (N=42)	38.90 ± 7.37[a]	39.39 ± 11.00[a]	38.42 ± 7.53[a]
MW (N=31)	31.50 ± 7.95[a]	27.03 ± 9.94[a]	35.97 ± 10.17[a]
NA (N=33)	35.03 ± 6.52[a]	47.16 ± 8.86[a]	22.89 ± 8.84[a]
RP (N=37)	12.23 ± 6.59[b]	15.57 ± 10.59[b]	8.88 ± 7.13[b]
SB (N=37)	48.49 ± 6.27[a]	59.14 ± 8.83[a]	37.86 ± 8.27[a]
WH (N=36)	32.47 ± 6.40[a]	16.80 ± 9.21[b]	48.14 ± 8.16[a]

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

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

[2]Plasma melatonin concentration of male and female birds was measured at 32 wks of age using ELISA kit. Values are expressed as LSmeans of plasma melatonin ± SE.

Table 3. 5. The comparison of semen quality in heritage and commercial turkeys.

Turkey varieties[1]	Semen quality parameters[2]			
	Ejaculate volume (mL)	Sperm concentration ($x10^9$/mL)	Total number of sperm ($x10^8$/ejaculate)	Sperm Viability (%)
BR (N=13)	0.049 ± 0.02^{c}	1.754 ± 0.24^{a}	0.970 ± 0.55^{b}	$82.408 \pm 1.21^{a,b}$
BS (N=15)	$0.102 \pm 0.01^{b,c}$	2.488 ± 0.19^{a}	$2.514 \pm 0.44^{a,b}$	79.689 ± 0.96^{b}
CC (N=11)	$0.136 \pm 0.01^{a,b}$	2.212 ± 0.25^{a}	$3.200 \pm 0.58^{a,b}$	86.315 ± 1.27^{a}
MW (N=15)	$0.133 \pm 0.01^{a,b}$	2.532 ± 0.22^{a}	$3.285 \pm 0.52^{a,b}$	$83.724 \pm 1.13^{a,b}$
NA (N=16)	0.179 ± 0.01^{a}	2.231 ± 0.19^{a}	4.124 ± 0.43^{a}	$84.070 \pm 0.94^{a,b}$
RP (N=11)	$0.099 \pm 0.01^{b,c}$	2.151 ± 0.51^{a}	2.066 ± 0.49^{b}	$83.548 \pm 1.06^{a,b}$
SB (N=16)	$0.102 \pm 0.01^{b,c}$	2.028 ± 0.17^{a}	$2.165 \pm 0.44^{a,b}$	$83.085 \pm 0.96^{a,b}$
WH (N=15)	$0.096 \pm 0.01^{b,c}$	2.598 ± 0.19^{a}	$2.641 \pm 0.43^{a,b}$	84.521 ± 0.95^{a}

[a,b,c,d]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Seven different varieties of heritage turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) and Commercial (CC) turkeys were used for the present study. N = numbers of males from each variety used for semen collection.

[2]Semen volume, sperm concentration, total number of sperm per ejaculate and sperm viability as semen quality parameters were estimated. Values are expressed as LSmeans ± SE.

Table 3.6. The comparison of egg production traits in heritage and commercial turkeys.

Turkey varieties[1]	Egg production traits				
	AFE[2] (Days)	Cumulative egg production[3], eggs/hen		Average egg weight[4] (g)	
		6 wks	10 wks	6 wks	10 wks
BR (N=9)	246.35 ± 12.38[a]	1.96 ± 3.79[c]	6.85 ± 5.49[b]	74.30 ± 1.42[b,c]	75.21 ± 1.15[b,c]
BS (N=21)	238.20 ± 12.44[a]	7.19 ± 2.46[b,c]	10.28 ± 3.57[b]	74.29 ± 1.13[b,c]	74.72 ± 1.07[b,c]
CC (N=30)	184.62 ± 8.59[b]	12.99 ± 2.39[a,b,c]	20.64 ± 3.46[a,b]	90.73 ± 0.84[a]	91.31 ± 0.80[a]
MW (N=15)	228.20 ± 9.93[a]	24.36 ± 3.28[a]	33.95 ± 4.76[a]	73.39 ± 0.96[c]	74.21 ± 0.92[c]
NA (N=17)	232.37 ± 7.90[a]	17.53 ± 2.62[a,b]	25.74 ± 3.81[a,b]	77.65 ± 0.77[b]	77.75 ± 0.74[b]
RP (N=25)	249.00 ± 7.71[a]	9.23 ± 2.21[b,c]	14.64 ± 3.22[b]	77.45 ± 0.74[b]	78.12 ± 0.71[b]
SB (N=20)	233.45 ± 8.40[a]	10.31 ± 2.47[b,c]	15.20 ± 3.59[a,b]	75.42 ± 0.89[b,c]	75.16 ± 0.80[b,c]
WH (N=21)	258.10 ± 7.91[a]	6.19 ± 2.54[c]	12.36 ± 3.69[b]	77.37 ± 0.90[b]	77.63 ± 0.76[b]

[a,b,c]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Seven different varieties of heritage turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) and Commercial (CC) turkeys were used for the present study. N = numbers of females from each variety used for egg production.

[2]Age at first egg (AFE) was recorded for each hen in days. Values are expressed as LSmeans of AFE ± SE.

[3]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production. Values are expressed as LSmeans of egg number for 6 wks and 10 wks ± SE.

[4]Egg weight was individually measured and recorded. The average egg weight (g) was calculated for the period of 6 wks and 10 wks separately for each hen. Values are expressed as LSmeans of average egg weight for 6 wks and 10 wks ± SE.

CHAPTER 4

Polymorphisms of Circadian Clock Genes Associated with Growth, Reproductive Parameters and Plasma Melatonin in the Turkeys, *Meleagris gallopavo.*

4.1 Abstract

The biological clock controls a wide variety of activities including behavioral, physiological, and biochemical. Circadian genes are involved in regulating the circadian clock feedback loop. Here, we screened *turClock, turPeriod3, turCry1 and turCry2* genes for nucleotide variations and evaluated the relationships among its haplotypes (based on haplogroups) with growth, reproductive parameters and day time plasma melatonin levels. DNA sequences of 24.6 kb, 16.6 kb, 15.0 kb and 16.6 kb including *turClock, turPeriod-3*, *turCry1* and *turCry2* genes respectively, were screened by re-sequencing of the individual amplicon. A total of 41 SNPs were identified from the genes that screened. Five haplogroups developed from the 12 SNPs of *turClock* gene were significantly associated with body weight (BW) at 309 d of age, feed conversion ratio (FCR) for 34 - 68 d and 69 - 159 d, egg production and average egg weight ($P \leq 0.05$). Hap5 appeared to be most advantageous haplogroup for BW. Further, Hap1 appeared to be most efficient haplogroup for feed conversion, egg production and egg weight. *turPeriod-3* haplogroups were significantly associated with BW at 231 d of age, average daily gain (ADG) for 160 - 231 d of age, FCR for 69 - 159 d and 160 - 231 d, egg production traits, semen quality traits and plasma melatonin concentration ($P \leq 0.05$). Hap2 had significantly higher BW and ADG. Hap1 and Hap4 appeared to be most efficient haplogroup for feed conversion. Hap4, Hap5 and Hap6 appeared to be advantageous for semen quality, egg production and egg weight respectively. Seven haplogroups identified from *turCry1* gene were significantly associated with

ADG for 35 - 68 d of age, FCR for 160 - 231 d and 34 - 231 d, egg production and ejaculate volume ($P \leq 0.05$). Hap3 had significantly higher ADG and ejaculate volume ($P \leq 0.05$). Hap3 and Hap7 appeared to be most efficient haplogroup for feed conversion. Hap4 and Hap5 were advantageous for egg production. The haplogroups observed from *turCry2* were significantly associated with BW at 34, 68 and 231 days of age, ADG 160 - 231 d, FCR for34 - 68 d and average egg weight ($P \leq 0.05$). Hap1 and Hap4 appeared be advantageous haplogroups for BW and ADG. Hap3 had lower FCR and higher egg weight. These findings reveal that haplogroups of the clock genes are associated with some of the growth, reproductive parameters and plasma melatonin. Further, it showed that *turPeriod-3* and *turCry2* genes are good candidate genes for association studies in turkey. DNA sequence variations of the clock genes may have some regulatory role in the molecular mechanism of the circadian clock which may affect the overall mechanism of the circadian clock. In general, these data support our hypothesis that DNA sequence variations of clock genes at the nucleotide and haplotype levels are associated with the differences in performance traits.

4.2 Introduction

The biological clock controls a wide variety of activities including behavioral, physiological and biochemical circadian rhythms. The molecular components of the circadian clock have been identified in diverse animal species (Bailey et al., 2002). Transcriptional and translational feedback loops characterize the basic molecular mechanism of the circadian clock (Helfer et al., 2006). Several genes including *Clock, Bmal1, Period1* (*Per1*), *Period2 (Per2*), *Period3* (*Per3*), *Cryptochrome1* (*Cry1*) and *Cryptochrome2* (*Cry2*) are involved in the molecular mechanism of the circadian clock (Froy, 2007). The *Clock*, one of the core genes involved in generating endogenous rhythms, encodes the transcription factor CLOCK which dimerize with BMAL1 protein, to activate the transcription of target genes including *Per* and *Cry*. The CLOCK and BMAL1 produce rhythmic transcription activation that serves as the basic driving force for the circadian clock (Ko and Takahashi, 2006). The BMAL1 protein acts as a positive element along with CLOCK to activate the *Per* and *Cry* genes (Yu et al., 1999).

Clock was the first mammalian circadian gene identified using mutagenesis phenotypic screen in mice. A mutation of *Clock* in mice causes an abnormally long circadian period in behavior and become arrhythmic (Vitaterna et al., 1994). King et al. (1997) showed that an A to T transversion at the 5′ splice donor site of intron 19 of the *Clock* gene causes skipping, which reduces the transcriptional activity of the CLOCK protein. The mice homozygous for *Clock* gene mutation exhibit an altered feeding rhythm and thus develop metabolic syndrome and alteration in sleep homeostasis (Turek et al., 2005).

A SNP in a non-coding sequence flanking the *Clock* open reading frame was discovered in human by Steeves et al. (1999). The SNP T3111C is located at position 3111 of the *Clock*

mRNA 3′ untranslated region (3′ UTR) and does not alter the amino acid sequence of the CLOCK protein. The main role of this region is to regulate mRNA stability (Robilliard et al., 2002). The SNP in the 3′ UTR (T3111C) has been associated with diurnal preference in human where minor allele (C) was associated with the increased evening preference and delayed timing of the sleep wake cycle (Katzenberg et al., 1998; Mishima et al., 2005). Iwase et al. (2002) identified three novel SNPs in the coding region of human *Clock* gene. Two of them are located in exon 17 at nucleotide positions 1982 and 1955. The SNP at 1982 is an A to G change which alters coding sequence from a histidine to arginine at amino acid 542 while second SNP is a G to A change. The third variation, a C to T change which is located at 2121 in exon 18, is a silent mutation. Both SNPs that are located in exon 17 are missense SNPs which are equal to exon 19 in the mouse *Clock* gene. Two missense SNPs located in exon 17 and T3111C SNP did not associate with delayed sleep phase and N-24 sleep wake syndromes (Iwase et al., 2002). Pedrazzoli et al. (2007) reported a novel SNP located at position 257 (T to G) of the *Clock* mRNA 5' untranslated region (5' UTR). However, they did not detect any association between either polymorphism T3111C or T257G in the *Clock* gene with diurnal preference or delayed sleep phase and N-24 sleep wake syndromes. In addition, Leder et al. (2006) reported that *Clock* gene polymorphisms are associated with spawning time in the rainbow trout.

In birds, clock genes share 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 (Chong et al., 2000; Yoshimura et al., 2000; Yasuo et al., 2003; Helfer et al., 2006). Similar to other organisms, the transcriptional and translational feedback loop is involved in birds as the molecular mechanism of the circadian clock (Helfer et

al., 2006). Expression studies have shown that the *Clock* gene in quails is expressed throughout the day (Yoshimura et al., 2000). Some studies show that *Clock* gene does not exhibit pronounced rhythmicity in the chicken and quails (Chong et al., 2000; Yoshimura et al., 2001) while others show the rhythmic expression of *Clock* gene with peak values at different time points (Yoshimura et al., 2000; Yasuo et al., 2003). The *Clock* gene is expressed at higher levels during light in the pineal gland and eyes of quails and the brain of the house sparrow (Yoshimura et al., 2000; Helfer et al., 2006). It seems that expression of *Clock* gene has been inconsistent in the different species of avian suggesting that expression of *Clock* gene is species specific. In addition, two genes, *Bmal1* and *Bmal2* have been identified in the chicken under Bmal family and shown to be expressed in the chicken pineal gland with peak expression at day-night transition (Chong et al., 2000)

The *Clock* gene is highly conserved among vertebrates. However, the C terminal domain of the CLOCK protein contains a variable poly-glutamine (poly Q) repeats both among and within species (Saleem et al., 2001). Poly-Q repeat is a characteristic feature of many transcription factors. This region is believed to be important for the transcription activating potential of the protein. Therefore, it can affect both behavior and physiology related to circadian rhythms (Johnsen et al., 2007). Several studies reported an association between *Clock* genotype and breeding latitude in birds. Liedvogel et al. (2009) reported an association between *Clock* genotype and breeding phenology (clutch initiation data and incubation duration) within a wild blue tit population, but not in great tits (*Parus major*) (Liedvogel and Sheldon, 2010). The latitudinal variation in the *Clock* gene have been found in the Blue Tit (*Cyanistes caeruleus*), but did not detect any association for the Blue throat (*Luscinia svecica*) (Johnsen et al., 2007) or *Tachycineta* swallows (Dor et al., 2012). Dor et al. (2011) showed a very low variation in the

Clock poly-Q region among five breeding populations of Barn swallow (*Hirundo rustica*) suggesting that high diversity in clock poly-Q is not common across avian species. A recent study in the migratory Barn swallow disclosed that an association between breeding phenology (timing of breeding) and *Clock* genotype (Caprioli et al., 2012).

The *Period* (*Per*) gene is a central component of the molecular mechanism of the circadian clock and functions as the negative element in the transcriptional and translation feedback loop. In mammals, *Per* gene has three paralogues, *Per1*, *Per2* and *Per3* (von Schantz et al., 2006). In human, two SNPs have been reported in exon 18 of the *Per1* gene. Of the two SNPs, Katzenberg et al. (1999) reported the first SNP in exon 18 of the *Per1,* which is an A to G synonymous substitution at position 2548, which is not associated with diurnal preference in normal adults. But, the T2434C polymorphism, a synonymous substitution is associated with extreme diurnal preference (Carpen et al., 2006). The first mutation (A2106G) of the *Per2* has been reported by Toh et al. (2001) and linked with pathologically extreme morningness (DSPT). In addition, Carpen et al. (2005) reported three more polymorphisms in the *Per2* gene, one downstream of the transcription start site (C1228T), one in the 5′-untranslated region (C111G), and one missense mutation (G3853A) located in exon 23 and showed that *Per2* 111G allele associates with morning preference. In human *Per3* gene, Ebisawa et al. (2001) reported 20 sequence variations of which six variants changed amino acids. Of the total variants, two missense mutations (T1940G and C2590G) were significantly associated with DSPT. Johansson et al. (2003) also reported the T1940G mutation is associated with morning preference in a mixed European populations. Only two paralogs of *Per* gene have been identified in the quails and chicken, *Per2* and *Per3* (Fukada and Okano, 2002). Avian *Per2* and *Per3* are rhythmically expressed in the pineal gland, retina and hypothalamus in both light and dark conditions with

peak at early light phase (Helfer et al., 2006). It is believed that *Per2* gene in birds appears more similar to mammalian *Per1* than *Per2*.

The *Cry1* gene was first identified in *Arabidopsis*. Then, it was discovered in other plants and various animal species including insects, fish, amphibians, birds and mammals (Liedvogel and Mouritsen, 2010). In animals, Cryptochrome, one of the four groups of clock genes, that generate transcriptional and translation negative feedback loop, play a major role as a component of circadian clock. Under *Cry* family, basically there are two paralogues; *Cry1* and *Cry2*. In mice, it has been shown that mice lacking both *Cry* genes behave arrhythmically suggesting that proper functioning of *Cry* genes are crucial in generating of circadian rhythms (Bailey et al., 2002). In the transcriptional and translation model, *Crys* are activated via E-box enhancers by a positive element referred to as BMAL-CLOCK heterodimer and expression of *Crys* are suppressed by PERs and CRYs proteins (Yamamoto et al., 2001). The *Cry* genes have been identified in the chicken, house sparrow and quails and are reported to be rhythmically expressed. The expression of *Cry1* gene is high during the light phase while *Cry2* is shown to be expressed at high levels during late night (Yamamoto et al., 2001; Bailey et al., 2002; Helfer et al., 2006).

Even though many studies on polymorphisms of clock genes and its association have been carried out in human and wild birds, association studies of circadian clock genes are limited in poultry. Therefore, we used turkey, *Meleagris gallopavo,* second most important poultry meat in the United States of America for the present study. We believe that turkey genome sequence and genomic resources provide the tools that are required to improve the growth and reproductive traits in the turkey industry. In addition, the emerging turkey genome sequence

provides a unique opportunity to understand the DNA sequence variations of the clock genes. Here, I hypothesized that the differences in the DNA sequence variation of turkey clock genes may be associated with performance traits including growth and reproductive traits. The objectives of the current study, therefore, were to screen the several clock genes including *turClock, turPeriod3, turCry1 and turCry2* for DNA sequence variations and to evaluate the relationships among its haplotypes (based on haplogroups) with growth, reproductive traits and plasma melatonin of turkeys.

4.3 Materials and methods

The part of the methodology (Animal study) has been described in detail under chapter 3.

Blood collection and DNA isolation

Blood was collected from each bird at 10 of age for DNA isolation by brachial venipuncture. Whole blood was collected into tubes containing EDTA as anticoagulant. Genomic DNA were isolated using a standard salting out procedure (Guan et al., 2007). Purity and quantity of the DNA was checked using a NanoDrop 1000 (NanoDrop Technologies, Inc., Wilmington, DE).

Genotyping and screening the population

The DNA sequences of the *turClock, turPeriod-3, turCry1 and turCry2* genes as determined using the genome sequence, were used to design primers using Primer 3 software (Rozen and Skaletsky, 2000). The information for the primers including the sequences, annealing temperature, and expected sizes of the Polymerase Chain Reaction (PCR) amplicon are presented in Table 4. 1,Table 4. 18, Table 4. 35, Table 4. 52. 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. (2007).

Statistical analyses

Allele, genotype and haplotype frequencies were determined by standard counting. The computer program, Arlequin ver3.5 (Excoffier and Lischer, 2010) was used to estimate 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.

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

where Y is the trait measured and estimated in 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, $(L \times G)$ is the interaction between the turkey variety and genotype, $(G \times S)$ is the interaction between the sex and genotype and it was excluded from the model if its effect was $P \geq 0.05$ and e is the residual error. Multiple comparisons were analyzed using Tukey's test. The values were presented as least square means ± standard error. Results were considered significant at $P \leq 0.05$.

4.4 Results and discussion

turClock gene variation

The amplicons produced by the five primer-pairs spanned a 24.6 kb region that included the *turClock* gene (Table 4. 1). A total of 12 SNPs were detected in the sequences scanned and validated. The complete list of the SNPs, the sequence contexts, alleles, and GenBank identification (*dbSNP*) are presented in Table 4. 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.

Within the 290 birds screened, the minor alleles ranged in frequency from 0.05 to 0.49 with the observed heterozygosity of 0.09 and 0.49, respectively. The SNPs reported here were not in HWE ($P \leq 0.05$). Across all SNPs, D′ ranged from 0.05 to 1.00. The correlation coefficient (r^2) for the SNPs, ranged from 0.003 to 0.81 (Table 4. 3). The pairwise F_{ST} estimated for the eight turkey varieties ranged from 0.03 to 0.90. The highest F_{ST} (0.90) reported between BR and MW turkeys while lowest (0.03) between RP and NA turkeys. The F_{ST} reported here were significantly different ($P \leq 0.05$) (Table 4. 4). The relatively low F_{ST} estimate suggests low genetic differentiation between the two genetic lines and probably a reflection of their common ancestry.

The haplotypes observed from the 12 SNPs were grouped into five haplogroups. The haplogroups ranged in frequency from 0.03 to 0.93 in the turkey varieties (Table 4. 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 4. 5).

The turkey *Clock* 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). Nucleotide variants of the *turClock* gene have not been published yet in the *db*SNP, NCBI. We also compared the genetic structure of *turClock* gene 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, according to BLAST result (http://blast.ncbi.nlm.nih.gov/) suggesting that most of the nucleotides of the *Clock* gene are conserved within these birds. Further, we compared the genetic relatedness between CC and heritage turkeys using *Fst* which is used to measure the genetic differentiations between the turkeys. Small *Fst* values between RP and NA, CC and BR turkeys indicated that closer relatedness between those turkeys. Smith et al. (2005) showed the closer relatedness between RP and NA turkeys, which we also observed in the present study. In addition, we found that CC turkeys were more closely related with BR turkeys which were consistent with the previous reports by Kamara et al. (2007). The pairwise *Fst* estimates showed that BR turkeys were genetically closely related to CC turkeys while BS and SB turkeys were distantly related to CC turkeys. The CC turkeys have been developed from heritage turkeys using different selection and mating procedures. However, further improvement with respect to disease resistant and reproductive performances is essential to meet the demand of the turkey meat. The some of the genetic information in the present study would be useful to genetically improve CC turkeys population by introgression using heritage turkeys because heritage turkeys show good reproductive performances and are believed high resistance for many diseases.

Associations of turClock haplogroups with growth, reproductive parameters and plasma melatonin

Statistical analysis showed that there was significant association ($P \leq 0.05$) of haplogroups with BW at 309 d of age (Table 4. 6). The results showed that Hap4 had the highest BW at day one and 68 d of age while Hap5 had the highest values for BW at 34, 159, 231 and 309 d of age. The BW of Hap5 was significantly greater compared to Hap3 at 309 d of age ($P \leq 0.05$) (Table 4. 6). There was no significant association ($P \geq 0.05$) of haplogroups with ADG. However, Hap5 numerically appeared to be advantageous haplogroup for all the periods of age except 160 - 231 d where Hap2 had the highest (Table 4. 7). In general, Hap5 appeared to be most advantageous haplogroup for BW and ADG.

Haplogroups were significantly ($P \leq 0.05$) associated with FCR during the periods of 34 - 68 d and 69 - 159 d (Table 4. 8). During the period of 34 - 68 d, Hap2 had significantly lower FCR compared to Hap1. Hap1 had significantly lower FCR than that of Hap3 during the period of 69 - 159 d. Hap1 reported the lowest FCR for other two periods though not significant ($P \geq 0.05$) among haplogroups (Table 4. 8). The FCR numerically increased with the age of turkeys in all the haplogroups. Generally, Hap1 numerically appeared to be most efficient feed conversion haplogroup when compared to others within the different periods of age for the turkeys.

As shown in Table 4. 9, haplogroups were not significantly ($P \geq 0.05$) associated with semen quality traits. However, Hap1 had the highest sperm concentration and viability while Hap3 had the largest ejaculate volume and total sperm count per ejaculate. For the reproductive traits including AFE, egg production and average egg weight, haplogroups were significantly ($P \leq 0.05$) associated with egg production for both 6 and 10 wks periods and the average egg weight

for 6 wks period. The egg production of Hap1 for the periods of 6 and 10 wks was significantly greater than that of Hap4 and Hap5. The average egg weight of the Hap1 for the period of 6 wks 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 4. 10). In general, Hap1 appeared to be most advantageous haplogroup for egg production parameters. Plasma melatonin concentration did not associate with the haplogroups ($P \geq 0.05$), but Hap2 had the highest plasma melatonin concentration compared to Hap1, where was the lowest (Table 4. 11).

Table 4. 12 showed the associations between haplogroups of *turClock* gene and the BW at different ages within different varieties of turkeys. There was no significant association of haplogroups with BW at day one and 309 d of age within each turkey variety ($P \geq 0.05$). However, there was a significant association of haplogroups with BW at 34, 159 and 231 d of age within CC, NA and SB turkeys ($P \leq 0.05$). At the ages of 34, 68 and 159 d of age, Hap4 within CC turkeys had significantly higher BW than that of Hap1. At 68 d of age, Hap3 within NA turkeys had significantly greater BW compared to Hap1 ($P \leq 0.05$). At the age of 159 d, the BWs of Hap3 and Hap5 for NA and SB turkeys were significantly greater compared to Hap2 and Hap3 respectively. There was a significant association of haplogroups with ADG for the periods of 1 - 34 d, 35 - 68 d, 69 - 159 d and 1 - 309 d for CC, NA and SB turkeys ($P \leq 0.05$). The ADG within the haplogroups of CC turkeys was significantly different only during the periods between 1 - 34 d and 35 - 68 d. Of these two periods, Hap4 had significantly greater ADG than that of Hap1. The ADG of Hap1 within NA turkeys was significantly lower compared to other haplogroups where Hap3 had significantly higher ADG for the period of 35 - 68 d. In addition, the ADG within the haplogroups of SB turkeys was significantly different for the period of 1 - 309 d where Hap5 had significantly greater ADG compared to Hap3 ($P \leq 0.05$) (Table 4. 13).

The FCR was estimated for four different periods of age which is shown in (Table 4. 14). There was significant association of haplogroups with FCR for the periods of 34 - 68 d, 69 - 159 d, 160 - 231 d and 34 - 231 d for NA, SB and RP turkeys ($P \leq 0.05$). During the period of 34 - 68 d, FCR was significantly different only among the haplogroups of NA turkeys ($P \leq 0.05$) where Hap1 had significantly greater FCR than that of other haplogroups ($P \leq 0.05$). The haplogroups for the FCR only within SB turkeys were significantly different during the period of 69 - 159 d ($P \leq 0.05$) where Hap5 had significantly lower FCR compared to Hap3 and Hap4. The FCR between Hap1 and Hap3 was also significantly different within SB turkeys. Of the period between 160 - 231 d, the haplogroups for the FCR within SB turkeys were significantly different where Hap5 had significantly lower FCR compared to Hap3 and Hap4 ($P \leq 0.05$). When compared the haplogroups for the FCR within turkey varieties during the period of 34 and 231 d, the haplogroups were significantly different only within RP and SB turkeys where Hap1 and Hap5 had significantly lower FCR, respectively ($P \leq 0.05$).

Table 4. 15 showed the association between the haplogroups of *turClock* gene and the reproductive traits within each variety of turkeys. There was significant association of haplogroups with AFE only for NA turkeys ($P \leq 0.05$). The AFE was significantly different within haplogroups of NA turkeys ($P \leq 0.05$) where Hap3 had significantly lower AFE compared to Hap5. The egg production for the periods of 6 and 10 wks significantly differed among haplogroups only for MW turkeys ($P \leq 0.05$) where Hap4 had the maximum egg production compared to Hap2. The average egg weight for 6 wks was significantly different within haplogroups of CC and SB turkeys ($P \leq 0.05$) where Hap1 within CC and SB turkeys had significantly greater egg weight compared to Hap4. The average egg weight for 10 wks was

significantly different only within haplogroups of RP and SB turkeys ($P \leq 0.05$) where Hap5 and Hap1 had significantly greater average egg weights respectively ($P \leq 0.05$).

As shown in Table 4. 16, the ejaculate volume among haplogroups was significantly different only within MW and NA turkeys ($P \leq 0.05$) where Hap2 of MW turkeys had significantly greater ejaculate volume compared to Hap4. Hap3 of the NA turkeys had significantly higher ejaculate volume than that of Hap5 ($P \leq 0.05$). There was no significant association of haplogroups with sperm concentration within each turkey variety ($P \geq 0.05$). The total sperm number per ejaculate significantly differed among haplogroups of MW turkeys where Hap2 had significantly greater sperm number compared to Hap4 ($P \leq 0.05$). The sperm viability of NA turkeys differed significantly among its haplogroups where Hap1 had significantly higher semen viability than that of Hap5 ($P \leq 0.05$).

The plasma melatonin concentration differed significantly among haplogroups of BS, MW, NA, RP and WH turkeys ($P \leq 0.05$) (Table 4. 17). The Hap3 had significantly lower plasma melatonin concentration for BS turkey compared to other haplogroups while Hap2 had significantly higher plasma melatonin concentration for MW turkeys than that of Hap4 ($P \leq 0.05$). Within the haplogroups of NA turkeys, Hap1 and Hap5 had significantly lower plasma melatonin concentration compared to others. The significantly higher plasma melatonin concentration among haplogroups of RP turkeys reported in Hap1 compared to other haplogroups. The Hap4 of WH turkeys had significantly lower plasma melatonin compared to Hap2 and Hap3 ($P \leq 0.05$).

Based on our assumption discussed earlier, *turClock* gene was selected as a candidate gene to investigate associations of gene polymorphism with growth, reproductive traits and plasma melatonin of the turkey. This particular gene was chosen mainly because of its

involvement in molecular mechanism of circadian clock as a positive element. In the present study, I described new genetic variants in the *turClock* gene and used them to identify haplotypes and haplogroups that span this gene. Haplotypes were constructed with the 12 SNPs and were used to develop the haplogroups based on the Visual Haplotype (VH) output. I analyzed the association among haplogroups of the *Clock* gene with growth, reproductive parameters and plasma melatonin and also analyzed the association among haplogroups within each turkey variety with growth, reproductive parameters and plasma melatonin. The association analyses of *turClock* gene haplogroups supported the significant association on some of the BW, FCR and reproductive parameters. Variations within genes have important implications for economically important traits. The potential associations of haplogroups with BW at 309 d of age, FCR for the periods of 34 - 68 d and 69 - 159 d, egg production and average egg weight showed that the turkey *Clock* gene variations may be involved in regulation of feed intake and egg production through a molecular mechanism of the circadian clock which may lead the phenotypic variations of the turkeys. According to the association analysis among haplogroups of *turClock* within each turkey variety with growth, reproductive parameters and plasma melatonin showed that haplogroups within some varieties associated with some of the growth and reproductive parameters suggesting that the differences within variety still remain and would be valuable for further improvement of turkeys. Similarly, if birds with high performances could be genetically identified, that genetic information is more valuable to be incorporated in future breeding programs in turkey.

turPeriod-3 gene variation

The amplicons produced by the four primer-pairs spanned a 16.6 kb region that included the *turPeriod-3* gene (Table 4. 18). A total of 7 SNPs were detected in the sequences scanned

and validated. The complete list of the SNPs, the sequence contexts, alleles, and GenBank identification (*dbSNP*) are presented in Table 4. 19. Of the 7 SNPs identified, one and two SNPs were detected in exons 18 and 19 while one and three SNPs were detected in introns 5 and 6 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. The SNP detected in the exon 18 was synonymous while the two SNPs detected in the exon 19 were non-synonymous. Of the non-synonymous SNPs, one is involved an amino acid change from methionine to threonine at amino acid 953 while the other is involved in changing an amino acid from serine to phenylalanine at amino acid 955 positions of the protein transcribed from the gene.

Within the 290 birds screened, the minor alleles ranged in frequency from 0.03 to 0.49 with the observed heterozygosity of 0.07 and 0.50, respectively. About 42 % the SNPs reported here were in HWE ($P \geq 0.05$). Across all SNPs, D′ ranged from 0.03 to 1.00. The correlation coefficient (r^2) for the SNPs ranged from 0.002 to 0.89 (Table 4. 20). The pairwise F_{ST} estimated for the eight turkey varieties ranged from 0.01 to 0.43. The highest F_{ST} (0.43) reported between BR and CC turkeys while lowest (0.01) reported between RP and NA, CC and NA and CC and RP turkeys. Most of the F_{ST} were significantly different ($P \leq 0.05$) (Table 4. 21). The relatively low F_{ST} estimate suggests low genetic differentiation between the two genetic lines and probably a reflection of their common ancestry.

The haplotypes observed from the 7 SNPs were grouped into six haplogroups. The haplogroups ranged in frequency from 0.02 to 1.00 in the turkey varieties (Table 4. 22). The most common haplogroup identified for BS, CC, NA and RP turkeys was Hap6, with the frequencies of 0.44, 0.76, 0.43 and 0.56, respectively. Hap4 was predominant for MW and WH

turkeys with the frequencies of 0.42 and 0.41, respectively. Hap2 and Hap3 were the leading haplogroups for SB turkey with the frequency of 0.32. BR turkey had the only one haplogroup which was Hap1.

The turkey *Period-3* gene is located over a region of 15.84 kb of the chromosome 23 and contains 25 exons. Only one transcript of the *turPeriod-3* gene has been identified up to now. The length of the transcript has been estimated the 3447 bp, which translate into a protein of 1149 aa (www.ensembl.org). Similar to *turClock*, we compared the genetic structure of *turPeriod-3* gene with chicken and zebra finch genomes to identify the sequence identity between these species. We found that the genetic structure of the turkey *Period-3* gene has 90 and 83 % sequence similarity with chicken and zebra finch genome respectively, according to BLAST result (http://blast.ncbi.nlm.nih.gov/) suggesting that most of the nucleotides of the *Period-3* gene are conserved within these birds. Here, we also compared the genetic relatedness between CC and seven heritage turkey varieties using *Fst.* Small *Fst* values between RP and NA, CC and NA, and CC and RP turkeys indicated that closer relatedness between those turkeys. Smith et al. (2005) showed the closer relatedness between RP and NA turkeys which we also observed in the present study. In addition, we found that CC turkeys were more closely related with NA and RP turkeys which was inconsistent with the previous reports by Kamara et al. (2007) and Smith et al. (2005).

Associations of turPeriod-3 haplogroups with growth, reproductive parameters and plasma melatonin

Statistical analysis revealed that there was significant association of haplogroups with some of the growth and reproductive parameters of the turkeys. Haplogroups were significantly

associated with BW at 231 d of age ($P \leq 0.05$) where Hap2 had significantly higher BW than that of Hap3. Though not significant, Hap6 numerically appeared be the advantageous haplogroup for BW at 1, 34, 68 and 309 days of age while Hap2 was numerically greater at 159 days of age (Table 4. 23). As shown in Table 4. 24, haplogroups were significantly associated with ADG ($P \leq 0.05$) during the period of 160 - 231 d but not with other periods of age. The Hap2 had significantly higher ADG during the period of 160 - 231 d compared to Hap3. The Hap6 numerically appeared to be advantageous haplogroup for the periods of 1 - 34 d, 232 - 309 d and 1 - 309 d. Hap4 had higher ADG during the periods of 35 - 68 d and 69 -159 d though not significant ($P \geq 0.05$) (Table 4. 24).

The FCR was calculated for the different periods of age for turkeys. The statistical analysis showed that haplogroups were statistically associated with FCR for the periods of 69 - 159 d and 160 - 231 d ($P \leq 0.05$) (Table 4. 25). The Hap4 had significantly lower FCR compared to Hap5 for the period of 69 - 159 d while Hap1 had significantly lower FCR than that of Hap3 for the period of 160 - 231 d ($P \leq 0.05$). However, Hap1 had the lowest FCR for the periods of 34 - 68 d and 34-231 d though not significant ($P \geq 0.05$). In general, Hap1 numerically appeared to be advantageous haplogroup for FCR.

The AFE, egg production, and average egg weight were compared with the haplogroups of *turPeriod-3*. According to the statistical analysis, haplogroups were significantly associated with AFE, egg production for 10 wks and average egg weight ($P \leq 0.05$) (Table 4. 26). The Hap 6 had significantly lower AFE compared to Hap1. Hap5 produced more eggs for the both periods (6 and 10 wks) compared to Hap1 but, it was significantly different only for the egg production for 10 wks ($P \leq 0.05$). The average egg weight was significantly different among haplogroups where Hap6 had significantly greater average egg weight compared to Hap1 ($P \leq 0.05$). In

general, Hap5 appeared to be most advantageous haplogroup for egg production while Hap6 was advantageous for AFE and average egg weight.

We analyzed the association between the haplogroups and semen quality traits including ejaculate volume, sperm concentration, total number of sperm and sperm viability. There was significant association of haplogroups with ejaculate volume, sperm concentration and total number of sperm ($P \leq 0.05$). The Hap4 had significantly higher values for ejaculate volume, sperm concentration and total number of sperm ($P \leq 0.05$) compared to Hap1 (Table 4. 27). Overall, Hap4 appeared to be most advantageous haplogroup for semen quality traits of the turkeys. The haplogroups of *turPeriod-3* were significantly associated with plasma melatonin levels of the turkeys ($P \leq 0.05$) where Hap2 had significantly higher plasma melatonin level compared to other haplogroups except Hap3. Meantime, Hap1 reported the lowest plasma melatonin concentration (Table 4. 28).

We also analyzed the associations between haplogroups of *turPeriod-3* gene and BW at different ages within each variety of turkeys. The haplogroups were not associated with BW at 1, 34, 68, 159, 231 and 309 d of ages within each variety of turkeys except CC turkeys at 34 and 68 d of age. The haplogroups were associated with BW at 34 and 68 days of age within CC turkeys ($P \leq 0.05$). The Hap3 and Hap6 had significantly higher BW compared to Hap4 at 34 d of age while Hap3 had significantly higher BW than that of Hap5 at 68 d of age ($P \leq 0.05$) (Table 4. 29). The analysis of associations between haplogroups and ADG within each variety of turkey showed that haplogroups were not associated with ADG by the different periods of age within each variety of turkeys except CC turkeys for the periods of 35 - 68 d and 160 - 231 d. The Hap4 had significantly higher ADG compared to Hap5 for the period of 35 - 68 d within CC turkey ($P \leq 0.05$) while Hap2 had significantly higher ADG compared to Hap3 for the period of 160 - 231

d (Table 4. 30). The associations between haplogroups of *turPeriods-3* gene and FCR within each variety of turkeys are presented in Table 4. 31. Statistical analysis showed that haplogroups were not associated with FCR by different period of ages within each variety ($P \geq 0.05$) though FCR values were different.

The association analysis between haplogroups of *turPeriod-3* and reproductive parameters showed that haplogroups were not associated with reproductive parameters ($P \geq 0.05$) within each variety of turkeys except AFE where haplogroups within CC turkeys were significantly associated with AFE. The Hap5 had significantly lower AFE compared to Hap4 within CC turkeys ($P \leq 0.05$). Even though, the egg production and average egg weight were different among haplogroups within each variety, haplogroups were not associated with egg parameters ($P \geq 0.05$) within each variety (Table 4. 32).

The semen quality traits were analyzed within each variety of turkeys to find any association with haplogroups as shown in Table 4. 33. The analysis results showed that haplogroups were not associated with ejaculate volume within each variety of turkeys ($P \geq 0.05$). The haplogroups within WH turkeys were associated with sperm concentration where Hap4 had significantly higher sperm concentration compared to Hap3 ($P \leq 0.05$). The total sperm number per ejaculate was also associated with haplogroups within WH turkeys where Hap4 had significantly higher total sperm number compared to Hap3 ($P \leq 0.05$). The haplogroups were not associated with total number of sperms per ejaculate within other varieties ($P \geq 0.05$). The sperm viability did not show any significant association with haplogroups except within MW turkeys where Hap2 had significantly higher sperm viability ($P \leq 0.05$) compared to Hap5 and Hap6 (Table 4. 33). The associations between haplogroups of *turPeriod-3* gene and the plasma melatonin concentration within each variety of turkeys are given in Table 4. 34. According to the

statistical analysis, results showed that haplogroups were not associated with plasma melatonin concentration within each variety though different ($P \geq 0.05$).

According to our assumption discussed at the beginning, we selected *Period-3* gene as another candidate gene to investigate associations of gene polymorphism with growth, reproductive parameters and melatonin concentration of the turkeys. In the present study, we described new genetic variants in the *turPeriod-3* gene and used them to identify haplotypes and haplogroups that span this gene. Haplotypes were constructed with the reported SNPs and were used to categorize the haplogroups. We analyzed the association among haplogroups of the *Period-3* gene with growth, reproductive parameters and plasma melatonin. In addition, we analyzed the association among haplogroups within each turkey variety with growth, reproductive parameters and plasma melatonin. According to the association analyses of *turPeriod-3* gene haplogroups, it revealed the significant association of *turPeriod-3* gene haplogroups with some of the BW, FCR and AFE, egg production, semen quality traits and plasma melatonin concentration. The potential associations of the haplogroups with BW at 231 d of age, ADG for the period of 160 - 231 d, FCR for the periods of 69 - 159d and 160 - 231 d, AFE, egg production, semen quality traits and plasma melatonin concentration showed that the variations of turkey *Period-3* gene may be involved in regulation of growth, feed intake, reproduction, semen quality traits and plasma melatonin concentration through a molecular mechanism of the circadian clock. According to the association analysis among haplogroups of *turPeriod-3* within each turkey variety with growth, reproductive parameters and plasma melatonin of the turkeys showed the genetic variation within each variety. The haplogroups within some varieties associated with some of the growth and reproductive parameters suggesting that the differences within variety are present in addition to the differences between

turkey varieties and would be useful for further improvement of those turkey varieties in general. The results indicated that haplogroups of *turPer3* gene was statistically associated with most of the growth, reproductive parameters and plasma melatonin levels compared to *turClock* gene haplogroups. This shows that genetic variants of the *turPer3* gene is closely associated with growth, reproductive parameters and plasma melatonin levels suggesting that *turPer3* gene is a good candidate gene for association studies for growth, reproductive parameters and plasma melatonin levels than *turClock* gene in turkeys.

turCry1 gene variation

The amplicons produced by the three primer-pairs spanned a 15.0 kb region that included the *turCry1* gene (Table 4. 35). A total of 7 SNPs were detected in the sequences scanned and validated. The complete list of the SNPs, the sequence contexts, alleles, and GenBank identification (*dbSNP*) are presented in Table 4. 36. Of the 7 SNPs identified, four and three SNPs were detected in introns 3 and 6 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 *turCry1* gene.

Within the 290 birds screened, the minor alleles ranged in frequency from 0.13 to 0.46 with the observed heterozygosity of 0.23 and 0.50, respectively. The SNPs reported here were not in HWE ($P \leq 0.05$). Across all SNPs, D′ ranged from 0.48 to 0.89. The correlation coefficient (r^2) for the SNPs ranged from 0.03 to 0.42 (Table 4. 37). All the D′ and r^2 values reported here were significant ($P \leq 0.05$). The pairwise F_{ST} estimated for the eight turkey varieties ranged from 0.004 to 0.67. The highest F_{ST} reported between BR and RP turkeys (0.67) while the lowest reported between the MW and NA turkeys (0.004). Most of the F_{ST} values were

significantly different ($P \leq 0.05$) (Table 4. 38). The relatively low F_{ST} estimate suggests low genetic differentiation between the two genetic lines and probably a reflection of their common ancestry.

The haplotypes observed from the 7 SNPs were grouped into seven haplogroups based on the VH1 output. The haplogroups ranged in frequency from 0.02 to 0.96 in the turkey varieties (Table 4. 39). The most common haplogroup identified for the BR, NA and SB turkeys was Hap1, with the frequencies of 0.96, 0.82 and 0.46, respectively. The predominant haplogroup identified for the BS, CC, MW, RP and WH turkeys was Hap4, Hap3, Hap2, Hap6 and Hap5 with the frequencies of 0.67, 0.68, 0.81, 0.56 and 0.73, respectively.

The turkey *Cry1* gene is located over a 14.46 kb region of the chromosome 1 and contains 13 exons and 12 introns. Only one transcript of the *turCry1* gene has been identified so far with a length of 1761 bp (www.ensembl.org). However, nucleotide variants of the *turCry1* gene though detected in the introns, have not been published yet according to our knowledge since turkey genome sequence has been published recently. We compared the genetic structure of *turCry1* gene with chicken and zebra finch genome to verify the sequence identity. The genetic structure of the turkey *Cry1* gene has 90 and 91 % sequence similarity with chicken and zebra finch genome respectively according to BLAST result (http://blast.ncbi.nlm.nih.gov/) suggesting that most of the nucleotides of the *Cry1* gene are also conserved within these birds. Using *Fst,* we also compared the genetic relatedness between CC and heritage turkeys varieties as discussed previously. Small *Fst* between the MW and NA and SB and NA turkeys indicated that closer relatedness between those turkeys, which is inconsistent with the previous reports by Smith et al. (2005) and Kamara et al. (2007). Interestingly, here we found that CC turkeys are

not genetically related with heritage varieties of turkeys though they were developed from heritage varieties.

Associations of turCry1 haplogroups with growth, reproductive parameters and plasma melatonin

Statistical analysis revealed that there was significant association of haplogroups with some of the growth and reproductive parameters of the turkeys. Haplogroups were not significantly associated with BW at all ages ($P \geq 0.05$). However, Hap3 numerically appeared be the advantageous haplogroup for BW at 1, 34, and 68 d of ages while Hap4 reported the highest BW at 231 and 309 d of ages (Table 4. 40). As shown in Table 4. 41, haplogroups were significantly associated with ADG ($P \leq 0.05$) during the period of 35 - 68 d, but not with other periods of age. During the period of 35 - 68 d, ADG of Hap3 was significantly higher ($P \leq 0.05$) compared to Hap5 and Hap6. Though not significant, Hap3 numerically appeared to be the advantageous haplogroup for ADG during the periods of age between hatch - 34 d, 232 - 309 d and 1-309 d, while the Hap6 and Hap4 had highest ADG during the periods of age between 69 - 159 d and 160 - 231 d respectively (Table 4. 41).

The FCR was calculated for four different periods of the age for turkeys. The statistical analysis showed that haplogroups were statistically associated with FCR for the periods of age between 160 - 231 d and 34 - 231 d ($P \leq 0.05$) (Table 4. 42). The Hap3 had significantly lower FCR compared to Hap1, Hap2 and Hap5 for the period of 160 - 231 d, while Hap7 had significantly lower FCR than that of Hap1 for the period of 34 - 231 d ($P \leq 0.05$). However, Hap4 and Hap7 showed the lowest FCR for the periods of 34 - 68 d and 69 - 159 d, respectively though FCR was not significantly different among haplogroups ($P \geq 0.05$).

The AFE, egg production, and average egg weight were compared within the haplogroups identified for the *turCry1* gene. According to the statistical analysis, results showed that haplogroups were significantly associated only with egg production ($P \leq 0.05$) but not with AFE and average egg weight (Table 4. 43). The Hap5 and Hap4 had significantly higher egg production for the periods of 6 and 10 wks, respectively ($P \leq 0.05$). Though not significant, Hap2 had the lowest AFE while Hap3 had the highest average egg weight for both periods of 6 and 10 wks compared to other haplogroups.

The associations between the haplogroups and semen quality traits including ejaculate volume, sperm concentration, total number of sperm and sperm viability were analyzed. Results showed that haplogroups were significantly associated with ejaculate volume ($P \leq 0.05$), but not with other semen quality traits. The Hap3 had significantly higher volume of semen compared to Hap2 and Hap6 ($P \leq 0.05$). Though not significant, Hap1 and Hap4 had numerically higher value for sperm concentration and total number of sperm, respectively, while Hap5 numerically appeared to be advantageous haplogroup for sperm viability ($P \geq 0.05$) (Table 4. 44). The haplogroups of *turCry1* did not associate with plasma melatonin levels of the turkeys ($P \geq 0.05$). However, the Hap6 had numerically higher plasma melatonin level (45.24 ± 6.37 pg/ml) compared to other haplogroups where Hap2 reported the lower level of plasma melatonin (27.60 ± 2.49 pg/ml) (Table 4. 45).

The associations between haplogroups of *turCry1* gene and BW at different ages within each variety of turkey were also analyzed. The haplogroups were not associated with BW at 1, 34, 68, 159, 231 and 309 d of ages within each variety of turkey except RP, WH and CC turkeys. The haplogroups were significantly associated with BW at day one, 68 and 309 days of age within RP, CC and WH turkeys, respectively ($P \leq 0.05$). The Hap3, Hap6 and Hap7 had

significantly higher BW at day one compared to Hap2 for RP turkeys while the BW of Hap3, 4 and 7 was significantly higher than that of other haplogroups at 68 d of age for CC turkeys. Hap1, Hap5 and Hap6 for WH turkeys had significantly higher BW compared to Hap7 at 309 d of age ($P \leq 0.05$) (Table 4. 46). The analysis of associations between haplogroups and ADG within each variety of turkey showed that haplogroups were not associated with ADG by the different periods of age within each variety of turkey except BS turkeys for the period of 35 - 68 d and CC turkeys for the periods of 35 - 68 d and 160 - 231 d. The Hap1 for BS turkeys had significantly higher ADG ($P \leq 0.05$) compared to Hap6 for the period of 35 - 68 d. The Hap4 had the significantly highest ADG compared to Hap1, Hap5 and Hap6 for the period of 35 - 68 d within CC turkeys ($P \leq 0.05$) while ADG of Hap1 was significantly higher compared to Hap6 for the period of 160 - 231 d (Table 4. 47). The associations between haplogroups of *turCry1* gene and FCR within each variety of turkey are presented in Table 4. 48. Statistical analysis showed that haplogroups were associated with FCR for the periods of age between 34 - 68 d and 160 - 231 d within BS, CC, NA turkeys ($P \leq 0.05$). The haplogroups were significantly associated with FCR only within BS and CC turkeys for the period of 34 - 68 d. The Hap1 and Hap4 had significantly lower FCR ($P \leq 0.05$) within BS and CC turkeys, respectively. For the period of 160 - 231 d, haplogroups were significantly associated with FCR within each variety of turkey except BR, MW and RP turkeys ($P \leq 0.05$) (Table 4. 48).

The association analysis between haplogroups of *turCry1* and reproductive parameters showed that haplogroups were associated with egg production and average egg weight ($P \leq 0.05$) within some varieties of turkey (Table 4. 49). The AFE did not differ significantly within each variety of turkey though values were numerically different ($P \geq 0.05$). Haplogroups within BR, CC, NA and RP turkeys were associated with egg production for the periods of 6 and 10 wks.

Hap7 for BR turkeys had significantly higher egg production compared to Hap1 for the both periods. Hap3, 4 and 5 for CC turkeys produced significantly higher numbers of eggs than that of Hap6 for both periods. Hap1 for NA turkeys had significantly higher egg production compared to Hap6. The haplogroups within RP turkeys for both egg production periods were significantly different ($P \leq 0.05$). Haplogroups within SB turkeys were significantly associated with average egg weight only for the period of 10 wks, where Hap6 had significantly higher egg weight compared to others ($P \leq 0.05$) (Table 4. 49).

The semen quality traits were analyzed within each variety of turkey to find out any association with haplogroups as shown in Table 4. 50. The analysis results showed that haplogroups were not associated with semen quality traits within each variety of turkey ($P \geq 0.05$). The associations between haplogroups of *turCry1* gene and the plasma melatonin concentration within each variety of turkeys are given in Table 4. 51. According to the statistical analysis, results showed that haplogroups were not associated with plasma melatonin concentration except within SB turkeys, where Hap6 reported the higher plasma melatonin concentration ($P \geq 0.05$).

We selected another clock gene, which was *turCry1* gene, as a candidate gene in our study to investigate associations of gene polymorphism with growth, reproductive parameters and plasma melatonin of the turkeys. In the present study, we described new genetic variants in the *turCry1* gene and used them to identify haplotypes and haplogroups that span this gene. Haplotypes were constructed with the 7 SNPs and were used to develop the haplogroups. We analyzed the association among haplogroups of the *turCry1* gene with growth, reproductive parameters and plasma melatonin of turkeys. In addition, we further analyzed the association among haplogroups within each turkey variety with growth, reproductive parameters and plasma

melatonin. According to the association analyses of *turCry1* gene haplogroups, it showed a significant association of *turCry1* gene haplogroups with ADG, FCR, egg production and ejaculate volume. The potential associations of the haplogroups with ADG for the period of 35 - 68 d, FCR for the periods of 160 - 231 d and 34 - 231 d, egg production and ejaculate volume showed that the variations of turkey *Cry1* gene may be involved in regulation of growth, feed intake and egg production through a molecular mechanism similar to previously discussed. According to the association analysis among haplogroups of *turCry1* within each turkey variety with growth, reproductive parameters and plasma melatonin of the turkeys showed that haplogroups are associated with some of the growth and reproductive traits within some of the turkey varieties suggesting that differences within variety could be used for further improvement of the turkey varieties. In general, haplogroups of *turCry1* gene significantly associated with few growth and reproductive parameters compared to haplogroups of *turClock* and *turPer3* genes suggesting that *turCry1* gene is not a candidate gene to be studied for association study in turkey.

turCry2 gene variation

The amplicons produced by the four primer-pairs spanned a 16.6 kb region that included the *turCry2* gene (Table 4. 52). A total of 15 SNPs were detected in the sequences scanned and validated. The complete list of the SNPs, the sequence contexts, alleles, and GenBank identification (*dbSNP*) are presented in Table 4. 53. Of the 15 SNPs identified, two SNPs were detected in the promoter region while five SNPs detected each in exon 1 and intron 1 respectively. In addition, three more SNPs found in intron 3. 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 *turCry2* gene. Of the five SNPs detected in the exon 1, one was

synonymous while rests were non-synonymous. Of the non-synonymous SNPs, the SNP located at the nucleotide position of 24670558, is involved an amino acid change from leucine to proline at amino acid 7, while the other SNP located at the position 24670553, is involved in changing an amino acid from aspartic acid to asparagine at amino acid 9 positions of the protein. The other two SNPs located at positions 24670551 and 24670510, are involved in changing amino acids from aspartic acid to glutamic acid and valine to glycine at 9 and 23 aa positions of the protein, respectively (Table 4. 53).

Within the 290 birds screened, the minor alleles ranged in frequency from 0.03 to 0.18 with the observed heterozygosity of 0.03 and 0.29, respectively. The SNPs reported here were not in HWE ($P \leq 0.05$) (Table 4. 53). Across all SNPs, D´ ranged from 0.01 to 1.00. The correlation coefficient (r^2) for the SNPs ranged from 0.001 to 0.92 (Table 4. 54). The pairwise F_{ST} estimated for the eight turkey varieties ranged from 0.01 to 0.30. The highest F_{ST} (0.30) reported between the WH and BS, RP and BS and RP and SB turkeys while lowest (0.01) reported between the CC and MW and RP and WH turkeys. Most of the F_{ST} values were significantly different ($P \leq 0.05$) (Table 4. 55). The relatively low F_{ST} estimate suggests low genetic differentiation between the two genetic varieties and probably a reflection of their common ancestry. The haplotypes observed from the 15 SNPs were grouped into four haplogroups based on the VH output. The haplogroups ranged in frequency from 0.02 to 0.86 in the turkey varieties (Table 4. 56). The most common haplogroup identified for the BR, BS, MW, NA, SB and WH turkeys was Hap1, with the frequencies of 0.78, 0.81, 0.51, 0.82, 0.86 and 0.42, respectively. Hap4 and Hap2 were predominant for the CC and RP turkeys with the frequencies of 0.60 and 0.39, respectively.

The turkey *Cry2* gene located over a region of 16.1 kb of the chromosome 5 which contains 11 exons. Only one transcript of the *turCry2* gene has been identified up to now. The length of the transcript has been estimated the 1747 bp which translates into a protein of 521 aa (www.ensembl.org). We compared the genetic structure of *turCry2* gene with chicken and zebra finch genome to identify the sequence identity between these species. We found that the genetic structure of the turkey *Cry2* gene has 91 and 90 % sequence similarity with chicken and zebra finch genome, respectively according to BLAST result (http://blast.ncbi.nlm.nih.gov/) suggesting that most of the nucleotides of the *Cry2* gene are conserved within these birds. In addition, we looked at the genetic relatedness between CC and seven heritage turkey varieties using *Fst.* Smallest *Fst* values between the CC and MW, and RP and WH turkeys suggest the closer relatedness between those turkeys. Here we reported that MW turkey was genetically closely related to CC turkeys while RP turkeys are related with the WH turkey. Therefore, the information gathered here would be useful to genetically improve the CC population by introgression using heritage turkeys.

Associations of turCry2 haplogroups with growth, reproductive parameters and plasma melatonin

Statistical analysis revealed that there was significant association of *turCry2* haplogroups with some of the growth and reproductive traits of the turkeys. Haplogroups were significantly associated with BW at 34 d, 68 d and 231 d of age ($P \leq 0.05$). At 34 and 68 d of age, Hap1 had significantly higher BW compared to Hap4. At 231 d of age, Hap4 had significantly higher BW than that of Hap2 ($P \leq 0.05$). Though not significant, Hap2 numerically appeared be the advantageous haplogroup at 1 and 309 d of age while Hap3 had numerically higher BW at 159 d

of age (Table 4. 57). As shown in Table 4. 58, haplogroups were significantly associated with ADG ($P \leq 0.05$) during the age between 160 and 231 d but not with other periods of age. During the period of 160 - 231 d, Hap4 had significantly higher ADG ($P \leq 0.05$) compared to other haplogroups. Though not significant, Hap1 numerically appeared to be the advantageous haplogroup during the periods of hatch - 34 d and 35 - 68 d while Hap2 had better ADG during the periods of 69 - 159 d and 232 - 309 d (Table 4. 58).

The statistical analysis showed that the haplogroups were statistically associated with FCR only for the period of age between 34 - 68 d ($P \leq 0.05$) (Table 4. 59) where Hap3 had significantly lower FCR compared to Hap4 for the period of 34 - 68 d ($P \leq 0.05$). However, Hap2 had lower FCR for the periods of 69 - 159 d and 34 - 231 d, while Hap4 showed better FCR for the period of 160 - 231 d though not significant ($P \geq 0.05$). Associations between haplogroups and reproductive parameters showed that the haplogroups were significantly associated only with average egg weight ($P \leq 0.05$) but not with AFE and egg production (Table 4. 60). The Hap3 had significantly higher average egg weight for both the periods of 6 and 10 wks ($P \leq 0.05$) compared to Hap4. Though not significant ($P \geq 0.05$), Hap2 reported the least AFE while Hap3 had numerically higher egg production for both periods of 6 and 10 wks (Table 4. 60).

Semen quality traits including ejaculate volume, sperm concentration, total number of sperm and sperm viability were not associated with the haplogroups ($P \geq 0.05$). However, Hap4 had numerically higher values for ejaculate volume and sperm viability while Hap3 numerically appeared to be advantageous haplogroup for sperm concentration and total number of sperm (Table 4. 61). The haplogroups of *turCry2* did not associate with the plasma melatonin levels of the turkeys ($P \geq 0.05$). However, the Hap1 had numerically higher plasma melatonin level (36.54

± 3.04 pg/ml) compared to other haplogroups while Hap3 reported the lowest plasma melatonin (27.57 ± 7.26 pg/ml) (Table 4. 62).

We also analyzed the associations between haplogroups of *turCry2* gene and BW at different ages within each variety of turkey. The haplogroups were not associated with BW at day one within each variety of turkey. But, haplogroups were significantly associated with BW at 34 d of age only within CC turkeys and at 68 d of age within CC and NA turkeys ($P \leq 0.05$). The Hap1 within CC turkeys had significantly higher BW compared to Hap3 and Hap4 at 34 d of age while Hap1 reported the significantly higher BW at 68 d of age within CC and NA turkeys. At the age of 309 d, Hap3 within NA turkeys had significantly higher BW compared to Hap2 (Table 4. 63). The analysis of associations between haplogroups and ADG within each variety of turkey showed that haplogroups within CC turkeys were significantly associated with ADG for the periods of age between 1-34 d, 35-68 d, 69-159 d and 160-231 d ($P \leq 0.05$). The haplogroups within MW turkeys were also significantly associated with ADG only for the period of age between 35 - 68 d. The Hap1 had significantly higher ADG for the period of 1 - 34 d and 35 - 68 d within CC turkeys ($P \leq 0.05$), while Hap3 and Hap4 reported significantly higher ADG for the periods of 69 - 159 d and 160 - 231 d, respectively. For MW turkeys, Hap4 had significantly higher ADG compared to other haplogroups ($P \leq 0.05$) (Table 4. 64).

The associations between haplogroups of *turCry2* gene and FCR within each variety of turkey are presented in Table 4. 65. Statistical analysis showed that haplogroups were significantly associated with FCR by different period of ages within some of the turkey varieties ($P \leq 0.05$). The FCR within MW turkeys was associated with haplogroups for the period of age between 69 - 159 d where Hap2 had significantly better feed conversion compared to Hap4. For the period of 160 - 231 d, haplogroups within CC turkeys were significantly different for FCR

where Hap4 had better FCR than Hap2 ($P \leq 0.05$). For the period of 34 - 231 d, the haplogroups within MW and RP turkeys differed significantly ($P \leq 0.05$) where Hap2 reported the better feed conversion (Table 4. 65).

The association analysis between haplogroups of *turCry2* and reproduction showed that haplogroups were significantly associated with reproductive parameters ($P \leq 0.05$) within some of the varieties of turkey. The haplogroups within CC and NA turkeys were significantly associated with AFE where Hap3 within CC turkeys and Hap1 within NA turkeys had significantly lower AFE ($P \leq 0.05$). The haplogroups only within BR and MW turkeys differed significantly with egg production for the period of 6 wks ($P \leq 0.05$), but did not vary with egg production for the 10 wks period. In both varieties, Hap3 reported the highest egg production which was significantly different ($P \leq 0.05$). The average egg weight for 6 and 10 wks was associated with haplogroups only within CC and SB turkeys. The Hap1 within CC turkeys and Hap3 within SB turkeys had significantly higher average egg weight for both periods of 6 and 10 wks (Table 4. 66).

The semen quality traits were analyzed within each variety of turkey to find any association with haplogroups as shown in Table 4. 67. The analysis results showed that haopogroups were significantly associated with ejaculate volume, sperm concentration and sperm viability within some of the varieties of turkey ($P \leq 0.05$). The haplogroups within NA turkey were significantly associated with ejaculate volume where Hap1 had the significantly higher semen volume compared to Hap2 and Hap3 ($P \leq 0.05$). The sperm concentration significantly differed with the haplogroups within WH turkeys, where Hap3 reported significantly higher sperm concentration compared to Hap1 ($P \leq 0.05$). The haplogroups were also associated with sperm viability only within NA turkeys, where Hap4 had significantly

higher sperm viability compared to Hap2 ($P \leq 0.05$) (Table 4. 67). The associations between haplogroups of *turCry2* gene and the plasma melatonin concentration within each variety of turkey are given in Table 4. 68. According to the statistical analysis, results showed that haplogroups were significantly associated with plasma melatonin concentration within WH turkeys where Hap4 had significantly higher level of plasma melatonin compared to others ($P \leq 0.05$).

The association of gene polymorphisms with economically important traits including growth and reproductive traits in farm animals is one of the areas of interest of geneticists to predict the genetic change on the phenotypic variations (Rothschild and Soller, 1997). Based on our assumption discussed earlier, *turCry2* gene was selected as another candidate gene to investigate associations of gene polymorphism with growth and reproductive traits of the turkeys. In the present study, we described new genetic variants in the *turCry2* gene and used them to identify haplotypes and haplogroups that span this gene. Haplotypes were constructed with 15 SNPs and were used to develop the haplogroups. We analyzed the association among haplogroups of the *turCry2* gene with growth, reproductive traits and plasma melatonin concentration of the turkeys. In addition, we also analyzed the associations among haplogroups within each turkey variety with growth, reproductive traits and plasma melatonin. According to the association analyses of *turCry2* gene haplogroups, it revealed that DNA sequence variations of *turCry2* gene were significantly associated with some of the BW, ADG, FCR and egg weight. The potential associations of the haplogroups with BW at 34, 68, and 231 d of age, ADG for the period of 160 - 231 d, FCR for the period of 34 - 68 d and egg weight showed that the DNA sequence variations of turkey *Cry2* gene may be involved in a molecular mechanism of the circadian clock which may regulate the growth and reproduction of the birds. According to the

association analysis among haplogroups of *turCry2* within each turkey variety with growth, reproductive traits and plasma melatonin showed that haplogroups are associated with some of the growth, reproductive traits and plasma melatonin within some of the turkey varieties suggesting that differences within varieties are still obvious and could be used for further improvement of turkeys. The haplogroups of *turCry2* gene were mainly associated with growth related traits suggesting that *turCry2*gene may be a good candidate gene for studying growth parameters in the turkey.

The circadian clock mechanism in birds is composed of several proteins including CLOCK, BMAL1, PER2, PER3, CRY1 and CRY2. Many of these proteins are transcription factors. These proteins work together to activate or inhibit its own transcription. In the transcriptional and translation feedback loop, CLOCK and BMAL1 proteins form a complex which acts as a positive feedback loop by activating the transcription of *Per* and *Cry* genes. Similarly, PER and CRY proteins form the protein complex which act as a negative feedback loop by inhibiting the transcriptional activity of CLOCK/BMAL1 complex. These protein components are important to initiate circadian rhythms at molecular level and lead for the proper functioning of the circadian clock. If there are any alterations that occur in gene expression of the circadian clock genes and/or protein translation, it may affect the total mechanism of the circadian clock. In addition, previous studies as discussed earlier have shown that genetic variants of clock genes are associated with reproductive traits, feeding, sleeping pattern and behavior patterns in various species of animals. Therefore, we selected *Clock*, *Bmal1*, *Per3*, *Cry1* and *Cry2* genes for the current study by considering their connection in the molecular mechanism of the circadian clock and associations in other animals.

About 0.6 million SNPs have been identified within the reference genome assembly of turkey (Dalloul et al., 2010). The whole genome SNP discovery study resulted in the detection of 5.49 million putative SNPs compared to the reference genome in the turkey. Of the total 5.49 million SNPs discovered, 75,254 were located in exonic regions, including 23,795 non-synonymous and 52,506 synonymous SNPs (Aslam et al., 2012). Most of the SNPs found in the clock genes were A-G/ C-T transitions as expected and 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, random mating ect. A deviation from HWE may be due to natural selection, population admixture, inbreeding, experimental errors and duplication (Cox and Kraft, 2006). 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 (Goodswen et al., 2010). *Fst* is used to measure the genetic differentiations between turkey populations. *Fst* is a measure of population differentiation that ranges from 0 to 1 with 0 indicating no differentiation and 1 indicating highly differentiated populations. The strong genetic differentiation between two populations is confirmed by the large *Fst* values (Hayes et al., 2008). The candidate gene approach is a very powerful method to investigate associations of gene polymorphisms with economically important traits in farm animals. It is important to note that regulatory and coding SNPs are of particular interest to molecular association studies. 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 (Rothschild and Soller, 1997). The association studies have been used to find any association between the genetic variants and phenotype of the animals. With the discovery of large number

of SNPs, it has been increasing interests in genetic associations with closely linked SNPs (Sha et al., 2007). In the present study, haplogroups of *turPeriod-3* and *turCry2* genes were significantly associated with most of the growth, reproductive parameters and plasma melatonin compared to *turClock* and *turCry1* genes suggesting that *turPeriod-3* and *turCry2* genes are good candidate genes for further association studies in turkey. 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, are naturally interpreted as genetic polymorphisms of SNP alleles on the same chromosome and tend to be conserved by evolutionary processes (Yang et al., 2008).

In summary, I tested the hypothesis that DNA sequence variation in the clock genes at the nucleotide and haplotype levels may be associated with differences in performance traits in the turkey. A total of 41 novel genetic variants in the four clock genes were reported in the present study and used them to develop the haplogroups for each gene in 8 turkey populations. The data presented here represent, to our knowledge, the first investigation of the genetic variations of clock genes in the turkey. The haplogroups developed for each gene were used to conduct association studies with growth, reproductive parameters, and plasma melatonin of the turkey. The association study revealed that haplogroups of the different genes are associated with some of the growth, reproductive parameters and plasma melatonin. In addition, haplogroups within some of the varieties were associated with growth, reproductive parameters and plasma melatonin. Further, it showed that *turPeriod-3* and *turCry2* are two potential genes for further association studies in turkey. DNA sequence variations of the clock 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 will be needed to show the value of our genetic data in genotype:phenotype correlations in the turkey .

Table 4. 1. Primer sequences, expected sizes of amplicons and PCR characteristics for *turClock* gene.

Primer ID	Primers[1]	Sequences	Tm[2] (^{0}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 4. 2. Characteristics of single nucleotide polymorphisms (SNPs) 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	ss538301101	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	ss538301102	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	ss538301103	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	ss538301104	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	ss538301105	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	ss538301106	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	ss538301107	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	ss538301108	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	ss538301109	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	ss538301110	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	ss538301111	G/G	51.4	0.42	0.00*
					G/A	12.8		
					A/A	35.8		
clk2-12	Intron 16	47798954	GTTTA(*G*/A)AAACA	ss538301112	A/A	65.9	0.33	0.00*
					A/G	2.1		
					G/G	32.0		

[1]Position of the SNP in Genbank 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]*ss* prefix indicates novel SNPs detected here and submitted for *rs* assignment in *dbSNP*, NCBI.

[4]Minor allele frequency (MAF) of 12 SNPs markers.

[5]Significance of deviation from HWE for the 12 SNPs. * refers to significant at $P < 0.05$.

Table 4. 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 (*clk-5 – clk-12*).

NS in the table indicates non-significant ($P \geq 0.05$) *D'* and r^2 values.

D' values are listed in upper right section, and r^2 values are listed in lower left section.

Table 4. 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	0.0							
BS	0.16	0.0						
NA	0.56	0.38	0.0					
MW	0.90	0.74	0.30	0.0				
SB	0.16	0.10	0.37	0.74	0.0			
WH	0.68	0.51	0.19	0.26	0.48	0.0		
RP	0.51	0.34	0.03	0.25	0.34	0.12	0.0	
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 Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) and Commercial (CC) turkeys were used for the present study.

Table 4. 5. Haplogroup frequencies of *turClock* gene in eight turkey populations.

Turkey Varieties[1]	Haplogroups					
	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 Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) and Commercial (CC) turkeys were used for the present study.

N = numbers of birds from each variety used for calculation of haplogroup frequencies.

Table 4. 6. Associations between haplogroups of *turClock* gene and body weight (BW) at different ages of turkeys.

Haplogrops	BW[1] (kg)					
	1 d	34 d	68 d	159 d	231 d	309 d*
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 4. 7. Associations between haplogroups of *turClock* gene and average daily gain (ADG) by periods of age for turkeys.

Haplogroups	ADG[1] (kg)					
	1 – 34 d	35 – 68 d	69 – 159 d	160 – 231 d	232 – 309 d	1 – 309 d
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.

Table 4. 8. Associations between haplogroups of *turClock* gene and feed conversion ratio (FCR) by periods of age for turkeys.

Haplogroups	FCR[1]			
	34 – 68 d*	69 – 159 d*	160 – 231 d	34 – 231 d
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 4. 9. Associations between haplogroups of *turClock* gene and semen quality traits for turkeys.

Haplogroups	Semen quality traits[1]			
	Ejaculate volume (mL)	Sperm Concentration ($x10^9$/mL)	Total number of sperm ($x10^8$/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	$\underline{0.10 \pm 0.01}^a$	2.23 ± 0.20^a	$\underline{2.37 \pm 0.45}^a$	84.13 ± 0.98^a
Hap5	0.11 ± 0.01^a	$\underline{2.15 \pm 0.20}^a$	2.58 ± 0.45^a	$\underline{82.32 \pm 0.99}^a$

[a]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 10. Associations between haplogroups of *turClock* gene and egg production traits for turkeys.

Haplogroups	Egg production traits[1]				
	AFE[4] (d)	Egg production[2]		Average egg weight[3] (g)	
		6 wks*	10 wks*	6 wks*	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 ($P \leq 0.05$).

[1]Least square means ± SE. *$P \leq 0.05$.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks 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.

Table 4. 11. Associations between haplogroups of *turClock* gene and plasma melatonin concentration for turkeys.

Haplogroups	Plasma melatonin[1] (pg/ml)
Hap1	29.87 ± 5.01[a]
Hap2	**40.04 ± 5.55[a]**
Hap3	35.15 ± 6.13[a]
Hap4	32.35 ± 6.19[a]
Hap5	33.39 ± 7.52[a]

[a]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE.

Bold values represent the highest plasma melatonin haplogroups while underline values represent the lowest.

Table 4. 12. Associations between haplogroups of *turClock* gene and body weight (BW) at different ages within each variety of turkeys.

Haplo-	BW[1*] (kg)							
groups	BR	BS	CC	MW	NA	RP	SB	WH
Day 1								
Hap1	0.042 ±0.001	0.049±0.001	0.056 ± 0.001	-	0.046±0.003	0.047±0.003	0.046±0.001	-
Hap2	-	-	-	0.041 ±0.001	0.046±0.001	0.045±0.001	0.051±0.004	0.043 ± 0.001
Hap3	0.041 ±0.003	0.046±0.002	-	-	0.046±0.001	0.044 ±0.001	0.044 ±0.002	0.045 ± 0.001
Hap4	-	0.049±0.002	0.055 ± 0.001	0.041 ±0.003	0.045±0.004	0.046±0.002	0.050±0.001	0.044 ± 0.002
Hap5	-	0.046±0.001	-	-	0.047±0.002	0.045 ±0.002	0.045±0.003	-
Day 34								
Hap1	0.59 ± 0.03	0.60 ± 0.04	1.29 ± 0.02[a]	-	0.70 ± 0.08	0.54 ± 0.08	0.64 ± 0.03	-
Hap2	-	-	-	0.60 ± 0.02	0.64 ± 0.03	0.57 ± 0.03	0.62 ± 0.14	0.61 ± 0.04
Hap3	0.61 ± 0.09	0.62 ± 0.08	-	-	0.68 ± 0.05	0.55 ± 0.04	0.59 ± 0.07	0.63 ± 0.03
Hap4	-	0.54 ± 0.07	1.42 ± 0.05[b]	0.60 ± 0.09	0.61 ± 0.13	0.63 ± 0.07	0.64 ± 0.04	0.61 ± 0.05
Hap5	-	0.61 ± 0.03	-	-	0.69 ± 0.06	0.59 ± 0.06	0.74 ± 0.09	-

Day 68								
Hap1	1.68 ± 0.08	1.60 ± 0.01	4.43 ± 0.06[a]	-	1.45 ± 0.21[a]	1.39 ± 0.23	1.70 ± 0.08	-
Hap2	-	-	-	1.59 ± 0.07	1.83 ± 0.09[a,b]	1.62 ± 0.09	1.68 ± 0.38	1.74 ± 0.10
Hap3	1.77 ± 0.02	1.62 ± 0.21	-	-	2.02 ± 0.13[b]	1.54 ± 0.12	1.56 ± 0.20	1.70 ± 0.10
Hap4	-	1.70 ± 0.19	4.93 ± 0.14[b]	1.44 ± 0.25	1.68 ± 0.37[a,b]	1.73 ± 0.19	1.69 ± 0.11	1.69 ± 0.13
Hap5	-	1.62 ± 0.09	-	-	1.93 ± 0.18[a,b]	1.67 ± 0.16	1.84 ± 0.24	-
Day 159								
Hap1	5.24 ± 0.21	5.24 ± 0.27	17.21 ± 0.16[a]	-	5.63 ± 0.56[a,b]	4.45 ± 0.62	5.10 ± 0.22[a]	-
Hap2	-	-	-	4.74 ± 0.18	5.37 ± 0.23[a]	4.23 ± 0.24	4.51 ± 1.00[a,b]	5.55 ± 0.27
Hap3	5.02 ± 0.67	5.10 ± 0.56	-	-	6.13 ± 0.34[b]	4.12 ± 0.33	3.93 ± 0.53[b]	5.00 ± 0.26
Hap4	-	4.75 ± 0.49	17.86 ± 0.38[b]	4.25 ± 0.67	5.77 ± 0.99[a,b]	4.52 ± 0.49	4.76 ± 0.30[a,b]	5.43 ± 0.34
Hap5	-	5.05 ± 0.25	-	-	5.90 ± 0.48[a,b]	4.51 ± 0.43	5.94 ± 0.64[a]	-
Day 231								
Hap1	7.50 ± 0.24	7.31 ± 0.32	20.44 ± 0.18	-	7.59 ± 0.65[b]	6.57 ± 0.72[b]	7.37 ± 0.26[b]	-
Hap2	-	-	-	6.47 ± 0.20	7.79 ± 0.27	6.30 ± 0.28	6.56 ± 1.16[a,b]	7.16 ± 0.32
Hap3	7.71 ± 0.78	7.13 ± 0.65	-	-	7.92 ± 0.39	5.98 ± 0.40	5.27 ± 0.61[a]	6.58 ± 0.30

Hap4	-	6.86 ± 0.57	20.88 ± 0.45	6.02 ± 0.78[b]	8.02 ± 1.15	6.35 ± 0.78	6.61 ± 0.35[a,b]	7.30 ± 0.40
Hap5	-	7.16 ± 0.29	-	-	7.44 ± 0.55	6.31 ± 0.50	8.93 ± 0.74[b]	-
Day 309								
Hap1	7.83 ± 0.24	7.48 ± 0.32	20.92 ± 0.21	-	8.03 ± 0.64	6.39 ± 0.72	7.72 ± 0.26	-
Hap2	-	-	-	6.68 ± 0.20	7.70 ± 0.27	6.19 ± 0.28	7.62 ± 1.16	7.45 ± 0.32
Hap3	7.78 ± 0.78	6.99 ± 0.64	-	-	8.43 ± 0.39	5.83 ± 0.39	6.35 ± 0.61	6.87 ± 0.30
Hap4	-	7.14 ± 0.57	21.33 ± 0.46	6.11 ± 0.78	8.66 ± 1.15	6.69 ± 0.78	7.32 ± 0.35	7.52 ± 0.40
Hap5	-	7.51 ± 0.28	-	-	7.94 ± 0.55	6.79 ± 0.50	8.68 ± 0.74	-

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[*]Means within columns without any superscripts are not significantly different ($P \geq 0.05$).

[1]BW, body weight (kg) was measured at 1, 34, 68, 159, 231 and 309 days (d). Least square means ± SE.

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

Table 4. 13. Associations between haplogroups of *turClock* gene and average daily gain (ADG) by period of age within each variety of turkeys.

	ADG[1*] (kg)							
	BR	BS	CC	MW	NA	RP	SB	WH
1 – 34 d								
Hap1	0.017±0.001	0.017±0.001	0.037±0.001[a]	-	0.020±0.002	0. 015± 0.003	0.018±0.001	-
Hap2	-	-	-	0.017±0.001	0.018±0.001	0.016 ± 0.001	0.018± 0.004	0.017± 0.001
Hap3	0.018±0.003	0.017± 0.002	-	-	0.019±0.001	0.015 ± 0.001	0.016± 0.002	0.018± 0.001
Hap4	-	0.015 ± 0.001	0.041±0.002[b]	0.017±0.003	0.017±0.004	0.018 ± 0.002	0.018± 0.001	0.017± 0.001
Hap5	-	0.017 ± 0.001	-	-	0.019±0.002	0.017 ± 0.002	0.021± 0.003	-
35 – 68 d								
Hap1	0.032±0.002	0.029 ± 0.002	0.092±0.001[a]	-	0.022±0.005[a]	0.025±0.005	0.031±0.002	-
Hap2	-	-	-	0.029±0.002	0.035±0.002[b]	0.031±0.002	0.031±0.009	0.033±0.002
Hap3	0.034±0.006	0.030 ± 0.005	-	-	0.039±0.003[b]	0.029±0.003	0.028±0.005	0.031±0.002
Hap4	-	0.034 ± 0.004	0.103±0.003[b]	0.025±0.006	0.031±0.009[b]	0.032±0.004	0.030±0.003	0.032±0.003
Hap5	-	0.030 ± 0.002	-	-	0.037±0.004[b]	0.032±0.004	0.032±0.006	-

69–159 d								
Hap1	0.039±0.002	0.040±0.003	0.140±0.002	-	0.046±0.006	0.034 ± 0.007	0.037 ± 0.002[b]	-
Hap2	-	-	-	0.035±0.002	0.039±0.003	0.029 ± 0.003	0.031 ± 0.011[b]	0.042 ± 0.003
Hap3	0.036±0.007	0.038±0.006	-	-	0.045±0.004	0.028 ± 0.004	0.026 ± 0.006[a]	0.036 ± 0.003
Hap4	-	0.034±0.005	0.142±0.004	0.031±0.007	0.045±0.011	0.031 ± 0.006	0.034 ± 0.003[b]	0.041 ± 0.004
Hap5	-	0.038±0.003	-	-	0.044±0.005	0.031 ± 0.005	0.045 ± 0.007[b]	-
160-231d								
Hap1	0.032±0.003	0.029±0.004	0.046±0.002	-	0.027±0.007	0.030±0.008	0.032±0.003	-
Hap2	-	-	-	0.024±0.002	0.034±0.003	0.029±0.003	0.029±0.013	0.022±0.004
Hap3	0.038±0.009	0.028±0.007	-	-	0.025±0.004	0.026±0.005	0.018±0.007	0.023±0.003
Hap4	-	0.030±0.006	0.039±0.005	0.025±0.009	0.032±0.013	0.025±0.009	0.026±0.004	0.026±0.005
Hap5	-	0.029±0.003	-	-	0.022±0.006	0.025±0.006	0.041±0.008	-
232-309d								
Hap1	0.004±0.003	0.002±0.004	0.005±0.002	-	0.006±0.008	-0.003±0.009	0.004±0.003	-
Hap2	-	-	-	0.003 ±0.002	-0.001±0.003	-0.001±0.003	0.013±0.014	0.004±0.004
Hap3	0.001±0.009	-0.002±0.008	-	-	0.006±0.005	-0.002±0.005	0.014±0.007	0.004±0.004

Hap4	-	0.004±0.007	0.005±0.005	0.001 ±0.009	0.009±0.014	0.004±0.009	0.009±0.004	0.003±0.005
Hap5	-	0.004±0.003	-	-	0.006±0.007	0.006 ± 0.006	-0.003±0.009	-
1 – 309 d								
Hap1	0.025±0.001	0.024±0.001	0.068±0.001	-	0.026±0.002	0.021±0.002	0.025±0.001[a.b]	-
Hap2	-	-	-	0.022±0.001	0.025±0.001	0.020±0.001	0.025±0.004[a,b]	0.024±0.001
Hap3	0.026±0.002	0.023±0.002	-	-	0.027±0.001	0.019±0.001	0.020±0.002[a]	0.022±0.001
Hap4	-	0.023±0.002	0.069±0.001	0.020±0.001	0.028±0.004	0.022±0.003	0.024±0.001[a,b]	0.024±0.001
Hap5	-	0.024±0.001	-	-	0.026±0.002	0.022±0.002	0.028±0.002[b]	-

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[*]Means within columns without any superscripts are not significantly different ($P \geq 0.05$).

[1]ADG, average daily gain (kg) was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 14. Associations between haplogroups of *turClock* gene and feed conversion ratio (FCR) by period of age within each variety of turkeys

Haplogroups	FCR[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
34 – 68 d								
Hap1	3.08 ± 0.19	3.38 ± 0.24	2.03 ± 0.14	-	7.04 ± 0.51[a]	2.59 ± 0.53	2.95 ± 0.20	-
Hap2	-	-	-	3.20 ± 0.15	2.39 ± 0.21[b]	2.44 ± 0.21	2.29 ± 0.87	2.65 ± 0.25
Hap3	2.89 ± 0.61	3.16 ± 0.51	-	-	2.20 ± 0.30[b]	2.55 ± 0.29	2.82 ± 0.46	2.87 ± 0.22
Hap4	-	3.05 ± 0.44	1.90 ± 0.31	3.87 ± 0.61	3.40 ± 0.88[b]	2.46 ± 0.44	2.97 ± 0.26	2.73 ± 0.31
Hap5	-	3.44 ± 0.22	-	-	2.32 ± 0.44[b]	2.30 ± 0.39	3.42 ± 0.56	-
69 – 159 d								
Hap1	5.40 ± 0.18	4.16±0.24	3.31 ± 0.14	-	4.51 ± 0.48	4.82±0.54	4.39±0.19[a,c]	-
Hap2	-	-	-	4.43 ±0.15	4.88 ± 0.20	5.58 ± 0.21	4.78± 0.87[a,b]	3.81 ± 0.24
Hap3	5.55 ± 0.58	4.10±0.48	-	-	4.39 ± 0.29	5.38 ± 0.28	5.81 ± 0.46[b]	4.42 ± 0.22
Hap4	-	4.79±0.43	3.23 ± 0.33	4.68 ±0.58	3.94 ± 0.86	5.20 ± 0.43	4.93± 0.26[b,c]	3.84 ± 0.30
Hap5	-	4.41±0.21	-	-	4.80 ± 0.41	4.91 ± 0.37	3.61 ± 0.55[a]	-

160 – 231 d								
Hap1	10.27 ± 0.90	12.04±1.17	13.26±0.68	-	11.07 ± 2.39	11.43±2.69	9.25 ± 0.96[a]	-
Hap2	-	-	-	13.47 ± 0.76	10.19 ± 0.99	11.34±1.04	9.57 ± 4.30[a]	12.58 ± 1.18
Hap3	8.17 ± 2.89	10.21±2.39	-	-	13.28 ± 1.44	11.86±1.47	15.12±2.27[b]	13.54 ± 1.10
Hap4	-	10.60±2.12	15.03±1.68	14.06 ± 2.89	8.62 ± 4.27	9.98±2.89	13.18±1.29[b]	10.82 ± 1.47
Hap5	-	11.91±1.06	-	-	14.16 ± 2.04	11.00±1.84	7.72±2.74[a]	-
34 – 231d								
Hap1	7.68 ± 0.27	7.08±0.35	5.90 ± 0.21	-	7.33 ± 0.73	7.47±0.81[a]	6.75±0.29[a]	-
Hap2	-	-	-	8.32 ± 0.23	7.07 ± 0.30	7.81±0.31[a]	7.42±1.30[a,b]	6.86 ± 0.36
Hap3	6.92 ± 0.88	6.48±0.72	-	-	6.90 ± 0.44	8.02±0.44[a]	9.57± 0.69[b]	7.22 ± 0.33
Hap4	-	7.25±0.64	5.72 ± 0.51	8.36 ± 0.88	6.33 ± 1.29	10.28± 0.88[b]	8.18±0.39[b]	6.64 ± 0.45
Hap5	-	7.33±0.32	-	-	7.70 ± 0.62	7.67±0.56[a]	5.83 ±0.83[a]	-

[a,b,c]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

[*]Means within columns without any superscripts are not significantly different ($P \geq 0.05$).

[1]FCR, feed conversion ratio was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 15. Associations between haplogroups of *turClock* gene and egg production traits within each variety of turkeys

Haplogroups	Egg production traits[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
AFE[2] (d)								
Hap1	243.13±10.40	233.50±20.81	184.05±6.42	-	225.50±20.81[a]	246.50±20.81	224.38±10.40	-
Hap2	-	-	-	223.92±8.16	226.75±10.40[a]	242.75±10.41	218.00±29.43	257.75±14.71
Hap3	-	261.00±29.43	-	-	214.00±14.71[a]	248.00±13.16	235.67±16.98	249.54±8.16
Hap4	-	-	161.33±16.99	212.00±29.43	256.00±29.42[a,b]	239.00±29.43	239.33±16.98	257.33±16.98
Hap5	-	251.00±14.71	-	-	291.00±29.43[b]	255.00±29.43	-	-
Egg production[3]								
6 wks								
Hap1	6.00 ± 3.64	9.70 ± 3.25	17.37 ± 2.10	-	28.50 ± 7.28	3.67 ± 5.94	14.11 ± 3.43	-
Hap2	-	-	-	25.21 ± 2.75[a]	19.12 ± 3.64	11.50 ± 2.97	21.00 ± 10.30	4.83 ± 4.21
Hap3	-	-	-	-	19.75 ± 5.15	15.40 ± 4.61	5.60 ± 4.61	8.92 ± 2.85
Hap4	-	2.00 ±5.95	8.28 ± 3.89	29.00 ± 10.30[b]	17.00 ± 10.30	7.33 ± 5.94	12.20 ± 4.61	4.00 ± 5.95
Hap5	-	7.00 ± 3.89	-	-	2.50 ± 7.28	1.00 ± 7.28	-	-

Egg weight[4] (6 wks)								
Hap1	76.01 ± 1.25	75.33 ± 1.40	92.61 ± 0.61[a]	-	76.75 ± 1.98	78.36 ± 1.98	77.85 ± 0.99[a]	-
Hap2	-	-	-	73.39 ± 0.78	77.57 ± 0.99	77.90 ± 0.93	75.95 ± 2.80[a,b]	78.34±1.16
Hap3	-	-	-	-	78.86 ± 1.40	76.00 ± 1.25	74.17 ± 2.80[a,b]	76.14±0.93
Hap4	-	74.18 ± 2.80	88.12± 1.62[b]	74.62 ± 2.80	78.37 ± 2.80	77.72 ± 2.80	72.14 ± 1.62[b]	76.87±1.98
Hap5	-	75.26 ± 1.98	-	-	75.36 ± 2.80	78.85 ± 2.80	-	-
Egg production								
10 wks								
Hap1	12.87±5.29	13.50±4.73	26.46 ±3.05	-	40.00 ± 10.57	6.33 ± 8.63	20.22 ± 4.98	-
Hap2	-	-	-	35.28 ± 3.99[a]	28.00 ± 5.29	17.92 ± 4.32	35.00 ±14.95	10.17 ± 6.10
Hap3	-	-	-	-	29.75 ± 7.48	24.80 ± 6.69	9.60 ± 6.68	17.15 ± 4.15
Hap4	-	4.33 ±8.63	14.57 ±5.65	41.00± 14.95[b]	26.00 ± 14.95	11.67 ± 8.63	17.20 ± 6.68	10.00 ± 8.63
Hap5	-	8.71 ± 5.65	-	-	4.51 ± 10.57	2.00 ± 10.57	-	-
Egg weight (10 wks)								
Hap1	76.07 ± 0.96	75.50 ± 1.36	92.07 ± 0.58	-	76.51 ± 1.92	78.61 ± 1.92[a,b]	76.81 ± 0.90[a]	-
Hap2	-	-	-	73.70 ± 0.75	77.19 ± 0.96	77.77 ± 0.90[a,b]	75.40 ± 2.71[a,b]	77.38 ± 1.36

Hap3	-	74.66 ± 2.71	-	-	78.53 ± 1.36	76.52 ± 1.21[a]	73.45 ± 1.92[a,b]	76.32 ± 0.75
Hap4	-	-	91.40 ± 1.57	74.77 ± 2.71	77.92 ± 2.71	77.27 ± 2.71[a,b]	72.46 ± 1.57[b]	77.94 ± 1.57
Hap5	-	75.81 ± 1.92	-	-	76.13 ± 2.71	82.25 ± 2.71[b]	-	-

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$)

[*]Means within columns without any superscripts are not significantly different ($P \geq 0.05$).

[1]Least square means ± SE.

[2]AFE, age at first egg was recorded and given in days (d).

[3]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[4]The average egg weight (g) was calculated for the period of 6 wks and 10 wks separately for each hen.

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

Table 4. 16. Associations between haplogroups of *turClock* gene and semen quality traits within each variety of turkeys.

Haplogroups	Semen quality traits[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
Ejaculate volume (mL)								
Hap1	0.06±0.01	0.08±0.02	0.14±0.01	-	0.18±0.05[a,b]	-	0.11±0.02	-
Hap2	-	-	-	0.14±0.01[a]	0.17±0.02[a,b]	0.10±0.02	-	0.08 ± 0.02
Hap3	0.01 ± 0.05	0.12±0.03	-	-	0.22 ± 0.02[a]	0.11±0.03	-	0.10 ± 0.02
Hap4	-	0.06±0.05	0.13±0.05	0.04±0.05[b]	-	0.08±0.05	0.09±0.02	0.11 ± 0.02
Hap5	-	0.12±0.02	-	-	0.14±0.03[b]	0.11±0.03	0.08±0.03	-
Sperm concentration (x10^9/mL)								
Hap1	1.78 ± 0.19	2.69 ± 0.33	2.20 ± 0.21	-	2.31 ± 0.66	-	2.14 ± 0.22	-
Hap2	-	-	-	2.63 ± 0.17	2.26 ± 0.22	1.99 ± 0.30	-	2.87 ± 0.27
Hap3	2.13 ± 0.66	2.21 ± 0.47	-	-	2.22 ± 0.33	2.41 ± 0.38	-	2.65 ± 0.33
Hap4	-	2.69 ± 0.66	2.84 ± 0.66	1.83 ± 0.66	-	2.43 ± 0.66	2.23 ± 0.33	2.29 ± 0.30
Hap5	-	2.35 ± 0.24	-	-	2.95 ± 0.47	2.07 ± 0.47	1.46 ± 0.38	-
Total sperm number (x10^8/ejaculate)								

Hap1	1.06 ± 0.45	2.03 ± 0.78	3.13 ± 0.49	-	4.19 ± 1.56	-	2.38 ± 0.52	-
Hap2	-	-	-	3.54 ± 0.40[a]	4.06 ± 0.52	1.95 ± 0.70	-	2.76 ± 0.64
Hap3	0.28 ± 1.56	2.59 ± 1.10	-	-	4.67 ± 0.78	2.51 ± 0.90	-	2.54 ± 0.78
Hap4	-	1.70 ± 1.56	3.74 ± 1.56	0.64 ± 1.56[b]	-	1.98 ± 1.56	2.16 ± 0.78	2.59 ± 0.70
Hap5	-	2.82 ± 0.55	-	-	4.04 ± 1.10	2.10 ± 1.10	1.19 ± 0.90	-
Sperm viability (%)								
Hap1	82.67± 0.97	78.25± 1.68	86.80 ±1.07	-	89.50 ±3.37[a]	-	81.75 ±2.38	-
Hap2	-	-	-	83.27 ±0.87	83.67±1.12[a,b]	83.50 ±1.51	-	84.83 ±1.37
Hap3	82.50± 3.37	81.75± 2.38	-	-	84.50±1.68[a,b]	82.33 ±1.94	-	85.38 ±1.68
Hap4	-	83.50± 3.37	84.50 ±3.37	87.50 ±3.37	-	85.50 ±3.37	83.88 ±1.68	84.20 ±1.51
Hap5	-	78.63 ±1.19	-	-	81.00 ±2.38[b]	83.75 ±2.38	82.33 ±1.94	-

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[*]Means within columns without any superscripts are not significantly different ($P \geq 0.05$).

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

Table 4. 17. Associations between haplogroups of *turClock* gene and plasma melatonin concentration within each variety of turkeys.

Haplogroups	Plasma melatonin[1*] (pg/ml)							
	BR	BS	CC	MW	NA	RP	SB	WH
Hap1	38.39 ± 7.68	30.88 ± 10.84[a]	35.82 ± 6.35	-	18.61 ± 2.44[b]	30.17 ± 3.19[a]	39.18 ± 8.21	-
Hap2	-	-	-	38.47±6.47[a]	43.58 ± 8.24[a]	11.01 ± 8.84[b]	-	41.46 ± 10.05[a]
Hap3	31.73±4.48	16.24 ± 2.42[b]	-	-	45.43± 13.22[a]	18.70 ± 1.95[b]	37.61 ± 9.80	33.16 ± 9.56[a]
Hap4	-	38.74 ± 8.12[a]	31.19 ± 4.46	14.08±4.63[b]	66.17 ± 6.39[a]	15.56 ± 4.63[b]	50.64 ± 1.01	27.12 ± 12.53[b]
Hap5	-	32.32 ± 9.01[a]	-	-	9.25 ± 7.41[b]	13.61 ± 5.71[b]	79.44 ± 3.50	-

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[*]Means within columns without any superscripts are not significantly different ($P \geq 0.05$).

[1]Least square means ± SE.

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

Table 4. 18. Primer sequences, expected sizes of amplicons and PCR characteristics for *turPeriod-3* gene.

Primer ID	Primers[1]	Sequences	Tm[2] (^{0}C)	Amplicon length[3] (bp)
Per_1	For(281658)	5′-GTGTCTGACTACAGATAGCATTAAAACAAAT-3′	62.0	4800
	Rev(276851)	5′-AATAGTTTACGACTGTTTACAGCTGCATTA -3′	61.9	
Per_2	For(277117)	5′-CTGTAATTTTGTAATGTCTCTTTGAGTTACTG-3′	62.1	4000
	Rev(273122)	5′-CAATTACGTTACTTTACCTTCTAGTACTTCTG-3′	63.4	
Per_3	For(273542)	5′-TACGAGAGTGGTGTTATTAACTTCTGTAGG-3′	64.6	4800
	Rev(268757)	5′-GGGAGACAGAGGGAGTAATTAGAAAAGTAT-3′	64.6	
Per_4	For(269236)	5′-GCTAAATGTGTAAGTAACAGAGATTTTTAGTG-3′	62.1	3000
	Rev(266155)	5′-CTTTAGAAAGGAACAAGGACATATCATTTA-3′	60.5	

[1]*For, forward primer; Rev, reverse primer.* Primer-binding sites in the turkey genome (GenBank accession No: LOC100008578) 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 4. 19. Characteristics of single nucleotide polymorphisms (SNPs) identified in the *turPeriod-3* gene in eight divergent turkey varieties.

SNP	Location	Nucleotide position[1]	Sequence Context[2]	*dbSNP* Identification[3]	Genotype	Genotype Frequency %	MAF[4]	HWE[5]
Per3-1	Exon 19	269002	ATAAA(*A*/G)AAAGC	ss538305370	A/A	25.17	0.49	NS
					A/G	48.28		
					G/G	26.55		
Per3-2	Exon 19	269008	AAAGC(A/*G*)TTGCC	ss538305371	A/A	91.38	0.05	0.00*
					A/G	6.90		
					G/G	1.72		
Per3-3	Exon 18	269119	TCAAA(C/*T*)GTAGT	ss538305372	C/C	91.38	0.05	0.00*
					C/T	6.90		
					T/T	1.72		
Per3-4	Intron 6	276755	CACTA(A/*T*)CAAAT	ss538305373	A/A	87.93	0.07	0.01*
					A/T	10.34		
					T/T	1.73		
Per3-5	Intron 6	276883	CACAC(*A*/T)CCTCA	ss538305374	A/A	1.03	0.04	0.01*

					A/T	6.56		
					T/T	92.41		
Per3-6	Intron 6	276891	CTCACA(A/*G*)TCCTT	ss538305375	A/A	90.34	0.05	NS
					A/G	8.96		
					G/G	0.70		
Per3-7	Intron 5	277055	ACTTA(A/*G*)TTCTG	ss538305376	A/A	93.79	0.03	NS
					A/G	5.52		
					G/G	0.69		

[1]Position of the SNP in GenBank on the forward strand of chromosome 23 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]*ss* prefix indicates novel SNPs detected here and submitted for *rs* assignment in *dbSNP*, NCBI.

[4]Minor allele frequency (MAF) of 7 SNPs markers.

[5]Significance of deviation from HWE for the 7 SNPs. NS indicates non-significant ($P > 0.05$) while * refers to significant at $P < 0.05$.

Table 4. 20. Linkage disequilibrium as measured by *D´* and r^2 between the 7 segregating SNPs in the *turpPeriod-3* gene.

SNPs[1]	*Per3-1*	*Per3-2*	*Per3-3*	*Per3-4*	*Per3-5*	*Per3-6*	*Per3-7*
Per3-1		0.69	0.68	0.66	0.76	NS	NS
Per3-2	0.03		0.96	NS	0.12	0.37	0.34
Per3-3	0.03	0.89		NS	0.11	0.37	0.34
Per3-4	0.04	NS	NS		NS	NS	NS
Per3-5	0.03	0.01	0.01	NS		0.15	0.13
Per3-6	NS	0.13	0.13	NS	0.02		0.91
Per3-7	NS	0.09	0.09	NS	0.02	0.66	

[1]SNP identification (*Per3-1 – Per3-7*).

NS in the table indicates non-significant ($P \geq 0.05$) *D'* and r^2 values.

D' values are listed in upper right section, and r^2 values are listed in lower left section.

Table 4. 21. The pairwise fixation index (*Fst*) estimated among turkey varieties using *turPeriod-3* SNPs**.**

Turkey varieties[1]	BR	BS	NA	MW	SB	WH	RP	CC
BR	0.0							
BS	0.41	0.0						
NA	0.40	0.06	0.0					
MW	0.39	0.37	0.23	0.0				
SB	0.39	0.03	0.15	0.40	0.0			
WH	0.37	0.18	0.05	0.07	0.25	0.0		
RP	0.41	0.06	0.01*	0.22	0.15	0.04	0.0	
CC	0.43	0.05	0.01*	0.24	0.15	0.06	0.01*	0.0

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

* $P \geq 0.05$

Table 4. 22. Haplogroup frequencies of *turPeriod-3* gene in eight turkey populations.

Turkey Varieties		Haplogroups					
	N	Hap1	Hap2	Hap3	Hap4	Hap5	Hap6
BR	23	1.00	0.00	0.00	0.00	0.00	0.00
BS	36	0.03	0.22	0.31	0.00	0.00	0.44
CC	50	0.00	0.04	0.10	0.08	0.02	0.76
MW	31	0.00	0.03	0.03	0.42	0.39	0.13
NA	33	0.00	0.00	0.27	0.21	0.09	0.43
RP	39	0.00	0.03	0.15	0.18	0.05	0.59
SB	37	0.00	0.32	0.32	0.03	0.08	0.25
WH	37	0.00	0.00	0.05	0.41	0.30	0.24

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

N = numbers of birds from each variety used for calculation of haplogroup frequencies.

Table 4. 23. Associations between haplogroups of *turPeriod-3* gene and body weight (BW) at different ages of turkeys.

Haplogroups	BW[1] (kg)					
	1 d	34 d	68 d	159 d	231 d*	309 d
Hap1	0.046 ± 0.004[a]	0.63 ± 0.12[a]	1.97 ± 0.32[a]	6.45 ± 0.85[a]	8.65 ± 0.99[a,b]	8.79 ± 0.98[a]
Hap2	0.046 ± 0.001[a]	0.68 ± 0.03[a]	1.98 ± 0.09[a]	6.66 ± 0.25[a]	**8.99 ± 0.28[a]**	9.12 ± 0.28[a]
Hap3	0.046 ± 0.001[a]	0.71 ± 0.03[a]	2.02 ± 0.07[a]	6.56 ± 0.19[a]	8.42 ± 0.22[b]	8.76 ± 0.22[a]
Hap4	0.046 ± 0.001[a]	0.69 ± 0.03[a]	2.05 ± 0.08[a]	**6.75 ± 0.20[a]**	8.91 ± 0.20[a]	9.10 ± 0.23[a]
Hap5	0.045 ± 0.001[a]	0.70 ± 0.03[a]	2.01 ± 0.09[a]	6.39 ± 0.23[a]	8.52 ± 0.27[a,b]	8.83 ± 0.26[a]
Hap6	**0.047 ± 0.001[a]**	**0.73 ± 0.03[a]**	**2.07 ± 0.06[a]**	6.56± 0.16[a]	8.78 ± 0.18[a,b]	**9.14 ± 0.18[a]**

[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 4. 24. Associations between haplogroups of *turPeriod-3* gene and average daily gain (ADG) by periods of age for turkeys.

Haplogroups	ADG[1] (kg)					
	1-34 d	35 – 68 d	69 – 159 d	160 – 231 d*	232 – 309 d	1 – 309 d
Hap1	0.018 ± 0.003[a]	0.039 ± 0.007[a]	0.049 ± 0.009[a]	0.031 ± 0.011[a,b]	0.002 ± 0.012[a]	0.028 ± 0.003[a]
Hap2	0.019 ± 0.001[a]	0.038 ± 0.002[a]	0.051 ± 0.003[a]	**0.033 ± 0.003[a]**	0.002 ± 0.003[a]	0.029 ± 0.001[a]
Hap3	0.020 ± 0.001[a]	0.039 ± 0.002[a]	0.050 ± 0.002[a]	0.026 ± 0.002[b]	**0.004 ± 0.003[a]**	0.028 ± 0.001[a]
Hap4	0.019 ± 0.001[a]	**0.040 ± 0.002[a]**	**0.052 ± 0.002[a]**	0.029 ± 0.002[a,b]	0.002 ± 0.003[a]	0.029 ± 0.001[a]
Hap5	0.020 ± 0.001[a]	0.039 ± 0.002[a]	0.048 ± 0.002[a]	0.030 ± 0.003[a,b]	**0.004 ± 0.003[a]**	0.029 ± 0.001[a]
Hap6	**0.021 ± 0.001[a]**	0.039 ± 0.001[a]	0.049 ± 0.002[a]	0.031 ± 0.002[a,b]	**0.004 ± 0.002[a]**	**0.030 ± 0.001[a]**

[a,b]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. *$P \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 25. Associations between haplogroups of *turPeriod-3* gene and feed conversion ratio (FCR) by periods of age for turkeys.

Haplogroups	FCE[1]			
	34 – 68 d	69 – 159 d*	160 – 231 d*	34 - 231
Hap1	**2.77 ± 0.88[a]**	4.54 ± 0.75[a,b]	**10.65 ± 3.64[a]**	**6.99 ± 1.16[a]**
Hap2	2.85 ± 0.25[a]	4.44 ± 0.22[a,b]	11.72 ± 1.05[a]	7.06 ± 0.33[a]
Hap3	2.87 ± 0.20[a]	4.57 ± 0.17[a,b]	13.36 ± 0.81[b]	7.61 ± 0.26[a]
Hap4	2.81 ± 0.21[a]	**4.39 ± 0.18[a]**	11.49 ± 0.86[a]	7.31 ± 0.27[a]
Hap5	2.80 ± 0.23[a]	4.77 ± 0.20[b]	12.58 ± 0.98[a]	7.58 ± 0.31[a]
Hap6	2.88 ± 0.17[a]	4.52 ± 0.14[a,b]	11.61 ± 0.68[a]	7.27 ± 0.22[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 4. 26. Associations between haplogroups of *turPeriod-3* gene and egg production traits for turkeys.

Haplogroups	Egg production traits[1]				
	AFE[4] (d)*	Egg production[2]		Average egg weight[3] (g)	
		6 wks	10 wks*	6 wks*	10 wks*
Hap1	243.13 ± 13.01[a]	5.91 ± 9.43[a]	10.30 ± 5.15[a]	76.01 ± 3.01[a]	76.05 ± 2.27[a]
Hap2	240.00 ± 16.46[a,b]	13.00 ± 3.84[a]	12.30 ± 5.15[a]	77.69 ± 3.01[a]	77.68 ± 2.87[a]
Hap3	227.55 ± 8.23[a,b]	14.58 ± 2.48[a]	20.07 ± 3.08[a,b]	77.81 ± 1.51[a]	77.86 ± 1.37[a]
Hap4	238.25 ± 8.23[a,b]	12.81 ± 2.82[a]	19.87 ± 3.40[a,b]	77.27 ± 1.55[a]	76.79 ± 1.40[a]
Hap5	231.33 ± 9.50[a,b]	**15.78 ± 3.30[a]**	**27.94 ± 4.08[b]**	76.16± 1.87[a]	76.07 ± 1.66[a]
Hap6	**217.58 ± 5.06[b]**	12.91 ± 1.97[a]	19.34 ± 1.91[a]	**82.90 ± 0.96[b]**	**82.89 ± 0.89[b]**

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE. *$P \leq 0.05$.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks 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.

Table 4. 27. Associations between haplogroups of *turPeriod-3* gene and semen quality traits for turkeys.

Haplogroups	Semen quality traits[1]			
	Ejaculate volume (mL)*	Sperm Concentration ($x10^9$/mL)*	Total number of sperm ($x10^8$/ejaculate)*	Sperm viability (%)
Hap1	0.05 ± 0.01[a]	1.81 ± 0.18[a]	1.00 ± 0.44[a]	82.65 ± 1.04[a]
Hap2	0.12 ± 0.01[b]	2.19 ± 0.17[a]	2.66 ± 0.42[b,c,d]	83.00 ± 1.00[a]
Hap3	0.11 ± 0.01[b]	2.18 ± 0.16[a]	2.58 ± 0.38[c]	83.88 ± 0.91[a]
Hap4	**0.14 ± 0.01[b]**	**2.65 ± 0.14[b]**	**3.68 ± 0.34[d]**	**84.18 ± 0.80[a]**
Hap5	0.12 ± 0.01[b]	2.30 ± 0.16[b]	2.63 ± 0.39[c]	83.19 ± 0.94[a]
Hap6	0.12 ± 0.01[b]	2.35 ± 0.12[b]	2.79 ± 0.28[c]	82.89 ± 0.67[a]

[a,b,c,d]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE. *$P \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 28. Associations between haplogroups of *turPeriod-3* gene and plasma melatonin concentration for turkeys.

Haplogroups	Plasma melatonin[1] (pg/ml)*
Hap1	25.01 ± 7.47[b]
Hap2	**52.84 ± 8.80[a]**
Hap3	41.60 ± 6.86[a]
Hap4	31.22 ± 7.19[b]
Hap5	26.86 ± 8.25[b]
Hap6	37.23 ± 5.73[b]

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE. *$P \leq 0.05$.

Bold values represent the highest plasma melatonin haplogroups while underline values represent the lowest.

Table 4. 29. Associations between haplogroups of *turPeriod-3* gene and body weight (BW) at different ages within each variety turkeys.

Haps[2]	BW[1*] (kg)							
	BR	BS	CC	MW	NA	RP	SB	WH
Day 1								
Hap1	0.041±0.001	0.047±0.004	-	-	-	-	-	-
Hap2	-	0.047±0.001	0.057±0.003	0.049±0.004	-	0.047±0.004	0.046±0.001	-
Hap3	-	0.048±0.001	0.055±0.002	0.041±0.004	0.044±0.001	0.042±0.002	0.046±0.001	0.049±0.003
Hap4	-	-	0.057±0.002	0.040±0.001	0.048±0.002	0.046±0.002	0.045±0.004	0.043±0.001
Hap5	-	-	0.052±0.004	0.041±0.001	0.042±0.002	0.044±0.003	0.047±0.002	0.044±0.001
Hap6		0.047±0.001	0.056±0.001	0.042±0.002	0.047±0.001	0.045±0.001	0.047±0.001	0.045±0.001
Day 34								
Hap1	0.61 ± 0.03	0.50 ± 0.13	-	-	-	-	-	-
Hap2	-	0.56 ± 0.05	1.21 ±0.09[a,b]	0.53 ± 0.13	-	0.66 ± 0.13	0.64 ± 0.04	-
Hap3	-	0.62 ± 0.04	1.32 ± 0.06[b]	0.59 ± 0.13	0.64 ± 0.04	0.54 ± 0.05	0.63 ± 0.04	0.65 ± 0.09
Hap4	-	-	1.13 ± 0.06[a]	0.62 ± 0.04	0.67 ± 0.05	0.56 ± 0.05	0.64 ± 0.13	0.61 ± 0.03

Hap5	-	-	1.30 ± 0.13[b]	0.58 ± 0.04	0.63 ± 0.07	0.58 ± 0.09	0.63 ± 0.07	0.62 ± 0.04
Hap6	-	0.61 ± 0.03	1.32 ± 0.02[b]	0.62 ± 0.06	0.67 ± 0.03	0.58 ± 0.03	0.67 ± 0.04	0.63 ± 0.04
Day 68								
Hap1	1.72 ± 0.08	1.48 ± 0.37		-	-	-	-	-
Hap2	-	1.68 ± 0.13	4.45 ± 0.26[a]	1.41 ± 0.37	-	1.79 ± 0.37	1.64 ± 0.10	-
Hap3	-	1.55 ± 0.11	4.52 ± 0.16[a]	1.31 ± 0.37	1.82 ± 0.12	1.56 ± 0.15	1.69 ± 0.10	1.71 ± 0.26
Hap4	-	-	4.25 ± 0.18[a]	1.60 ± 0.10	1.97 ± 0.14	1.58 ± 0.14	1.80 ± 0.37	1.68 ± 0.09
Hap5	-	-	3.15 ± 0.37[b]	1.58 ± 0.11	1.95 ± 0.21	1.59± 0.26	1.56 ± 0.21	1.73 ± 0.11
Hap6	-	1.64 ± 0.09	4.31 ± 0.07[a]	1.67 ± 0.18	1.79 ± 0.10	1.64 ± 0.08	1.77± 0.12	1.76 ± 0.13
Day 159								
Hap1	5.22 ± 0.20	4.98 ± 0.98	-	-	-	-	-	-
Hap2	-	5.19 ± 0.34	17.78 ± 0.70	5.18 ± 0.99	-	4.88± 0.99	4.89 ± 0.28	-
Hap3	-	5.29 ± 0.30	17.18 ± 0.43	4.37 ± 0.99	5.55 ± 0.32	4.07± 0.40	4.95 ± 0.28	5.37 ± 0.69
Hap4	-	-	17.54 ± 0.49	4.88 ± 0.27	6.11 ± 0.37	4.22 ± 0.37	4.35 ± 0.98	5.42 ± 0.25
Hap5	-	-	17.59 ± 0.98	4.50 ± 0.28	5.53 ± 0.56	4.03 ± 0.69	4.02 ± 0.56	5.27 ± 0.29
Hap6	-	4.94 ± 0.24	17.25 ± 0.17	4.72 ± 0.42	5.49 ± 0.26	4.37 ± 0.23	5.40 ± 0.33	5.16 ± 0.34

Day 231								
Hap1	7.53 ± 0.23	7.09 ± 1.11		-	-	-	-	-
Hap2	-	7.71 ± 0.78	22.54 ± 0.79	6.39 ± 1.11	-	7.01 ± 1.11	7.10 ± 0.32	-
Hap3	-	7.15 ± 0.33	19.57 ± 0.49	6.23 ± 1.11	7.26 ± 0.36	6.23 ± 0.45	6.76 ± 0.32	7.08 ± 0.77
Hap4	-	-	21.59 ± 0.63	6.42 ± 0.31	8.53 ± 0.41	6.10 ± 0.41	7.25 ± 1.11	7.07 ± 0.27
Hap5	-	-	20.64 ± 1.11	6.48 ± 0.32	7.16 ± 0.63	5.03 ± 1.11	6.05 ± 0.63	6.94 ± 0.33
Hap6	-	7.04 ± 0.27	20.38 ± 0.20	6.45 ± 0.55	7.81 ± 0.29	6.35 ± 0.26	7.92 ± 0.37	7.07 ± 0.38
Day 309								
Hap1	7.83 ± 0.23	7.19 ± 1.09		-	-	-	-	-
Hap2	-	7.72 ± 0.38	21.71 ± 0.79	6.74 ± 1.10	-	7.05 ± 1.10	7.37 ± 0.31	-
Hap3	-	7.43 ± 0.33	19.58 ± 0.48	6.50 ± 1.10	7.77 ± 0.36	5.84 ± 0.45	7.52 ± 0.31	7.34 ± 0.76
Hap4	-	-	20.48 ± 0.63	6.60± 0.30	8.72 ± 0.41	6.13 ± 0.41	7.98 ± 1.09	7.37 ± 0.29
Hap5	-	-	20.14 ± 1.10	6.73 ± 0.31	7.63 ± 0.63	6.15 ± 1.10	6.27 ± 0.63	7.25 ± 0.33
Hap6	-	7.24 ± 0.27	21.25 ± 0.23	6.57 ± 0.54	7.77 ± 0.29	6.37 ± 0.26	8.32 ± 0.36	7.21 ± 0.37

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]BW, body weight (kg) was measured at 1, 34, 68, 159, 231 and 309 days (d). Least square means ± SE.

[2]Haps = haplogroups.

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

Table 4. 30. Associations between haplogroups of *turPeriod-3* gene and average daily gain (ADG) by periods of age within each variety of turkeys.

Haplogroups	ADG[1]* (kg)							
	BR	BS	CC	MW	NA	RP	SB	WH
1 – 34 d								
Hap1	0.017±0.001	0.015±0.004	-	-	-	-	-	-
Hap2	-	0.015±0.001	0.034±0.003	0.014±0.003	-	0.018±0.004	0.018±0.001	-
Hap3	-	0.017±0.001	0.038±0.002	0.017±0.004	0.018±0.001	0.015±0.002	0.018±0.001	0.018±0.003
Hap4	-	-	0.033±0.002	0.017±0.001	0.019±0.001	0.016±0.001	0.018±0.004	0.017±0.001
Hap5	-	-	0.039±0.004	0.016±0.001	0.018±0.002	0.017±0.003	0.017±0.002	0.018±0.001
Hap6	-	0.017±0.001	0.039±0.001	0.018±0.002	0.019±0.001	0.016±0.001	0.019±0.001	0.018±0.001
35 – 68 d								
Hap1	0.032±0.002	0.031±0.008		-	-	-	-	-
Hap2	-	0.033±0.003	0.082±0.006[a]	0.025±0.008	-	0.033±0.008	0.029±0.002	-
Hap3	-	0.028±0.003	0.097±0.004[a]	0.022±0.008	0.035±0.003	0.030±0.003	0.031±0.002	0.031±0.006
Hap4	-	-	0.098±0.004[a]	0.029±0.002	0.038±0.003	0.030±0.003	0.034±0.008	0.031±0.002

Hap5	-	-	0.067±0.008[b]	0.029±0.002	0.039±0.005	0.030±0.006	0.027±0.005	0.033±0.002
Hap6	-	0.030±0.002	0.094±0.001[a]	0.031±0.004	0.033±0.002	0.031±0.002	0.032±0.003	0.033±0.003
69 – 159 d								
Hap1	0.039±0.002	0.038±0.011	-	-	-	-	-	-
Hap2	-	0.039±0.004	0.152±0.008	0.042±0.011	-	0.034±0.011	0.036±0.003	-
Hap3	-	0.041±0.003	0.138±0.005	0.033±0.011	0.041±0.004	0.027±0.004	0.036±0.003	0.040±0.008
Hap4	-	-	0.144±0.005	0.036±0.003	0.046±0.004	0.029±0.004	0.039±0.011	0.041±0.003
Hap5	-	-	0.154±0.011	0.032±0.003	0.039±0.006	0.027±0.008	0.027±0.006	0.039±0.003
Hap6	-	0.036±0.003	0.140±0.002	0.034±0.005	0.041±0.003	0.030±0.002	0.040±0.036	0.037±0.004
160 – 231 d								
Hap1	0.032±0.003	0.030±0.013	-	-	-	-	-	-
Hap2	-	0.030±0.004	0.066±0.009[a]	0.017±0.013	-	0.029±0.013	0.031±0.004	-
Hap3	-	-	0.033±0.006[b]	0.026±0.013	0.024±0.004	0.030±0.005	0.025±0.004	0.024±0.009
Hap4	-	-	0.049±0.007[a,b]	0.021±0.004	0.034±0.005	0.026±0.005	0.027±0.013	0.023±0.003
Hap5	-	-	0.043±0.013[a,b]	0.027±0.004	0.023±0.007	0.019±0.013	0.028±0.007	0.023±0.004
Hap6	-	0.029 0.003	0.044±0.002[a,b]	0.024±0.006	0.032±0.003	0.027±0.003	0.035±0.004	0.026±0.004

232 – 309 d								
Hap1	0.004±0.003	0.001±0.013	-	-	-	-	-	-
Hap2	-	0.004±0.005	-0.010±0.009	0.004±0.013	-	0.000±0.013	0.004±0.004	-
Hap3	-	0.004±0.004	-0.000±0.006	0.004±0.013	0.007±0.004	-0.005±0.005	0.010±0.004	0.004±0.009
Hap4	-	-	-0.015± 0.008	0.002±0.004	0.003±0.005	0.000 ± 0.005	0.009±0.013	0.004±0.003
Hap5	-	-	-0.007± 0.013	0.003±0.004	0.006±0.007	0.015± 0.013	0.003±0.007	0.004±0.004
Hap6	-	0.003±0.003	0.010±0.003	0.002±0.006	-0.001±0.003	0.000± 0.003	0.005±0.004	0.002±0.004
1 – 309 d								
Hap1	0.025±0.001	0.023±0.004	-	-	-	-	-	-
Hap2	-	0.025±0.001	0.070±0.003	0.021±0.004	-	0.023±0.001	0.024±0.001	-
Hap3	-	0.024±0.001	0.063±0.002	0.021±0.004	0.025±0.001	0.019 ± 0.001	0.024±0.001	0.024±0.002
Hap4	-	-	0.067±0.002	0.021±0.001	0.028 ± 0.001	0.020 ± 0.001	0.026±0.004	0.024±0.001
Hap5	-	-	0.065±0.004	0.022±0.001	0.024 ± 0.002	0.020 ± 0.004	0.020±0.002	0.024±0.001
Hap6	-	0.023±0.001	0.069±0.008	0.021±0.002	0.022 ± 0.001	0.021 ± 0.001	0.027±0.001	0.023±0.001

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without superscripts are non-significant ($P \geq 0.05$).

[1]ADG, average daily gain (kg) was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 31. Associations between haplogroups of *turPeriod-3* gene and feed conversion ratio (FCR) by periods of age within each variety of turkeys.

Haplogroups	FCR[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
34 – 68 d								
Hap1	3.02 ± 0.21	3.33 ± 1.02	-	-	-	-	-	-
Hap2	-	3.07 ± 0.36	1.89 ± 0.72	2.98 ± 1.02	-	2.52 ± 1.02	3.12 ± 0.29	-
Hap3	-	3.61 ± 0.31	1.92 ± 0.46	3.97 ± 1.02	2.50 ± 0.34	2.65 ± 0.42	3.04 ± 0.29	2.71 ± 0.72
Hap4	-	-	2.06 ± 0.51	3.21 ± 0.28	2.20 ± 0.39	2.59 ± 0.39	2.98 ± 1.02	3.00 ± 0.26
Hap5	-	-	2.36 ± 1.02	3.23 ± 0.29	2.18 ± 0.59	2.45 ± 0.72	3.36 ± 0.59	2.67 ± 0.31
Hap6	-	3.33 ± 0.26	2.14 ± 0.17	2.92 ± 0.51	2.39 ± 0.27	2.63 ± 0.21	2.93 ± 0.34	2.84 ± 0.34
69 – 159 d								
Hap1	5.34 ± 0.20	4.80 ± 0.96	-	-	-	-	-	-
Hap2	-	4.14 ± 0.34	2.37 ± 0.68	3.28 ± 0.96	-	4.23 ± 0.96	4.76 ± 0.28	-
Hap3	-	4.33 ± 0.29	3.29 ± 0.43	4.88 ± 0.96	4.83 ± 0.32	5.93 ± 0.39	4.69 ± 0.28	4.00 ± 0.68
Hap4	-	-	3.62 ± 0.48	4.11 ± 0.27	4.19 ± 0.36	5.37 ± 0.36	4.23 ± 0.96	4.07 ± 0.25

Hap5	-	-	3.36 ± 0.96	4.86 ± 0.28	4.55 ± 0.56	5.56 ± 0.68	5.77 ± 0.56	3.99 ± 0.29
Hap6	-	4.55 ± 0.24	3.63 ± 0.16	4.31 ± 0.48	4.96 ± 0.26	5.08 ± 0.20	4.19 ± 0.32	4.57 ± 0.32
160 – 231 d								
Hap1	9.62 ± 1.05	14.08 ± 5.04	-	-	-	-	-	-
Hap2	-	12.65 ± 1.78	8.91 ± 3.56	10.03 ± 5.04	-	13.03 ± 5.04	9.64 ± 1.45	-
Hap3	-	13.99 ± 1.52	16.41 ± 2.25	19.88 ± 5.04	12.72 ± 1.68	12.30 ± 2.06	13.36 ± 1.45	10.29 ± 3.56
Hap4	-	-	12.64 ± 2.91	11.79 ± 1.40	10.01 ± 1.90	12.63 ± 1.90	14.14 ± 5.06	12.27 ± 1.30
Hap5	-	-	19.08 ± 5.04	14.64 ± 1.45	11.47 ± 2.91	9.12 ± 5.04	11.15 ± 2.91	12.43 ± 1.52
Hap6		11.14 ± 1.26	13.92 ± 0.84	13.71 ± 2.52	11.49 ± 1.35	13.18 ± 1.10	9.23 ± 1.68	14.73 ± 1.68
34 – 231 d								
Hap1	7.38 ± 0.39	8.26 ± 1.85	-	-	-	-	-	-
Hap2	-	6.84 ± 0.66	4.31 ± 1.31	5.90 ± 1.85	-	7.63± 1.85	7.27 ± 0.54	-
Hap3	-	7.61 ± 0.56	6.71 ± 0.83	7.09 ± 1.85	7.82 ± 0.62	8.15 ± 0.76	8.06 ± 0.54	6.57 ± 1.31
Hap4	-	-	5.50 ± 1.07	7.98 ± 0.51	6.45 ± 0.70	8.38 ± 0.70	7.95 ± 1.85	7.07 ± 0.48
Hap5	-	-	6.00 ± 1.85	8.71 ± 0.54	7.31 ± 1.07	7.70 ± 1.85	8.41 ± 1.07	6.87 ± 0.56
Hap6	-	7.58 ± 0.46	6.31 ± 0.31	7.92 ± 0.93	7.01 ± 0.50	8.67 ± 0.40	6.66 ± 0.62	7.74 ± 0.62

*Means within columns without superscripts are non-significant ($P \geq 0.05$).

[1]FCR, feed conversion ratio was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 32. Associations between haplogroups of *turPeriod-3* gene and egg production traits within each variety of turkeys.

Haps	Egg production traits*							
	BR	BS	CC	MW	NA	RP	SB	WH
AFE[1] (d)								
Hap1	243.13±10.24	-	-	-	-	-	-	-
Hap2	-	231.00± 28.95	-	-	-	230.00±28.95	246.33±16.71	-
Hap3	-	276.00± 28.95	156.00±20.47[b]	212.00±28.95	226.80±12.95	257.00±14.47	223.67±11.82	247.00±28.95
Hap4	-	-	253.00±28.95[a]	211.00±12.95	245.00±16.71	243.50±20.47	216.00±28.95	252.38±10.24
Hap5	-	-	154.00±28.95[b]	230.43±10.94	251.00±28.95	239.00±28.95	254.00±28.95	239.75±14.47
Hap6	-	245.00 ± 12.95	181.50 ± 6.47[b]	243.00±28.95	220.00±10.94	242.78 ± 9.65	221.75±14.47	260.29±10.94
Egg production[2]								
6 wks								
Hap1	5.33 ± 3.56	-	-	-	-	-	-	-
Hap2	-	7.00 ± 5.35	-	-	-	25.00 ± 10.69	8.20 ± 4.78	-
Hap3	-	9.29 ± 4.04	15.67 ± 6.17	29.00 ± 10.69	17.60 ± 4.78	11.00 ± 5.35	14.71 ± 4.04	-
Hap4	-	-	19.00 ± 7.56	22.60 ± 4.78	13.00 ± 6.17	13.67 ± 6.17	4.00 ± 10.69	7.11 ± 3.56

Hap5	-	-	13.00 ± 10.69	29.43 ± 4.04	31.00 ± 10.69	22.00 ± 10.69	-	5.60 ± 4.78
Hap6	-	6.56 ± 3.56	15.08 ± 2.14	17.00 ± 7.56	19.13 ± 3.78	7.38 ± 2.67	14.83 ± 4.37	9.29 ± 4.04
Average egg weight[3] (g)								
6 wks								
Hap1	76.01 ± 1.32	-	-	-	-	-	-	-
Hap2	-	73.64 ± 2.94	-	-	-	81.50 ± 2.94	77.76 ± 1.70	-
Hap3	-	75.70 ± 1.70	92.51 ± 2.08	74.62 ± 2.94	77.30 ± 1.32	76.02 ± 1.47	75.76 ± 1.32	-
Hap4	-	-	94.32 ± 2.08	73.29 ± 1.32	75.82 ± 1.70	76.61 ± 2.08	78.63 ± 2.94	75.62 ± 1.20
Hap5	-	-	87.24 ± 2.94	73.65 ± 1.11	79.03 ± 2.94	77.72 ± 2.94	-	76.87 ± 1.70
Hap6	-	75.09 ± 1.70	92.02 ± 0.68	72.06 ± 2.94	78.61 ± 1.11	77.79 ± 0.93	74.66 ± 1.47	77.94 ± 1.32
Egg production[2]								
10 wks								
Hap1	11.44 ± 5.15	-	-	-	-	-	-	-
Hap2	-	8.50 ± 7.72	-	-	-	36.00 ± 15.44	10.60 ± 6.90	-
Hap3	-	13.43 ± 5.84	24.00 ± 8.91	41.00 ± 15.44	24.00 ± 6.90	19.50 ± 7.72	22.14 ± 5.84	2.00 ± 15.44
Hap4	-	-	17.00 ± 10.92	33.60 ± 6.90	19.33 ± 8.91	20.67 ± 8.91	7.00 ± 15.44	14.22 ± 5.15

Hap5		-	21.00 ± 15.44	40.57 ± 5.84	43.00 ± 15.44	35.00 ± 15.44	3.00 ± 15.44	12.20 ± 6.90
Hap6	-	9.00 ± 5.15	24.40 ± 3.09	21.00 ± 10.92	29.63 ± 5.46	11.63 ± 3.86	22.17 ± 6.30	17.57 ± 5.84
Average egg weight[3] (g)								
10 wks								
1	76.07 ± 0.96	-	-	-	-	-	-	-
2	-	74.26 ± 2.71	-	-	-	80.40 ± 2.71	77.91 ± 1.57	-
3	-	75.74 ± 1.57	92.28 ± 1.92	74.77 ± 2.31	77.00 ± 1.21	76.66 ± 1.36	75.92 ± 1.11	79.30 ± 2.71
4	-	-	89.18 ± 1.92	73.39 ± 1.21	75.50 ± 1.57	76.86 ± 1.92	76.96 ± 2.71	76.27 ± 0.96
5	-	-	88.42 ± 2.71	74.09 ± 1.03	78.62 ± 2.71	77.27 ± 2.71	73.30 ± 2.71	76.16 ± 1.36
6	-	75.60 ± 1.57	92.42 ± 0.61	72.47 ± 2.71	78.36 ± 1.03	78.13 ± 0.86	72.86 ± 1.36	77.35 ± 1.03

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]AFE, age at first egg was recorded and given in days (d). Least square means ± SE.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks separately for each hen.

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

Table 4. 33. Associations between haplogroups of *turPeriod-3* gene and semen quality traits within each variety of turkeys.

Haplogroups	Semen quality traits[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
Ejaculate volume (mL)								
Hap1	0.05 ± 0.01	-	-	-	-	-	-	-
Hap2	-	0.10 ± 0.02	0.17 ± 0.03	0.12 ± 0.05	-	-	0.11 ± 0.02	-
Hap3	-	0.09 ± 0.02	0.10 ± 0.03	-	-	0.10 ± 0.03	0.11 ± 0.02	0.07 ± 0.05
Hap4	-	-	0.14 ± 0.05	0.15 ± 0.02	0.18 ± 0.02	0.11 ± 0.03	-	0.10 ± 0.02
Hap5	-	-	-	0.10 ± 0.02	0.24 ± 0.03	0.05 ± 0.05	0.09 ± 0.03	0.11 ± 0.02
Hap6	-	0.11 ± 0.02	0.14 ± 0.02	0.12 ± 0.03	0.16 ± 0.02	0.10 ± 0.02	0.08 ± 0.03	0.06 ± 0.03
Sperm concentration (x10^9/mL)								
Hap1	1.80 ± 0.18	-	-	-	-	-	-	-
Hap2	-	2.36 ± 0.32	2.10 ± 0.45	3.02 ± 0.64	-	-	2.01 ± 0.24	-
Hap3	-	2.55 ± 0.32	2.14 ± 0.45	-	2.55 ± 0.32	1.39 ± 0.45	2.10 ± 0.32	1.23 ± 0.64[a]
Hap4	-	-	1.48 ± 0.64	2.58 ± 0.23	2.62 ± 0.32	2.25 ± 0.37	-	3.18 ± 0.26[b]
Hap5	-	-	-	2.32 ± 0.29	1.99 ± 0.45	2.52 ± 0.64	2.42 ± 0.45	2.31 ± 0.26[a,b]

Hap6	-	2.43 ± 0.24	2.49 ± 0.26	3.03 ± 0.45	2.14 ± 0.26	2.34 ± 0.29	1.75 ± 0.37	2.57 ± 0.45[b]
Total sperm number (x10^8/ejaculate)								
Hap1	1.00 ± 0.42		-	-	-	-	-	-
Hap2	-	2.44 ± 0.76	4.05 ± 1.08	3.62 ± 1.52	-	-	2.25 ± 0.58	-
Hap3	-	2.21 ± 0.76	2.18 ± 1.08	-	4.62 ± 0.76	1.29 ±1.08	2.35 ± 0.76	0.29 ± 1.52[a]
Hap4	-	-	2.00 ± 1.52	3.88 ± 0.54	4.87 ± 0.76	2.48 ± 0.88	-	3.50 ± 0.62[b]
Hap5	-	-	-	2.48 ± 0.68	4.55 ± 1.08	1.34 ± 1.52	2.04 ± 1.08	2.54 ± 0.62[b]
Hap6	-	2.71 ± 0.58	3.44 ± 0.62	3.35 ± 1.08	3.41 ± 0.62	2.41 ± 0.68	1.48 ± 0.88	1.58 ± 1.08[a,b]
Sperm viability (%)								
Hap1	82.65 ± 0.92	-	-	-	-	-	-	-
Hap2	-	79.25 ± 1.65	89.75 ± 2.33	89.50 ± 3.30[a]	-	-	82.29 ± 1.25	-
Hap3	-	79.00 ± 1.65	87.50 ± 2.33	-	85.75 ± 1.65	82.50 ± 2.33	85.13 ± 1.65	86.50 ± 3.30
Hap4	-	-	89.50 ± 3.29	84.25 ± 1.67[a,b]	84.38 ± 1.65	82.67 ± 1.91	-	83.83 ± 1.35
Hap5	-	-	-	82.30 ± 1.48[b]	80.25 ± 2.33	80.50 ± 3.30	83.25 ± 2.33	85.33 ± 1.35
Hap6	-	79.43 ± 1.25	84.75 ± 1.35	80.75 ± 2.33[b]	83.58 ± 1.35	84.80 ± 1.48	82.67 ± 1.91	85.00 ± 2.33

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]Least square means ± SE.

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

Table 4. 34. Associations between haplogroups of *turPeriod-3* gene and plasma melatonin concentration within each variety of turkeys.

Haplogroups	Plasma melatonin[1*] (pg/ml)							
	BR	BS	CC	MW	NA	RP	SB	WH
Hap1	38.01±7.34	5.24 ± 34.41	-	-	-	-	-	-
Hap2	-	26.41 ± 12.17	35.98 ± 24.33	31.26 ± 34.41	-	4.51 ± 34.41	73.50 ± 9.93	-
Hap3	-	32.98 ± 10.88	55.16 ± 15.39	14.50 ± 34.41	33.46 ± 11.47	15.36 ± 14.05	36.32 ± 10.38	31.17 ± 4.33
Hap4	-	-	22.83 ± 17.21	31.77 ± 9.54	50.15 ± 13.00	13.81 ± 13.01	6.79 ± 34.41	32.32 ± 9.20
Hap5	-	-	18.71 ± 34.41	37.38 ± 9.93	31.16 ± 19.87	7.71 ± 34.41	26.54 ± 19.87	26.69 ± 10.38
Hap6	-	32.91 ± 8.60	33.57 ± 6.28	57.90 ± 17.21	36.96 ± 9.20	13.44 ± 7.34	29.34 ± 11.47	43.17 ± 11.47

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]Least square means ± SE.

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

Table 4. 35. Primer sequences, expected sizes of amplicons and PCR characteristics for the *turCry1* gene.

Primer ID	Primers[1]	Sequences	Tm[2] (^{0}C)	Amplicon length[3] (bp)
Cry1_1	For(54615366)	5′-TACTGTAAGCTAAGTGTAACTAGGATGTGGAC-3′	65.9	5000
	Rev(54610288)	5′-TATGCTTTTAGTGGTAGGCTACTTCTCTACTC-3′	65.9	
Cry1_2	For(54610609)	5′-ATAGTACAATATTTCTTCTGTGTGTCTAGATG-3′	62.1	4500
	Rev(54606117)	5′-TACTAATTTCTACAAAGCAGGAAACACAAG-3′	61.9	
Cry1_3	For(54606311)	5′-TAATACAAATAGTAATGACCACAGAAGATGGT-3′	62.1	5500
	Rev(54600808)	5′-TTAAGTGGTATCTCCTCTTCTAATAATGCTAC-3′	63.4	

[1]*For, forward primer; Rev, reverse primer.* Primer-binding sites in the turkey genome (GenBank accession No: LOC100008577) 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 4. 36. Characteristics of single nucleotide polymorphisms (SNPs) identified in the *turCry1* gene in eight divergent turkey varieties.

SNP	Location	Nucleotide position[1]	Sequence Context[2]	*dbSNP* Identification[3]	Genotype	Genotype Frequency %	MAF[4]	HWE[5]
Cry1-1	Intron 6	54606291	GATGC(*A*/G)TGAAC	ss538786564	A/A	12.75	0.17	0.00*
					A/G	7.93		
					G/G	79.32		
Cry1-2	Intron 6	54606392	CTTCC(*A*/G)AAGCT	ss538786565	A/A	42.07	0.43	0.00*
					A/G	1.72		
					G/G	56.21		
Cry1-3	Intron 6	54606661	ATCAT(C/*T*)GTCAT	ss538786566	C/C	82.41	0.16	0.00*
					C/T	4.13		
					T/T	13.46		
Cry1-4	Intron 3	54609933	GAAGA(*C*/G)ATTTT	ss538786567	C/C	13.10	0.13	0.01*
					C/G	0.00		
					G/G	86.90		
Cry1-5	Intron 3	54610176	CCATT(*C*/T)AATCA	ss538786568	C/C	41.38	0.46	0.01*

					C/T	9.31		
					T/T	49.31		
Cry1-6	Intron 3	54610182	AATCA(C/*A*)AACAA	ss538786569	C/C	73.45	0.21	0.00*
					C/A	11.38		
					A/A	15.17		
Cry1-7	Intron 3	54610265	GCTTT(*C*/T)AGTGG	ss538786570	C/C	43.45	0.46	0.01*
					C/T	4.82		
					T/T	51.73		

[1]Position of the SNP in GenBank on the forward strand of chromosome 1 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]*ss* prefix indicates novel SNPs detected here and submitted for *rs* assignment in *dbSNP*, NCBI.

[4]Minor allele frequency (MAF) of 7 SNPs markers.

[5]Significance of deviation from HWE for the 7 SNPs. NS indicates non-significant ($P > 0.05$) while * refers to significant at $P < 0.05$.

Table 4. 37. Linkage disequilibrium as measured by *D′* and r^2 between the 7 segregating SNPs in the *turCry1* gene.

SNPs[1]	*Cry1-1*	*Cry1-2*	*Cry1-3*	*Cry1-4*	*Cry1-5*	*Cry1-6*	*Cry1-7*
Cry1-1		0.74	0.70	0.61	0.48	0.50	0.83
Cry1-2	0.19		0.66	0.52	0.73	0.56	0.83
Cry1-3	0.41	0.18		0.65	0.74	0.69	0.71
Cry1-4	0.31	0.08	0.29		0.56	0.67	0.82
Cry1-5	0.03	0.23	0.10	0.04		0.89	0.73
Cry1-6	0.17	0.16	0.39	0.25	0.17		0.72
Cry1-7	0.14	0.42	0.12	0.12	0.38	0.16	

[1]SNP identification (*Cry1-1 – Cry1-7*).

All *D'* and r^2 values are significant ($P \leq 0.05$).

D' values are listed in upper right section, and r^2 values are listed in lower left section.

Table 4. 38. The pairwise fixation index (*Fst*) estimated for turkey varieties using the *turCry1* gene variants.

Turkey varieties[1]	BR	BS	NA	MW	SB	WH	RP	CC
BR	0.0							
BS	0.50	0.0						
NA	0.08	0.29	0.0					
MW	0.06	0.34	0.004*	0.0				
SB	0.17	0.20	0.02*	0.05	0.0			
WH	0.50	0.25	0.22	0.28	0.20	0.0		
RP	0.67	0.33	0.49	0.54	0.39	0.51	0.0	
CC	0.66	0.22	0.47	0.52	0.38	0.45	0.16	0.0

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

* indicates non-significant *Fst* ($P \geq 0.05$).

Table 4. 39. Haplogroup frequencies of *turCry1* gene in eight turkey populations.

Turkey Varieties		Haplogroups						
	N	Hap1	Hap2	Hap3	Hap4	Hap5	Hap6	Hap7
BR	23	0.96	0.00	0.00	0.00	0.00	0.00	0.04
BS	36	0.17	0.00	0.00	0.67	0.00	0.05	0.11
CC	50	0.02	0.00	0.68	0.02	0.08	0.12	0.08
MW	31	0.00	0.81	0.00	0.00	0.00	0.03	0.16
NA	33	0.82	0.00	0.00	0.00	0.00	0.15	0.03
RP	39	0.03	0.05	0.08	0.00	0.00	0.56	0.28
SB	37	0.46	0.00	0.00	0.00	0.00	0.11	0.43
WH	37	0.08	0.00	0.00	0.00	0.73	0.05	0.14

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

N = numbers of birds from each variety used for calculation of haplogroup frequencies.

Table 4. 40. Associations between haplogroups of the *turCry1* gene and body weight (BW) at different ages of turkeys.

Haplogroups	BW[1] (kg)					
	1 d	34 d	68 d	159 d	231 d	309 d
Hap1	0.046 ± 0.001[a]	0.69 ± 0.02[a]	2.01 ± 0.05[a]	6.64 ± 0.15[a]	8.73 ± 0.18[a]	9.03 ± 0.18[a]
Hap2	0.045 ± 0.001[a]	0.71 ± 0.05[a]	1.98 ± 0.13[a]	6.42 ± 0.35[a]	8.66 ± 0.41[a]	8.82 ± 0.40[a]
Hap3	**0.048 ± 0.001[a]**	**0.74 ± 0.03[a]**	**2.23 ± 0.09[a]**	6.56 ± 0.25[a]	8.55 ± 0.30[a]	9.14 ± 0.31[a]
Hap4	0.045 ± 0.001[a]	0.73 ± 0.04[a]	2.12 ± 0.11[a]	6.55 ± 0.30[a]	**8.91 ± 0.35[a]**	**9.21 ± 0.35[a]**
Hap5	0.044 ± 0.001[a]	0.66 ± 0.04[a]	1.94 ± 0.10[a]	6.41 ± 0.27[a]	8.47 ± 0.32[a]	8.69 ± 0.32[a]
Hap6	0.047 ± 0.001[a]	0.70 ± 0.02[a]	1.95 ± 0.06[a]	6.56 ± 0.17[a]	8.75 ± 0.21[a]	9.11 ± 0.20[a]
Hap7	0.047 ± 0.001[a]	0.71 ± 0.02[a]	2.07 ± 0.06[a]	**6.65 ± 0.15[a]**	8.86 ± 0.18[a]	8.90 ± 0.18[a]

[a]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.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 41. Associations between haplogroups of *turCry1* gene and average daily gain (ADG) by periods of age for turkeys.

Haplogroups	ADG[1] (kg)					
	1-34 d	35 – 68 d*	69 – 159 d	160 – 231 d	232 – 309 d	1 – 309 d
Hap1	0.019 ± 0.001[a]	0.039 ± 0.001[a,b]	0.050 ± 0.002[a]	0.029 ± 0.002[a]	0.004 ± 0.002[a]	0.029 ± 0.001[a]
Hap2	0.020 ± 0.001[a]	0.038 ± 0.003[a,b]	0.049 ± 0.004[a]	0.031 ± 0.005[a]	0.002 ± 0.005[a]	0.029 ± 0.001[a]
Hap3	**0.021 ± 0.001[a]**	**0.044 ± 0.002[a]**	0.048 ± 0.003[a]	0.029 ± 0.003[a]	**0.005 ± 0.004[a]**	**0.031 ± 0.001[a]**
Hap4	0.020 ± 0.001[a]	0.041 ± 0.003[a,b]	0.048 ± 0.003[a]	**0.033 ± 0.004[a]**	0.004 ± 0.004[a]	0.030 ± 0.001[a]
Hap5	0.018 ± 0.001[a]	0.036 ± 0.002[b]	0.047 ± 0.003[a]	0.028 ± 0.004[a]	0.003 ± 0.004[a]	0.028 ± 0.001[a]
Hap6	0.020 ± 0.001[a]	0.037 ± 0.001[b]	**0.051 ± 0.002[a]**	0.030 ± 0.002[a]	0.004 ± 0.002[a]	0.029 ± 0.001[a]
Hap7	0.020 ± 0.001[a]	0.040 ± 0.001[a,b]	0.050 ± 0.002[a]	0.031 ± 0.002[a]	0.001 ± 0.002[a]	0.029 ± 0.001[a]

[a,b]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. *$P \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 42. Associations between haplogroups of *turCry1* gene and feed conversion ratio (FCR) by periods of age for turkeys.

Haplogroups	FCE[1]			
	34 – 68 d	69 – 159 d	160 – 231 d*	34 – 231d*
Hap1	2.85 ± 0.16[a]	4.47 ± 0.13[a]	13.18 ± 0.66[a]	7.57 ± 0.21[a]
Hap2	3.02 ± 0.36[a]	4.66 ± 0.31[a]	12.08 ± 1.49[a]	7.51 ± 0.47[a]
Hap3	2.73 ± 0.26[a]	4.60 ± 0.22[a]	**10.12 ± 1.10[b]**	7.32 ± 0.35[a,b]
Hap4	**2.64 ± 0.31[a]**	4.71 ± 0.27[a]	11.21 ± 1.29[a,b]	7.19 ± 0.41[a,b]
Hap5	2.94 ± 0.28[a]	4.58 ± 0.24[a]	12.05 ± 1.16[a]	7.17 ± 0.37[a,b]
Hap6	2.96 ± 0.18[a]	4.46 ± 0.15[a]	10.99 ± 0.75[b]	7.22 ± 0.24[a,b]
Hap7	2.70 ± 0.15[a]	**4.45 ± 0.13[a]**	11.24 ± 0.64[a,b]	**6.92 ± 0.20[b]**

[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 4. 43. Associations between haplogroups of *turCry1* gene and egg production traits for turkeys.

Haplogroups	Egg production traits[1]				
	AFE[4] (d)	Egg production[2]		Average egg weight[3] (g)	
		6 wks*	10 wks*	6 wks	10 wks
Hap1	229.99 ± 7.67[a]	9.51 ± 2.10[a,b]	14.96 ± 3.06[a,b]	77.92 ± 0.86[a]	77.77 ± 0.72[a]
Hap2	**219.11 ± 17.05[a]**	14.73 ± 4.62[a]	22.95 ± 6.73[b]	78.25 ± 1.71[a]	78.33 ± 1.58[a]
Hap3	249.35 ± 13.28[a]	15.09 ± 3.55[a]	23.26 ± 5.18[b]	**79.00 ± 1.44[a]**	**79.49 ± 1.24[a]**
Hap4	232.25 ± 19.86[a]	16.51 ± 3.95[a]	**25.60 ± 5.75[b]**	77.03 ± 1.78[a]	77.71 ± 1.65[a]
Hap5	233.45 ± 11.08[a]	**17.91 ± 3.63[a]**	24.68 ± 5.29[b]	78.36 ± 1.27[a]	78.02 ± 1.03[a]
Hap6	235.90 ± 8.99[a]	5.96 ± 2.41[b]	10.81 ± 3.52[a]	78.17 ± 0.93[a]	78.24 ± 0.82[a]
Hap7	226.96 ± 7.88[a]	15.81 ± 2.10[a]	24.31 ± 3.06[b]	78.10 ± 0.75[a]	77.85 ± 0.66[a]

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1]Least square means ± SE. *$P \leq 0.05$.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks 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

Table 4. 44. Associations between haplogroups of *turCry1* gene and semen quality traits for turkeys.

Haplogroups	Semen quality traits[1]			
	Ejaculate volume (mL)*	Sperm Concentration ($x10^9$/mL)	Total number of sperm ($x10^8$/ejaculate)	Sperm viability (%)
Hap1	$0.12 \pm 0.01^{a,b}$	**2.48 ± 0.18^{a}**	3.14 ± 0.40^{a}	84.18 ± 0.90^{a}
Hap2	$\underline{0.07 \pm 0.03^{a}}$	2.12 ± 0.41^{a}	2.24 ± 0.93^{a}	$\underline{81.20 \pm 2.08^{a}}$
Hap3	**0.14 ± 0.02^{b}**	2.00 ± 0.34^{a}	2.78 ± 0.77^{a}	83.01 ± 1.73^{a}
Hap4	$0.13 \pm 0.02^{a,b}$	2.18 ± 0.36^{a}	**3.18 ± 0.82^{a}**	84.36 ± 1.83^{a}
Hap5	$0.11 \pm 0.02^{a,b}$	$\underline{1.89 \pm 0.32^{a}}$	2.31 ± 0.72^{a}	**84.74 ± 1.61^{a}**
Hap6	0.09 ± 0.01^{a}	2.34 ± 0.20^{a}	$\underline{2.15 \pm 0.45^{a}}$	82.58 ± 1.00^{a}
Hap7	$0.11 \pm 0.01^{a,b}$	2.42 ± 0.17^{a}	2.87 ± 0.38^{a}	83.01 ± 0.85^{a}

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE. *$P \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 45. Associations between haplogroups of *turCry1* gene and plasma melatonin concentration for turkeys.

Haplogroups	Plasma melatonin[1] (pg/ml)
Hap1	28.25 ± 5.44[a]
Hap2	27.60 ± 2.49[a]
Hap3	40.39 ± 9.44[a]
Hap4	31.43 ± 1.19[a]
Hap5	43.94 ± 9.83[a]
Hap6	**45.24 ± 6.37[a]**
Hap7	32.58 ± 5.43[a]

[a]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE.

Bold values represent the highest plasma melatonin haplogroups while underline values represent the lowest.

Table 4. 46. Associations between haplogroups of *turCry1* gene and body weight (BW) at different ages within each variety turkeys.

Haps	BW[1*] (kg)							
	BR	BS	CC	MW	NA	RP	SB	WH
Day 1								
Hap1	0.041±0.001	0.048±0.002	0.056±0.004	-	0.046±0.001	0.043±0.004[a,b]	0.046±0.001	0.043±0.002
Hap2	-	-	-	0.041±0.001	-	0.037±0.003[a]	-	-
Hap3	-	-	0.056±0.001	-	-	0.046±0.002[b]	-	-
Hap4	-	0.047±0.001	0.053±0.004	-	-	-	-	-
Hap5	-	-	0.057±0.002	-	-	-	-	0.044±0.001
Hap6	-	0.048±0.003	0.056±0.002	0.042±0.004	0.046±0.002	0.046±0.001[b]	0.047±0.002	0.048±0.003
Hap7	0.046 ±0004	0.049±0.002	0.056±0.002	0.040±0.002	0.048±0.004	0.044±0.001[b]	0.048±0.001	0.047±0.002
Day 34								
Hap1	0.60 ± 0.03	0.54 ± 0.06	1.12 ± 0.13	-	0.66 ± 0.02	0.58 ± 0.13	0.64 ± 0.03	0.62 ± 0.07
Hap2	-	-	-	0.60 ± 0.03	-	0.58 ± 0.09	-	-
Hap3	-	-	1.32 ± 0.02	-	-	0.64 ± 0.07	-	-
Hap4	-	0.61 ± 0.03	1.40 ± 0.13	-	-	-	-	-

Hap5	-	-	1.20 ± 0.06	-	-	-	-	0.62 ± 0.03
Hap6	-	0.65 ± 0.09	1.29 ± 0.05	0.66 ± 0.13	0.62 ± 0.06	0.57 ± 0.03	0.68 ± 0.06	0.52 ± 0.09
Hap7	0.57 ± 0.13	0.62 ± 0.06	1.35 ± 0.06	0.58 ± 0.06	0.74 ± 0.13	0.56 ± 0.04	0.64 ± 0.03	0.67 ± 0.06
Day 68								
Hap1	1.69 ± 0.08	1.71 ± 0.15	3.72 ± 0.36[a]	-	1.85 ± 0.07	1.53 ± 0.36	1.66 ± 0.09	1.79 ± 0.21
Hap2	-	-	-	1.56 ± 0.07	-	1.65 ± 0.26	-	-
Hap3	-	-	4.56 ± 0.06[b]	-	-	1.74 ± 0.21	-	-
Hap4	-	1.64 ± 0.07	5.06 ± 0.36[b]	-	-	-	-	-
Hap5	-	-	4.18 ± 0.18[a]	-	-	-	-	1.70 ± 0.07
Hap6	-	1.37 ± 0.25	4.16 ± 0.15[a]	1.70 ± 0.36	1.81 ± 0.16	1.59 ± 0.08	1.69 ± 0.18	1.57 ± 0.26
Hap7	1.60 ± 0.34	1.61 ± 0.18	4.58 ± 0.18[b]	1.64 ± 0.16	2.19 ± 0.36	1.64 ± 0.08	1.71 ± 0.09	1.80 ± 0.16
Day 159								
Hap1	5.25 ± 0.21	5.41 ± 0.40	16.84 ± 0.98	-	5.69 ± 0.19	4.15 ± 0.99	4.84 ± 0.24	5.48 ± 0.56
Hap2	-	-	-	4.64 ± 0.19	-	4.41± 0.71	-	-
Hap3	-	-	17.30 ± 0.18	-	-	4.34± 0.56	-	-
Hap4	-	5.07 ± 0.20	17.41 ± 0.98	-	-	-	-	-

Hap5	-	-	17.10 ± 0.49	-	-	-	-	5.26 ± 0.19
Hap6	-	4.44 ± 0.69	17.52 ± 0.41	4.89 ± 0.99	5.15 ± 0.44	4.27 ± 0.22	5.23 ± 0.49	5.62 ± 0.71
Hap7	4.71 ± 1.00	5.17 ± 0.49	17.32 ± 0.49	4.99 ± 0.44	6.70 ± 0.99	4.26 ± 0.30	5.04 ± 0.24	5.35 ± 0.45
Day 231								
Hap1	7.53 ± 0.24	7.40 ± 0.46	21.39 ± 1.14	-	7.71 ± 0.22	-	6.79 ± 0.27	7.80 ± 0.65
Hap2	-	-	-	6.40 ± 0.23	-	6.20 ± 0.82	-	-
Hap3	-	-	20.43 ± 0.21	-	-	6.27 ± 0.65	-	-
Hap4	-	7.21 ± 0.23	20.29 ± 1.14	-	-	-	-	-
Hap5	-	-	20.31 ± 0.57	-	-	-	-	6.95 ± 0.22
Hap6	-	6.13 ± 0.80	20.44 ± 0.51	6.70 ± 1.14	8.08 ± 0.50	6.13 ± 0.26	7.70 ± 0.56	7.40 ± 0.82
Hap7	7.39 ± 1.15	6.88 ± 0.57	21.23 ± 0.56	6.59 ± 0.50	7.27 ± 1.14	6.41 ± 0.35	7.27 ± 0.28	6.82 ± 0.52
Day 309								
Hap1	7.86 ± 0.24	7.80 ± 0.46	21.21 ± 1.13	-	7.89 ± 0.21	-	7.42 ± 0.27	7.99 ± 0.64[a]
Hap2	-	-	-	6.64 ± 0.22	-	5.86 ± 0.81	-	-
Hap3	-	-	21.03 ± 0.23	-	-	6.45 ± 0.64	-	-
Hap4	-	7.46 ± 0.23	21.48 ± 1.13	-	-	-	-	-

Hap5	-	-	20.27 ± 0.56	-	-	-	-	7.26 ± 0.22[a]
Hap6	-	6.31 ± 0.78	20.81 ± 0.51	6.85 ± 1.13	8.41 ± 0.50	6.31 ± 0.26	8.02 ± 0.55	7.50 ± 0.81[a]
Hap7	7.25 ± 1.14	7.03 ± 0.56	21.50 ± 0.64	6.66 ± 0.50	7.69 ± 1.13	6.06 ± 0.35	7.62 ± 0.28	6.95 ± 0.51[b]

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]BW, body weight (kg) was measured at 1, 34, 68, 159, 231 and 309 days (d). Least square means ± SE

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

Table 4. 47. Associations between haplogroups of *turCry1* gene and average daily gain (ADG) by period of age within each variety of turkeys.

Haplogroups	ADG[1]* (kg)							
	BR	BS	CC	MW	NA	RP	SB	WH
1 – 34 d								
Hap1	0.017±0.001	0.015±0.002	0.033±0.004	-	0.019±0.001	0.016±0.004	0.018±0.001	0.017±0.002
Hap2	-	-	-	0.017±0.001	-	0.016±0.003	-	-
Hap3	-	-	0.038±0.001	-	-	0.018±0.002	-	-
Hap4	-	0.017±0.001	0.041±0.004	-	-	-	-	-
Hap5	-	-	0.035±0.002	-	-	-	-	0.017±0.001
Hap6	-	0.018±0.003	0.037±0.002	0.019±0.004	0.017±0.002	0.016±0.001	0.019±0.002	0.014±0.003
Hap7	0.016±0.004	0.017±0.002	0.039±0.002	0.017±0.002	0.021±.004	0.016±0.001	0.018±0.001	0.019±0.002
35 – 68 d								
Hap1	0.032±0.002	0.034±0.003[a]	0.077±0.008[a]	-	0.035±0.002	0.028±0.008	0.030±0.002	0.034±0.005
Hap2	-	-	-	0.028±0.002	-	0.031±0.006	-	-
Hap3	-	-	0.096±0.001[b]	-	-	0.032±0.005	-	-

Hap4	-	0.030±0.002[a,b]	0.108±0.008[b]	-	-	-	-	-
Hap5	-	-	0.088±0.004[a]	-	-	-	-	0.032±0.002
Hap6	-	0.022±0.006[b]	0.084±0.003[a]	0.031±0.008	0.035±0.004	0.030±0.002	0.030±0.004	0.031±0.006
Hap7	0.030±0.008	0.029±0.004[a,b]	0.095±0.004[b]	0.031±0.004	0.043±0.008	0.031±0.003	0.031±0.002	0.033±0.004
69 – 159 d								
Hap1	0.039±0.002	0.041±0.004	0.144±0.011	-	0.042±0.002	0.029±0.011	0.035±0.003	0.040±0.006
Hap2	-	-	-	0.034±0.002	-	0.030±0.008	-	-
Hap3	-	-	0.140±0.002	-	-	0.029±0.006	-	-
Hap4	-	0.038±0.002	0.135±0.011	-	-	-	-	-
Hap5	-	-	0.142±0.005	-	-	-	-	0.039±0.002
Hap6	-	0.034±0.008	0.147±0.005	0.035±0.011	0.037±0.005	0.029±0.002	0.039±0.005	0.044±0.008
Hap7	0.034±0.011	0.039±0.005	0.140±0.005	0.037±0.005	0.050±0.011	0.029±0.003	0.037±0.003	0.039±0.005
160 – 231 d								
Hap1	0.032±0.003	0.028±0.005	0.063±0.013[a]	-	0.028±0.002[a]	0.024±0.006	0.027±0.003	0.032±0.007
Hap2	-	-	-	0.024±0.003	-	0.025±0.009	-	-
Hap3	-	-	0.044±0.002[a,b]	-	-	0.027±0.007	-	-

Hap4	-	0.030±0.003	0.040±0.013[a,b]	-	-	-	-	-
Hap5	-	-	0.044±0.006[a,b]	-	-	-	-	0.023±0.002
Hap6	-	0.024±0.009	0.036±0.006[b]	0.025±0.013	0.041±0.006[b]	0.026±0.003	0.034±0.006	0.025±0.009
Hap7	0.038±0.013	0.024±0.006	0.055±0.006[a]	0.022±0.006	0.012±0.003[a]	0.030±0.004	0.031±0.003	0.020±0.006
232 – 309 d								
Hap1	0.004±0.003	0.005±0.005	-0.004±0.013	-	0.002±0.003	0.002±0.002	0.008±0.003	0.002±0.008
Hap2	-	-	-	0.003±0.003	-	-0.005±0.010	-	-
Hap3	-	-	0.005±0.003	-	-	0.002±0.008	-	-
Hap4	-	0.003±0.003	0.010±0.007	-	-	-	-	-
Hap5	-	-	-0.001±0.007	-	-	-	-	0.004±0.003
Hap6	-	0.002±0.009	0.004±0.006	0.001±0.013	0.004±0.006	0.002±0.003	0.004±0.007	0.001±0.010
Hap7	-0.002±0.014	0.002±0.007	0.011±0.008	0.001±0.006	0.005±0.013	-0.005±0.004	0.004±0.003	0.001±0.006
1 – 309 d								
Hap1	0.025±0.001	0.025±0.001	0.069±0.004	-	0.026±0.001	0.020±0.002	0.024±0.001	0.026±0.002
Hap2	-	-	-	0.021±0.001	-	0.019±0.003	-	-
Hap3	-	-	0.068±0.001	-	-	0.021±0.002	-	-

Hap4	-	0.024±0.001	0.070±0.004	-	-	-	-	-
Hap5	-	-	0.066±0.002	-	-	-	-	0.023±0.001
Hap6	-	0.021±0.003	0.067±0.002	0.022±0.004	0.027±0.002	0.020±0.001	0.026±0.002	0.024±0.003
Hap7	0.023±0.004	0.023±0.002	0.070±0.002	0.021±0.002	0.025±0.004	0.020±0.001	0.025±0.001	0.022±0.002

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]ADG, average daily gain (kg) was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 48. Associations between haplogroups of *turCry1* gene and feed conversion ratio (FCR) by periods of age within each variety of turkeys.

Haplogroups	FCR[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
34 – 68 d								
Hap1	3.06 ± 0.21	2.95 ± 0.41[a]	2.51 ± 1.00[a]	-	2.87 ± 0.19	2.73 ± 1.01	3.13 ± 0.24	2.58 ± 0.57
Hap2	-	-	-	3.32 ± 0.20	-	2.33 ± 0.72	-	-
Hap3	-	-	1.97 ± 0.18[a,b]	-	-	2.38 ± 0.57	-	-
Hap4	-	3.30 ± 0.20[a]	1.61 ± 1.00[b]	-	-	-	-	-
Hap5	-	-	2.09 ± 0.50[a,b]	-	-	-	-	2.81 ± 0.19
Hap6	-	4.90 ± 0.70[b]	2.26 ± 0.42[a]	2.85 ± 1.01	2.53 ± 0.44	2.52 ± 0.22	3.08 ± 0.49	2.84 ± 0.72
Hap7	3.28 ± 1.01	3.40 ± 0.50[a]	1.97 ± 0.49[a,b]	2.94 ± 0.44	1.69 ± 1.00	2.35 ± 0.31	2.92 ± 0.23	2.56 ± 0.45
69 – 159 d								
Hap1	5.36 ± 0.18	3.98 ± 0.35	3.08 ± 0.86	-	4.64 ± 0.16	5.38 ± 0.87	4.92 ± 0.21	3.86 ± 0.49
Hap2	-	-	-	4.52 ± 0.17	-	5.03 ± 0.62	-	-
Hap3	-	-	3.30 ± 0.15	-	-	5.59 ± 0.49	-	-

Hap4	-	4.40 ± 0.17	3.43 ± 0.86	-	-	-	-	-
Hap5	-	-	3.14 ± 0.43	-	-	-	-	4.12 ± 0.17
Hap6	-	4.75 ± 0.60	3.08 ± 0.36	4.09 ± 0.87	5.16 ± 0.38	5.26 ± 0.19	4.47 ± 0.43	3.66 ± 0.62
Hap7	6.50 ± 0.87	4.10 ± 0.43	3.55 ± 0.43	4.11 ± 0.38	4.08 ± 0.86	5.52 ± 0.27	4.43 ± 0.21	4.05 ± 0.39
160 – 231 d								
Hap1	10.24 ± 0.86	11.77 ± 1.64[a]	8.73 ± 4.02a	-	11.98 ± 0.77[a]	-	12.77 ± 0.97[a]	13.03 ± 2.31[a]
Hap2	-	-	-	13.48 ± 0.80	-	14.96 ± 2.90	-	-
Hap3	-	-	13.21 ± 0.73[b]	-	-	9.81 ± 2.31	-	-
Hap4	-	11.17 ± 0.81[a]	11.97 ± 4.02[b]	-	-	-	-	-
Hap5	-	-	14.82 ± 2.01[b]	-	-	-	-	12.48 ± 0.78[a]
Hap6	-	12.40 ± 2.81[a]	15.72 ± 1.82[b]	15.02 ± 4.04	7.04 ± 1.78[b]	11.60 ± 0.91	8.36 ± 1.99[b]	11.81 ± 2.90[a,b]
Hap7	6.80 ± 4.07	15.50 ± 2.02[b]	11.91 ± 1.99[b]	13.33 ± 1.78	13.30 ± 4.03[a]	10.97 ± 1.24	9.42 ± 0.99[b]	11.53 ± 1.83[b]
34 – 231 d								
Hap1	7.60 ± 0.28	6.98 ± 0.53	4.82 ± 1.31	-	7.22 ± 0.25	-	9.16 ± 0.32	6.42 ± 0.75
Hap2	-	-	-	8.46 ± 0.26	-	7.92± 0.94	-	-
Hap3	-	-	5.95 ± 0.24	-	-	7.93 ± 0.75	-	-

Hap4	-	7.15 ± 0.26	6.34 ± 1.31	-	-	-	-	-
Hap5	-	-	5.59 ± 0.65	-	-	-	-	7.00 ± 0.25
Hap6	-	8.51 ± 0.91	5.82 ± 0.59	7.59 ± 1.31	6.39 ± 0.58	8.24 ± 0.30	6.68 ± 0.65	6.54 ± 0.94
Hap7	7.51 ± 1.32	7.22 ± 0.66	5.79 ± 0.65	7.79 ± 0.58	7.69 ± 1.31	7.50 ± 0.40	6.84 ± 0.32	7.33 ± 0.59

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]FCR, feed conversion ratio was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 49. Associations between haplogroups of *turCry1* gene and egg production traits within each variety of turkeys.

Haps	Egg production traits*							
	BR	BS	CC	MW	NA	RP	SB	WH
AFE[1](d)								
Hap1	245.00±10.92	236.00±28.90	-	-	224.00±8.01	-	237.71±10.92	247.50±20.43
Hap2	-	-	-	221.67±8.34	-	236.00±28.90	-	-
Hap3	-	-	184.28±6.81	-	-	-	-	-
Hap4	-	246.80±12.93	159.00±28.90	-	-	-	-	-
Hap5	-	-	189.67±16.68	-	-	-	-	249.71±7.22
Hap6	-	-	155.33±16.68	-	249.33±16.68	247.30±9.14	260.00±28.90	-
Hap7	230.00±28.92	241.25±10.26	-	231.50±20.43	-	243.33±11.80	216.29±10.92	264.00±14.45
Egg production[2]								
6 wks								
Hap1	2.63 ± 3.50[a]	6.75 ± 4.95	-	-	19.00 ± 2.65[a]	-	9.30 ± 3.13	5.50 ± 7.00
Hap2	-	-	-	26.50 ± 2.86	-	11.00 ± 7.00[a]	-	-
Hap3	-	-	17.05 ± 2.16[a]	-	-	-	-	-

Hap4	-	8.69 ± 2.75	24.00 ± 9.90[a]	-	-	-	-	-
Hap5	-	-	20.67 ± 5.71[a]	-	-	-	-	8.00 ± 2.47
Hap6	-	-	7.75 ± 4.95[b]	-	15.00 ± 5.71[b]	7.64 ± 2.65[b]	8.50 ± 4.00	-
Hap7	27.00 ± 9.90[b]	4.00 ± 5.71	-	32.00 ± 7.00	-	15.13 ± 3.50[a]	17.88 ± 3.50	4.50 ± 4.95
Average Egg Weight[3] (g)								
6 wks								
Hap1	75.71 ± 1.49	76.18 ± 2.97	-	-	77.92 ± 0.82	-	74.90 ± 1.33	79.03 ± 2.97
Hap2	-	-	-	73.53 ± 0.86	-	77.18 ± 2.97	-	-
Hap3	-	-	92.28 ± 0.70	-	-	-	-	-
Hap4	-	75.18 ± 1.33	88.72 ± 2.97	-	-	-	-	-
Hap5	-	-	91.81 ± 1.72	-	-	-	-	76.76 ± 0.94
Hap6	-	-	92.00 ± 2.10	-	76.77 ± 1.72	77.26 ± 0.94	80.80 ± 2.97	-
Hap7	77.20 ± 2.97	73.90 ± 2.97	-	73.15 ± 2.10	-	77.79 ± 1.12	76.29 ± 1.12	75.82 ± 1.72
Egg production[2]								
10 wks								
Hap1	8.38 ± 5.11[a]	9.25 ± 7.23	-	-	27.86 ± 3.86[a]	-	13.20 ± 4.57	12.00± 10.22

Hap2	-	-	-	37.50 ± 4.17	-	15.00± 10.22[a]	-	-
Hap3	-	-	26.48 ± 3.16[a]	-	-	-	-	-
Hap4	-	12.00 ± 4.01	39.00± 14.46[a]	-	-	-	-	-
Hap5	-	-	27.00 ± 8.35[a]	-	-	-	-	15.19 ± 3.61
Hap6	-	-	15.25 ± 7.23[b]	-	22.67 ± 8.35[b]	12.57 ± 3.86[a]	10.50 ± 10.22	-
Hap7	36.00± 14.46[b]	5.33 ± 8.35	-	42.50± 10.22	-	23.88 ± 5.11[b]	27.25 ± 5.11	11.75 ± 7.23
Average Egg Weight[3] (g)								
10 wts								
Hap1	75.92 ± 1.04	76.34 ± 2.75	-	-	77.58 ± 0.76	-	74.41 ± 1.12[a]	77.77 ± 1.95
Hap2	-	-	-	73.88 ± 0.79	-	77.45 ± 2.75	-	-
Hap3	-	-	92.39 ± 0.65	-	-	-	-	-
Hap4	-	75.55 ± 1.23	90.06 ± 2.75	-	-	-	-	-
Hap5	-	-	91.63 ± 1.59	-	-	-	-	76.68 ± 0.74
Hap6	-	-	90.59 ± 1.59	-	76.69 ± 1.59	77.88 ± 0.87	80.80 ± 2.75[b]	-
Hap7	77.13 ± 2.75	74.20 ± 2.75	-	73.09 ± 1.95	-	77.58 ± 1.04	75.46 ± 0.97[a]	76.62 ± 1.38

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]AFE, age at first egg was recorded and given in days (d). Least square means ± SE.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks separately for each hen.

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

Table 4. 50. Associations between haplogroups of *turCry1* gene and semen quality traits among different varieties of turkeys.

Haplogroups	Semen quality traits[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
Ejaculate volume (mL)								
Hap1	0.05 ± 0.01	0.10 ± 0.03	-	-	0.18 ± 0.01	-	0.11 ± 0.02	0.10 ± 0.05
Hap2	-	-	-	0.12 ± 0.01	-	-	-	-
Hap3	-	-	0.15 ± 0.02	-	-	0.12 ± 0.05	-	-
Hap4	-	0.11 ± 0.05	-	-	-	-	-	-
Hap5	-	-	0.10 ± 0.05	-	-	-	-	0.10 ± 0.01
Hap6	-	0.04 ± 0.05	0.08 ± 0.05	-	0.17 ± 0.03	0.10 ± 0.02	0.08 ± 0.03	0.06 ± 0.03
Hap7	-	0.11 ± 0.05	0.14 ± 0.03	0.16 ± 0.03	0.17 ± 0.05	0.09 ± 0.03	0.10 ± 0.02	0.14 ± 0.05
Sperm concentration (x10^9/mL)								
Hap1	1.81 ± 0.18	2.52 ± 0.46	-	-	2.42 ± 0.18	-	2.11 ± 0.27	2.40 ± 0.65
Hap2	-	-	-	2.52 ± 0.18	-	-	-	-
Hap3	-	-	2.25 ± 0.25	-	-	1.23 ± 0.65	-	-
Hap4	-	2.38 ± 0.20	-	-	-	-	-	-

Hap5	-	-	1.78 ± 0.65	-	-	-	-	2.51 ± 0.20
Hap6	-	2.80 ± 0.65	1.60 ± 0.65	-	2.05 ± 0.46	2.47 ± 0.25	1.40 ± 0.46	2.96 ± 0.46
Hap7	-	2.66 ± 0.65	2.88 ± 0.46	2.83 ± 0.38	1.85 ± 0.65	1.75 ± 0.38	2.14 ± 0.23	3.37 ± 0.65
Total sperm number (x10^8/ejaculate)								
Hap1	1.00 ± 0.42	2.38 ± 1.06	-	-	4.44 ± 0.42	-	2.44 ± 0.61	2.24 ± 1.51
Hap2	-	-	-	3.05 ± 0.42	-	-	-	-
Hap3	-	-	3.42 ± 0.57	-	-	1.46 ±1.51	-	-
Hap4	-	2.60 ± 0.45	-	-	-	-	-	-
Hap5	-	-	1.78 ± 1.51	-	-	-	-	2.67 ± 0.45
Hap6	-	1.21 ± 1.51	1.28 ± 1.51	-	3.33 ± 1.06	2.43 ± 0.57	1.05 ± 1.06	1.70 ± 1.06
Hap7	-	3.01 ± 1.51	4.04 ± 1.06	4.69 ± 0.87	3.11 ± 1.51	1.66 ± 0.87	2.11 ± 0.53	4.71 ± 1.51
Sperm viability (%)								
Hap1	82.65 ± 0.93	78.00 ± 2.36	-	-	83.96 ± 0.93	-	84.83 ± 1.36	85.00 ± 3.34
Hap2	-	-	-	83.19 ± 0.93	-	-	-	-
Hap3	-	-	86.71 ± 1.26	-	-	82.00 ± 3.34	-	-
Hap4	-	79.50 ± 1.01	-	-	-	-	-	-

Hap5	-	-	88.50 ± 3.34	-	-	-	-	85.18 ± 1.01
Hap6	-	75.50 ± 3.34	89.00 ± 3.34	-	85.00 ± 2.36	83.57 ± 1.26	79.00 ± 2.36	82.50 ± 2.36
Hap7	-	83.00 ± 3.34	84.00 ± 2.36	85.00 ± 1.93	81.00 ± 3.34	83.50 ± 1.93	83.00 ± 1.18	84.50 ± 3.34

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]Least square means ± SE.

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

Table 4. 51. Associations between haplogroups of *turCry1* gene and plasma melatonin concentration within each variety of turkeys.

Haplogroups	Plasma melatonin[1*] (pg/ml)							
	BR	BS	CC	MW	NA	RP	SB	WH
Hap1	39.01±7.54	30.79±14.10	41.11±34.71	-	35.15±6.71	30.63±34.76	36.77±8.35[a]	19.87±19.85
Hap2	-	-	-	34.56±6.84	-	16.14±24.98	-	-
Hap3	-	-	35.97±7.01	-	-	11.84±19.85	-	-
Hap4	-	29.81±7.00	47.07±34.71	-	-	-	-	-
Hap5	-	-	29.90±17.35	-	-	-	-	38.65±6.77
Hap6		7.93±34.72	36.55±15.71	16.45±34.76	55.48±13.96	16.03±7.87	103.54±17.10[b]	25.95±24.94
Hap7	20.93±35.01	40.62±17.36	29.57±17.10	52.80±15.34	27.66±34.72	12.04±10.70	40.80±8.84[a]	24.57±15.73

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]Least square means ± SE.

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

Table 4. 52. Primer sequences, expected sizes of amplicons and PCR characteristics for the *turCry2* gene.

Primer ID	Primers[1]	Sequences	Tm[2] (^{0}C)	Amplicon length[3] (bp)
Cry2_1	For(24670732)	5′- ATAACTGTAGACAAATGAGGGATGAATAAG -3′	61.9	4833
	Rev(24665899)	5′- CTAAATCTATTAACCACATTAAGGACAGGAG -3′	63.3	
Cry2_2	For(24666256)	5′- CCATTACTGTAAGTTTAAGATCACATAGCAA -3′	62.0	4479
	Rev(24661777)	5′- CTATTATTCTGGAAACAAAATAAGCGAAGT -3′	60.5	
Cry2_3	For(24661580)	5′- CTGTAAAGATTTCTGTATCCAAAGTAAAAGAC -3′	62.1	3995
	Rev(24657585)	5′- AGAGACAGATAGATTTCAGCAGTAACTCAC -3′	64.6	
Cry2-4	For(24657975)	5′- GTAGGAACTCACGTTAGGTTAATAGTGGTA -3′	64.6	3285
	Rev(24654690)	5′- CTGCAGAAGAAAGTAGAAGAGAGTGTTTAC -3′	64.6	

[1]*For, forward primer; Rev, reverse primer.* Primer-binding sites in the turkey genome (GenBank accession No: LOC100008575) 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 4. 53. Characteristics of single nucleotide polymorphisms (SNPs) identified in the *turCry2* gene in eight divergent turkey varieties.

SNP	Location	Nucleotide position[1]	Sequence Context[2]	*dbSNP* Identification[3]	Genotype	Genotype Frequency %	MAF[4]	HWE[5]
Cry2-1	Promoter	24670588	GAAGG(A/*G*)AAAAG	ss538786531	A/A	94.48	0.04	0.00*
					A/G	0.0		
					G/G	5.52		
Cry2-2	Promoter	24670584	CAGAG(A/*G*)AGGAA	ss538786532	A/A	95.17	0.04	0.00*
					A/G	0.0		
					G/G	4.83		
Cry2-3	Exon 1	24670560	TCCAG(A/*G*)GACTG	ss538786533	A/A	87.59	0.08	0.00*
					A/G	8.28		
					G/G	4.13		
Cry2-4	Exon 1	24670558	CTTCC(A/*G*)GAGAC	ss538786534	A/A	85.86	0.14	0.01*
					A/G	0.00		
					G/G	14.14		
Cry2-5	Exon 1	24670553	CAGAT(C/*T*)TTCCA	ss538786535	C/C	84.48	0.15	0.01*

					C/T	0.00		
					T/T	15.52		
Cry2-6	Exon 1	24670551	TCCAG(A/*T*)TCTTC	ss538786536	A/A	82.76	0.18	0.00*
					A/T	0.00		
					T/T	17.24		
Cry2-7	Exon 1	24670510	GCACC(*C*/A)CAAAC	ss538786537	C/C	14.83	0.15	0.01*
					C/A	0.00		
					A/A	85.17		
Cry2-8	Intron 1	24670430	GTTTT(C/*T*)CTCAC	ss538786538	C/C	95.52	0.03	0.00*
					C/T	3.10		
					T/T	1.38		
Cry2-9	Intron 1	24670419	ATGGG(*C*/A)CATTA	ss538786539	C/C	14.83	0.15	0.01*
					C/A	0.00		
					A/A	85.17		
Cry2-10	Intron 1	24670340	GTACA(A/*G*)GAGTA	ss538786540	A/A	84.48	0.15	0.01*
					A/G	0.00		
					G/G	15.52		

Cry2-11	Intron 1	24670268	AAGCC(*C*/A)CCATC	ss538786541	C/C	13.79	0.13	0.00*
					C/A	0.00		
					A/A	86.21		
Cry2-12	Intron 1	24670204	CTGAG(C/*T*)AAAGG	ss538786542	C/C	92.41	0.06	0.00*
					C/T	4.14		
					T/T	3.45		
Cry2-13	Intron 3	24661558	TTCAC(A/*T*)CACAA	ss538786543	A/A	93.79	0.06	0.00*
					A/T	0.70		
					T/T	5.51		
Cry2-14	Intron 3	24661541	TGGCA(*A*/G)TTAAC	ss538786544	A/A	2.75	0.02	0.01*
					A/G	2.06		
					G/G	95.19		
Cry2-15	Intron 3	24661389	TCAAC(A/*G*)AAATG	ss538786545	A/A	96.21	0.02	0.00*
					A/G	1.72		
					G/G	2.07		

[1]Position of the SNP in GenBank on the forward strand of chromosome 5 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]*ss* prefix indicates novel SNPs detected here and submitted for *rs* assignment in *dbSNP*, NCBI.

[4]Minor allele frequency (MAF) of 15 SNPs markers.

[5]Significance of deviation from HWE for the 15 SNPs. NS indicates non-significant ($P > 0.05$) while * refers to significant at $P < 0.05$.

Table 4. 54. Linkage disequilibrium as measured by *D´* and r^2 between the 15 segregating SNPs in the *turCry2* gene.

SNPs[1]	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***
1		1.00	0.54	0.82	0.91	0.91	0.91	NS	0.86	0.86	0.73	NS	NS	NS	NS
2	0.92		0.59	0.81	1.00	1.00	0.90	NS	0.95	0.95	0.81	NS	NS	NS	NS
3	0.15	0.16		0.76	0.81	0.93	0.90	NS	0.93	0.83	0.81	NS	NS	NS	NS
4	0.20	0.18	0.35		0.82	0.89	0.91	NS	0.89	0.86	0.82	0.13	NS	NS	NS
5	0.22	0.24	0.34	0.58		0.88	0.81	NS	0.82	0.77	0.85	NS	NS	NS	NS
6	0.18	0.20	0.36	0.56	0.63		0.93	NS	0.94	0.86	0.92	NS	NS	NS	NS
7	0.22	0.20	0.43	0.74	0.64	0.69		NS	0.93	0.88	0.94	NS	NS	NS	0.35
8	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS
9	0.20	0.22	0.46	0.71	0.66	0.71	0.86	NS		0.95	0.97	NS	NS	NS	NS
10	0.20	0.22	0.36	0.64	0.59	0.61	0.75	NS	0.87		0.94	0.19	NS	NS	NS
11	0.16	0.18	0.38	0.66	0.65	0.63	0.81	NS	0.87	0.79		0.20	NS	NS	NS
12	NS	NS	NS	0.007	NS	NS	NS	NS	NS	0.01	0.02		NS	NS	NS
13	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		0.82	0.53
14	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.24		0.32
15	NS	NS	NS	NS	NS	NS	0.01	NS	NS	NS	NS	NS	0.08	0.08	

[1]SNP identification (*1-15 indicates Cry2-1 – Cry2-15*).

NS indicates the non-significant *D'* and r^2 values ($P \leq 0.05$).

D' values are listed in upper right section, and r^2 values are listed in lower left section.

Table 4. 55. The pairwise fixation index (*Fst*) estimated for turkey varieties using the *turCry2* gene variants.

Turkey varieties[1]	BR	BS	NA	MW	SB	WH	RP	CC
BR	0.0							
BS	0.03	0.0						
NA	0.02	0.03	0.0					
MW	0.07	0.14	0.11	0.0				
SB	0.03	0.04	0.03	0.14	0.0			
WH	0.20	0.30	0.25	0.04	0.29	0.0		
RP	0.22	0.30	0.26	0.07	0.30	0.01*	0.0	
CC	0.07	0.14	0.10	0.01*	0.12	0.04	0.07	0.0

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

* indicates non-significant *Fst* ($P \geq 0.05$).

Table 4. 56. Haplogroup frequencies of *turCry2* gene in eight turkey populations.

Turkey Varieties	Haplogroups				
	N	Hap1	Hap2	Hap3	Hap4
BR	23	0.78	0.04	0.14	0.04
BS	36	0.81	0.00	0.11	0.08
CC	50	0.18	0.20	0.02	0.60
MW	31	0.51	0.23	0.19	0.07
NA	33	0.82	0.03	0.06	0.09
RP	39	0.33	0.39	0.00	0.28
SB	37	0.86	0.00	0.14	0.00
WH	37	0.42	0.36	0.08	0.14

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

N = numbers of birds from each variety used for calculation of haplogroup frequencies.

Table 4. 57. Associations between haplogroups of the *turCry2* gene and body weight (BW) at different ages of turkeys.

Haplogroups	BW[1] (kg)					
	1 d	34 d*	68 d*	159 d	231 d*	309 d
Hap1	0.045 ± 0.001[a]	**0.712 ± 0.01[a]**	**2.07 ± 0.03[a]**	6.57 ± 0.08[a]	8.70 ± 0.10[a,b]	9.00 ± 0.10[a]
Hap2	**0.047 ± 0.001[a]**	0.708 ± 0.02[a]	2.02 ± 0.06[a,b]	6.65 ± 0.16[a]	8.51 ± 0.19[a]	**9.01 ± 0.18[a]**
Hap3	0.046 ± 0.001[a]	0.704 ± 0.03[a,b]	2.04 ± 0.08[a,b]	**6.61 ± 0.20[a]**	8.59 ± 0.23[a,b]	8.95 ± 0.23[a]
Hap4	0.046 ± 0.001[a]	0.656 ± 0.02[b]	1.93 ± 0.06[b]	6.43 ± 0.16[a]	**9.00 ± 0.18[b]**	8.91 ± 0.18[a]

[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 4. 58. Associations between haplogroups of *turCry2* gene and average daily gain (ADG) by period of age for turkeys.

Haplogroups	ADG[1] (kg)					
	1-34 d	35 – 68 d	69 – 159 d	160 – 231 d*	232 – 309 d	1 – 309 d
Hap1	**0.020 ± 0.001[a]**	**0.040 ± 0.001[a]**	0.050 ± 0.001[a]	0.030 ± 0.001[a,b]	0.004 ± 0.001[a]	0.029 ± 0.001[a]
Hap2	0.019 ± 0.001[a]	0.038 ± 0.001[a]	**0.051 ± 0.002[a]**	0.026 ± 0.002[a]	**0.007 ± 0.002[a]**	0.029 ± 0.001[a]
Hap3	0.019 ± 0.001[a]	0.039 ± 0.002[a]	0.050 ± 0.002[a]	0.027 ± 0.003[a]	0.006 ± 0.003[a]	0.028 ± 0.001[a]
Hap4	0.018 ± 0.001[a]	0.037 ± 0.001[a]	0.049 ± 0.002[a]	**0.036 ± 0.002[b]**	-0.002 ± 0.002[a]	0.028 ± 0.001[a]

[a,b]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. *$P \leq 0.05$.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 59. Associations between haplogroups of *turCry2* gene and feed conversion ratio (FCR) by periods of age for turkeys.

Haplogroups	FCE[1]			
	34 – 68 d*	69 – 159 d	160 – 231 d	34 – 231d
Hap1	2.74 ± 0.08[a]	4.51 ± 0.07a	12.16 ± 0.35[a]	7.36 ± 0.11[a]
Hap2	2.91 ± 0.16[a,b]	**4.47 ± 0.14a**	11.98 ± 0.69[a]	**7.17 ± 0.22[a]**
Hap3	**2.69 ± 0.20[a]**	4.48 ± 0.18a	11.64 ± 0.86[a]	7.43 ± 0.28[a]
Hap4	3.21 ± 0.16[b]	4.68 ± 0.14a	**10.96 ± 0.68[a]**	7.23 ± 0.22[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 4. 60. Associations between haplogroups of *turCry2* gene and egg production traits for turkeys.

Haplogroups	Egg production traits[1]				
	AFE[4] (d)	Egg production[2]		Average egg weight[3] (g)	
		6 wks	10 wks	6 wks*	10 wks*
Hap1	232.93 ± 3.67[a]	12.79 ± 1.17[a]	19.75 ± 1.69[a]	78.16 ± 0.39[a,b]	78.12 ± 0.33[a,b]
Hap2	**221.88 ± 6.75[a]**	12.40 ± 2.27[a]	20.00 ± 3.27[a]	77.65 ± 0.71[a,b]	78.01 ± 0.61[a,b]
Hap3	235.14 ± 9.52[a]	**14.60 ± 2.89[a]**	**22.43 ± 4.18[a]**	**79.78 ± 0.91[a]**	**79.78 ± 0.83[a]**
Hap4	232.82 ± 7.45[a]	10.48 ± 2.24[a]	15.44 ± 3.23[a]	76.68 ± 0.76[b]	76.63 ± 0.67[b]

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE. *$P \leq 0.05$.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks 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.

Table 4. 61. Associations between haplogroups of *turCry2* gene and semen quality traits for turkeys.

Haplogroups	Semen quality traits[1]			
	Ejaculate volume (mL)	Sperm Concentration ($x10^9$/mL)	Total number of sperm ($x10^8$/ejaculate)	Sperm viability (%)
Hap1	0.11 ± 0.01^a	2.24 ± 0.09^a	$\underline{2.58 \pm 0.22}^a$	83.03 ± 0.48^a
Hap2	0.11 ± 0.01^a	2.30 ± 0.19^a	2.71 ± 0.45^a	$\underline{82.57 \pm 0.98}^a$
Hap3	$\underline{0.10 \pm 0.02}^a$	$\mathbf{2.62 \pm 0.22^a}$	$\mathbf{2.78 \pm 0.50^a}$	83.12 ± 1.10^a
Hap4	$\mathbf{0.12 \pm 0.01^a}$	$\underline{2.22 \pm 0.19}^a$	2.73 ± 0.45^a	$\mathbf{84.93 \pm 0.97^a}$

[a]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE.

Bold values represent the advantageous haplogroups while underline values represent the low performing haplogroups.

Table 4. 62. Associations between haplogroups of *turCry2* gene and plasma melatonin concentration for turkeys.

Haplogroups	Plasma melatonin[1] (pg/ml)
Hap1	**36.54 ± 3.04[a]**
Hap2	29.11 ± 5.94[a]
Hap3	27.57 ± 7.26[a]
Hap4	34.50 ± 5.76[a]

[a]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

[1]Least square means ± SE.

Bold values represent the highest plasma melatonin haplogroups while underline values represent the lowest.

Table 4. 63. Associations between haplogroups of *turCry2* gene and body weight (BW) at different ages within each variety of turkeys.

Haplogroups	BW*[1] (kg)							
	BR	BS	CC	MW	NA	RP	SB	WH
Day 1								
Hap1	0.041±0.001	0.047±0.001	0.059±0.002	0.041±0.001	0.046±0.001	0.044±0.001	0.047±0.001	0.042±0.001
Hap2	0.043±0.004	-	0.057±0.001	0.042±0.002	0.044±0.004	0.046±0.001	-	0.044±0.001
Hap3	0.043±0.002	0.049±0.002	0.055±0.004	0.041±0.002	0.046±0.003	-	0.044±0.002	0.047±0.003
Hap4	0.047±0.004	0.046±0.002	0.055±0.001	0.039±0.003	0.047±0.002	0.046±0.001	-	0.048±0.002
Day 34								
Hap1	0.60 ± 0.03	0.61 ± 0.02	1.46 ± 0.05[a]	0.59 ± 0.03	0.66 ± 0.02	0.58 ± 0.04	0.64 ± 0.02	0.61 ± 0.03
Hap2	0.46 ± 0.13	-	1.37 ± 0.04[a]	0.60 ± 0.05	0.62 ± 0.13	0.58 ± 0.03	-	0.63 ± 0.04
Hap3	0.61 ± 0.07	0.59 ± 0.06	1.05 ± 0.13[b]	0.62 ± 0.05	0.63 ± 0.09	-	0.65 ± 0.06	0.69 ± 0.08
Hap4	0.60 ± 0.13	0.58 ± 0.07	1.26 ± 0.02[b]	0.57 ± 0.09	0.68 ± 0.07	0.55 ± 0.04	-	0.63 ± 0.06
Day 68								
Hap1	1.70 ± 0.08	1.64 ± 0.07	4.84 ± 0.13[a]	1.53 ± 0.09	1.90 ± 0.07[a]	1.61 ± 0.10	1.68 ± 0.06	1.71 ± 0.09

Hap2	1.56 ± 0.36	-	4.60 ± 0.11[a]	1.58 ± 0.13	1.74 ± 0.36[a,b]	1.62 ± 0.10	-	1.65 ± 0.10
Hap3	1.65 ± 0.21	1.71 ± 0.18	3.80 ± 0.36[b]	1.60 ± 0.14	1.87 ± 0.25a,b	-	1.70 ± 0.16	1.80 ± 0.21
Hap4	1.72 ± 0.36	1.40 ± 0.20	4.40 ± 0.07[a,b]	1.95 ± 0.26	1.44 ± 0.21[b]	1.61 ± 0.11	-	1.82 ± 0.16
Day 159								
Hap1	5.27 ± 0.23	5.15 ± 0.18	17.20 ± 0.35	4.61 ± 0.24	5.62 ± 0.18	4.27 ± 0.27	4.93 ± 0.17	5.43 ± 0.25
Hap2	5.00 ± 0.98	-	18.00 ± 031	4.82 ± 0.36	6.26 ± 0.97	4.38 ± 0.27	-	4.85 ± 0.27
Hap3	4.73 ± 0.56	4.88 ± 0.48	17.20 ± 0.97	4.83 ± 0.39	5.41 ± 0.68	-	5.22 ± 0.44	5.95 ± 0.58
Hap4	6.08 ± 0.98	4.91 ± 0.55	17.12 ± 0.18	4.63 ± 0.70	5.77 ± 0.55	4.17 ± 0.29	-	5.46 ± 0.43
Day 231								
Hap1	7.54 ± 0.26	7.20 ± 0.21	20.49 ± 0.41	6.48 ± 0.28	7.74 ± 0.21	6.09 ± 0.33	7.01 ± 0.20	7.11 ± 0.29
Hap2	7.66 ± 1.13	-	19.56 ± 0.37	6.65 ± 0.42	6.91 ± 1.12	6.40 ± 0.33	-	6.69 ± 0.32
Hap3	6.99 ± 0.64	6.82 ± 0.56	19.16 ± 1.12	6.04 ± 0.45	8.21 ± 0.78	-	7.67 ± 0.51	7.21 ± 0.67
Hap4	8.94 ± 1.13	7.05 ± 0.64	20.83 ± 0.21	6.55 ± 0.81	7.86 ± 0.64	6.19 ± 0.34	-	7.21 ± 0.50
Day 309								
Hap1	7.83 ± 0.26	7.45 ± 0.20	21.29 ± 0.43	6.73 ± 0.27	7.88 ± 0.21[a,b]	6.16 ± 0.32	7.46 ± 0.19	7.52 ± 0.30
Hap2	7.68 ± 1.12	-	21.44 ± 0.37	6.92 ± 0.41	6.49 ± 1.11[a]	6.45 ± 0.32	-	6.83 ± 0.31

Hap3	7.50 ± 0.63	6.92 ± 0.55	19.82 ± 1.11	6.19 ± 0.44	9.07 ± 0.77[b]	-	8.23 ± 0.50	7.34 ± 0.66
Hap4	9.08 ± 1.12	7.60 ± 0.63	20.78 ± 0.23	6.39 ± 0.80	8.42 ± 0.63[a,b]	6.07 ± 0.33	-	7.55 ± 0.49

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]BW, body weight (kg) was measured at 1, 34, 68, 159, 231 and 309 days (d). Least square means ± SE.

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

Table 4. 64. Associations between haplogroups of *turCry2* gene and average daily gain (ADG) by period of age within each variety of turkeys.

Haplogroups	ADG[1]* (kg/d)							
	BR	BS	CC	MW	NA	RP	SB	WH
1 – 34 d								
Hap1	0.017±0.001	0.017±0.001	0.043±0.001[a]	0.017±0.001	0.019±0.001	0.016±0.001	0.018±0.001	0.017±0.001
Hap2	0.013±0.004	-	0.040±0.001[a]	0.017±0.001	0.018±0.004	0.016±0.001	-	0.018±0.001
Hap3	0.017±0.002	0.016±0.002	0.030±0.004[b]	0.018±0.002	0.018±0.003	-	0.018±0.002	0.019±0.001
Hap4	0.017±0.004	0.016±0.002	0.036±0.001[b]	0.016±0.003	0.019±0.002	0.015±0.001	-	0.018±0.002
35 – 68 d								
Hap1	0.032±0.002	0.031±0.002	0.099±0.003[a]	0.027±0.002[a]	0.037±0.002	0.030±0.002	0.031±0.001	0.032±0.002
Hap2	0.032±0.008	-	0.095±0.003[a]	0.029±0.003[a]	0.033±0.008	0.031±0.002	-	0.030±0.002
Hap3	0.030±0.005	0.033±0.004	0.081±0.008[b]	0.029±0.003[a]	0.037±0.006	-	0.031±0.004	0.033±0.005
Hap4	0.032±0.008	0.025±0.005	0.092±0.002[a]	0.041±0.006[b]	0.032±0.005	0.031±0.003	-	0.035±0.004
69 – 159 d								
Hap1	0.039±0.002	0.039±0.002	0.136±0.004[b]	0.034±0.003	0.041±0.002	0.029±0.003	0.036±0.002	0.041±0.003

Hap2	0.038±0.011	-	0.147±0.003[a]	0.036±0.004	0.050±0.011	0.030±0.003	-	0.035±0.003
Hap3	0.034±0.006	0.035±0.005	0.148±0.011[a]	0.036±0.004	0.039±0.007	-	0.039±0.005	0.046±0.006
Hap4	0.048±0.011	0.039±0.006	0.140±0.002[a,b]	0.030±0.008	0.048±0.006	0.028±0.003	-	0.040±0.005
160 – 231 d								
Hap1	0.032±0.003	0.027±0.006	0.046±0.004[b]	0.026±0.003	0.029±0.002	0.025±0.003	0.029±0.002	0.023±0.003
Hap2	0.037±0.012	-	0.022±0.004[a]	0.025±0.004	0.029±0.012	0.028±0.003	-	0.026±0.003
Hap3	0.032±0.007	0.030±0.006	0.027±0.012[a]	0.017±0.005	0.039±0.008	-	0.034±0.005	0.018±0.007
Hap4	0.040±0.012	0.030±0.007	0.052±0.002[b]	0.027±0.009	0.029±0.007	0.028±0.004	-	0.024±0.005
232 – 309 d								
Hap1	0.004±0.003	0.003±0.002	0.010±0.005	0.003±0.003	0.002±0.002	0.001±0.004	0.006±0.002	0.005±0.003
Hap2	0.000±0.013	-	0.013±0.004	0.004±0.005	-0.006±0.013	0.001±0.004	-	0.002±0.004
Hap3	0.007±0.007	0.001±0.006	0.006±0.013	0.002±0.005	0.011±0.009	-	0.008±0.006	0.002±0.008
Hap4	0.002±0.013	0.007±0.007	-0.003±0.003	-0.002±0.009	0.007±0.017	-0.002±0.004	-	0.004±0.006
1 – 309 d								
Hap1	0.025±0.001	0.024±0.001	0.069±0.001	0.022±0.001	0.026±0.001	0.020±0.001	0.024±0.001	0.024±0.001
Hap2	0.025±0.004	-	0.070±0.001	0.022±0.001	0.021±0.004	0.021±0.001	-	0.022±0.001

Hap3	0.024±0.002	0.022±0.002	0.064±0.004	0.020±0.001	0.029±0.003	-	0.027±0.002	0.024±0.002
Hap4	0.030±0.004	0.024±0.002	0.067±0.001	0.021±0.003	0.027±0.002	0.020±0.001	-	0.025±0.002

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]ADG, average daily gain (kg) was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 65. Associations between haplogroups of *turCry2* gene and feed conversion ratio (FCR) by period of age within each variety of turkeys.

Haplogroups	FCR[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
34 – 68 d								
Hap1	3.05 ± 0.21	3.32 ± 0.16	1.88 ± 0.33	3.39 ± 0.22	2.38 ± 0.17	2.46 ± 0.25	3.04 ± 0.16	2.80 ± 0.23
Hap2	3.03 ± 0.90	-	1.94 ± 0.28	3.28 ± 0.33	2.25 ± 0.89	2.44 ± 0.25	-	2.87 ± 0.25
Hap3	3.21 ± 0.51	2.99 ± 0.45	2.36 ± 0.89	3.20 ± 0.36	2.26 ± 0.62	-	2.98 ± 0.40	2.72 ± 0.53
Hap4	2.99 ± 0.90	4.11 ± 0.51	2.06 ± 0.16	2.14 ± 0.64	2.91 ± 0.51	2.48 ± 0.27	-	2.50 ± 0.39
69 – 159 d								
Hap1	5.33 ± 0.20	4.29 ± 0.15	3.49 ± 0.31	4.48 ± 0.21[a]	4.77 ± 0.16	5.21 ± 0.24	4.71 ± 0.15	3.84 ± 0.22
Hap2	5.37 ± 0.85	-	3.06 ± 0.27	4.15 ± 0.31[a]	4.07 ± 0.84	5.09 ± 0.23	-	4.51 ± 0.24
Hap3	6.14 ± 0.48	4.60 ± 0.42	2.97 ± 0.84	4.22 ± 0.34[a]	5.07 ± 0.59	-	4.30 ± 0.38	3.58 ± 0.50
Hap4	4.52 ± 0.85	4.23 ± 0.48	3.32 ± 0.15	5.78 ± 0.61[b]	4.12 ± 0.48	5.82 ± 0.25	-	4.07 ± 0.37
160 – 231 d								
Hap1	10.32 ± 0.97	11.79 ± 0.76	13.34 ± 1.52[a,b]	13.07 ± 1.02	11.70 ± 0.79	11.84 ± 1.20	11.16 ± 0.72	13.26 ± 1.08

Hap2	9.20 ± 4.18	-	16.50 ± 1.38[a]	12.80 ± 1.55	18.39 ± 4.15	10.48 ± 1.20	-	11.88 ± 1.17
Hap3	9.17 ± 2.38	11.16 ± 2.07	14.09 ± 4.14[a,b]	15.67 ± 1.67	7.12 ± 2.87	-	8.62 ± 1.87	13.59 ± 2.47
Hap4	8.93 ± 4.18	12.60 ± 2.37	12.65 ± 0.78[b]	12.92 ± 2.99	9.87 ± 2.37	11.88 ± 1.25	-	10.53 ± 1.83
34 – 231 d								
Hap1	7.60 ± 0.31	7.16 ± 0.24	6.14 ± 0.48	8.24 ± 0.33[a,b]	7.21 ± 0.25	7.92 ± 0.38[a,b]	7.56 ± 0.23	6.93 ± 0.34
Hap2	7.37 ± 1.33	-	6.33 ± 0.44	7.61 ± 0.49[a]	7.58 ± 1.32	7.45 ± 0.38[a]	-	7.23 ± 0.37
Hap3	7.90 ± 0.75	7.74 ± 0.66	6.23 ± 1.31	9.15 ± 0.53[b]	6.48 ± 0.92	-	6.51 ± 0.59	6.83 ± 0.79
Hap4	6.70 ± 1.33	7.05 ± 0.75	5.68 ± 0.25	9.05 ± 0.95[a,b]	6.49 ± 0.75	8.61 ± 0.40[b]	-	6.73 ± 0.58

[a,b]Means within columns with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]FCR, feed conversion ratio was estimated at different periods of the age. Least square means ± SE.

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

Table 4. 66. Associations between haplogroups of *turCry2* gene and egg production traits within each variety of turkeys.

Haps	Egg production traits*							
	BR	BS	CC	MW	NA	RP	SB	WH
AFE[1](d)								
Hap1	242.83±11.38	250.50±11.38	209.33±11.38[a]	223.71±10.54	222.36±7.45[a]	244.33±11.38	228.25±8.05	253.56±9.29
Hap2	-	-	160.33±11.38[b]	219.00±16.10	-	244.14±10.54	-	248.78±9.29
Hap3	244.00±19.72	-	161.00±27.83[b]	214.00±11.10	291.00±27.88[b]	-	233.00±16.10	-
Hap4	-	232.00±27.83	177.67±8.05[b]	258.00±27.88	256.00±27.88[a,b]	248.50±13.94	-	263.00±19.72
Egg production[2]								
6 wks								
Hap1	2.00 ± 3.91[a]	7.88 ± 2.59	14.00 ± 3.66	26.43 ± 3.91[a]	19.27 ± 2.67	14.75 ± 3.66	10.94 ± 2.59	6.20 ± 3.27
Hap2	-	-	15.71 ± 3.91	29.00 ± 5.98[a]	-	7.90 ± 3.27	-	9.00 ± 3.45
Hap3	17.00 ± 7.32[b]	-	22.00 ± 10.35	30.33 ± 5.98[a]	5.00 ± 10.35	-	15.50 ± 5.18	-
Hap4	-	13.00 ± 7.32	15.40 ± 2.67	9.50 ± 7.32[b]	17.00 ± 10.35	7.57 ± 3.91	-	4.67 ± 5.98
Average Egg Weight[3] (g)								
6 wks								

Hap1	75.30 ± 1.53	75.18 ± 1.08	94.69 ± 1.08[a]	72.67 ± 1.00	77.82 ± 0.71	78.29 ± 1.00	74.39 ± 0.88[a]	77.38 ± 1.08
Hap2	-	-	90.31 ± 1.19[b]	76.79 ± 1.53	-	77.29 ± 1.00	-	76.49 ± 1.00
Hap3	77.08 ± 1.88	-	93.82 ± 2.65[a,b]	72.79 ± 1.53	75.36 ± 2.65	-	79.97 ± 1.33[b]	-
Hap4	-	74.91 ± 2.65	91.30 ± 0.77[b]	71.26 ± 2.65	78.37 ± 2.65	76.33 ± 1.33	-	74.34 ± 2.65
Egg production[2]								
10 wks								
Hap1	7.29 ± 5.79	11.13 ± 3.77	21.88 ± 5.33	36.00 ± 5.70	28.20 ± 3.89	22.00 ± 5.33	16.63 ± 3.77	13.10 ± 4.77
Hap2	-	-	26.14 ± 5.70	41.00 ± 8.70	-	13.30 ± 4.77	-	17.56 ± 5.02
Hap3	26.00 ± 10.66	-	37.00 ± 15.07	44.67 ± 8.70	9.00 ± 15.07	-	21.25 ± 7.54	-
Hap4	-	15.50 ± 10.66	22.80 ± 3.89	13.00 ± 10.66	26.00 ± 15.07	12.57 ± 5.70	-	8.33 ± 8.70
Average Egg Weight[3] (g)								
10 wts								
Hap1	75.89 ± 1.00	75.41 ± 1.00	95.45 ± 1.00[a]	72.96 ± 0.92	77.47 ± 0.65	77.93 ± 0.92	74.00 ± 0.74[a]	76.79 ± 0.81
Hap2	-	-	91.20 ± 1.00[b]	76.71 ± 1.41	-	77.97 ± 0.92	-	76.71 ± 0.81
Hap3	76.60 ± 1.73	-	93.98 ± 2.44[a,b]	73.32 ± 1.41	76.13 ± 2.44	-	79.24 ± 1.22[b]	-
Hap4	-	75.79 ± 2.44	90.49 ± 0.70[a,b]	71.99 ± 2.44	77.92 ± 2.44	77.00 ± 1.22	-	77.02 ± 1.73

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]AFE, age at first egg was recorded and given in days (d). Least square means ± SE.

[2]Egg production was individually recorded for a period of 10 wks starting from 30 to 40 wks of age. The total egg production for each hen was estimated for a period of 6 wks and 10 wks. The individual egg production of 6 wks was calculated excluding the first and last two weeks egg production from the period of 10 wks of egg production.

[3]The average egg weight (g) was calculated for the period of 6 wks and 10 wks separately for each hen.

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

Table 4. 67. Associations between haplogroups of *turCry2* gene and semen quality traits among different varieties of turkeys.

Haplogroups	Semen quality traits[1*]							
	BR	BS	CC	MW	NA	RP	SB	WH
Ejaculate volume (mL)								
Hap1	0.05 ± 0.01	0.09 ± 0.01	-	0.13 ± 0.02	0.20 ± 0.01[b]	0.10 ± 0.02	0.10 ± 0.01	0.08 ± 0.02
Hap2	0.07 ± 0.05	-	-	0.15 ± 0.02	0.10 ± 0.05[a]	0.11 ± 0.03	-	0.10 ± 0.02
Hap3	0.07 ± 0.05	0.16 ± 0.05	-	0.10 ± 0.03	0.10 ± 0.05[a]	-	0.10 ± 0.05	0.09 ± 0.03
Hap4	0.07 ± 0.05	0.17 ± 0.05	0.14 ± 0.01	-	0.15 ± 0.03[a,b]	0.09 ± 0.02	-	0.13 ± 0.03
Sperm concentration (x10^9/mL)								
Hap1	1.83 ± 0.21	2.44 ± 0.18	-	2.61 ± 0.22	2.35 ± 0.19	2.13 ± 0.33	2.00 ± 0.17	2.05 ± 0.29[a]
Hap2	2.00 ± 0.65	-	-	2.51 ± 0.33	2.95 ± 0.65	2.28 ± 0.38	-	2.37 ± 0.33[b]
Hap3	1.48 ± 0.65	2.51 ± 0.65	-	2.58 ± 0.38	1.96 ± 0.65	-	2.45 ± 0.65	3.69 ± 0.38[b]
Hap4	1.70 ± 0.65	2.38 ± 0.65	2.26 ± 0.20	-	2.15 ± 0.46	2.10 ± 0.33	-	2.70 ± 0.46[a,b]
Total sperm number (x10^8/ejaculate)								
Hap1	0.94 ± 0.48	2.26 ± 0.42	-	3.36 ± 0.50	4.70 ± 0.43	2.10 ± 0.75	2.08 ± 0.39	1.76 ± 0.67
Hap2	1.33 ± 1.51	-	-	3.93 ± 0.75	2.86 ± 1.51	2.44 ± 0.87	-	2.26 ± 0.75

Hap3	1.01 ± 1.51	2.06 ± 1.51	-	2.60 ± 0.87	2.03 ± 1.51	-	2.43 ± 1.51	3.96 ± 0.87
Hap4	1.24 ± 1.51	2.09 ± 1.51	3.19 ± 0.45	-	3.15 ± 1.06	1.93 ± 0.75	-	3.59 ± 1.07
Sperm viability (%)								
Hap1	82.15 ± 1.04	79.00 ± 0.91	-	84.39 ± 1.10	83.25 ± 0.95[a,b]	81.75 ± 1.65	83.53 ± 0.85	85.20 ± 1.47
Hap2	84.50 ± 3.29	-	-	81.13 ± 1.65	80.50 ± 3.29[a]	84.50 ± 1.90	-	84.63 ± 1.65
Hap3	86.00 ± 3.29	82.00 ± 3.29	-	84.17 ± 1.90	85.50 ± 3.29[a,b]	-	78.00 ± 3.29	83.33 ± 1.90
Hap4	82.50 ± 3.29	80.00 ± 3.29	86.59 ± 0.99	-	88.75 ± 2.33[b]	84.25 ± 1.65	-	85.75 ± 2.33

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]Least square means ± SE.

Seven different varieties of heritage turkeys including Bourbon Red (BR), Blue Slate (BS), Narragansett (NA), Royal Palm (RP), Spanish Black (SB), White Holland (WH) and Midget White (MW) and Commercial (CC) turkeys were used.

Table 4. 68. Associations between haplogroups of *turCry2* gene and plasma melatonin concentration within each variety of turkeys.

Haplogroups	Plasma melatonin[1*] (pg/ml)							
	BR	BS	CC	MW	NA	RP	SB	WH
Hap1	41.21±8.26	33.7±6.48	44.44±14.27	35.33±8.54	42.58±6.55	11.80±10.03	47.80±6.11	32.91±9.27[a]
Hap2	61.42±34.86	-	48.15±14.49	15.84±12.89	2.64±34.54	16.78±9.75	-	26.50±9.74[a]
Hap3	18.46±19.80	23.68±17.26	23.17±34.57	53.02±13.88	4.16±24.05	-	33.69±15.60	23.80±20.63[a]
Hap4	23.72±34.86	8.64±19.73	31.57±6.56	73.00±24.87	29.42±19.74	15.83±10.39	-	69.66±15.26[b]

[a,b]Means within columns and rows with different superscripts are significantly different ($P \leq 0.05$).

*Means within columns without any superscripts are non-significant ($P \geq 0.05$).

[1]Least square means ± SE.

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

CHAPTER 5

Summary and Conclusions

As proof of concept for the genotype:phenotype association, the *chLEAP-2* gene was first screened for DNA sequence variation and evaluated the relationships among its haplotypes (based on haplogroups) with gene expression levels, weight gain and lesion score in two *Eimeria maxima* challenged chicken lines. After establishing a working approach using the proof of concept, the primary focus of the dissertation was to test the hypothesis that the differences in DNA sequence variation of turkey clock genes may be associated with performance traits including growth and reproduction. Turkey genome sequences and genomic resources provide scientists with knowledge of specific genes that are essential in meat yield and quality, health and disease resistance, fertility and reproduction. The understanding of genetic variations in turkeys may be useful for the development of new tools that can be used to breed turkeys with desirable texture, flavor and leanness of the meat and grow turkey faster. In addition, the emerging turkey genome sequence provides a unique opportunity to understand DNA sequence variations of the clock genes and to evaluate the relationships among its haplotypes (based on haplogroups) with growth and reproductive traits of turkeys.

Specific findings:

1. In the preliminary investigation to test the proof of concept, a total of 16 SNPs were identified in the DNA sequence of *chLEAP-2* gene in the chicken. Of the 16 SNPs, seven each in the promoter and introns, and two in exons were detected. One of the exonic SNPs was non-synonymous, involving a cysteine to tyrosine codon change. D´

among the SNPs ranged from 0.02 to 1.00. The haplotypes observed from the 16 SNPs were assembled into 5 haplogroups which showed differences, though not significant in *chLEAP-2* expression levels. Similarly, differences among haplogroups for lesion score (LS) and weight gain were not statistically significant ($P \geq 0.05$). Based on LS, Hap4 appeared to be the haplogroup least susceptible to coccidiosis. At a minimum, the data does not strongly support an association between *chLEAP-2* DNA sequence variations and susceptibility to coccidiosis though some haplogroups deserve further studies. Additionally, the genomic reagents provide resources for evaluating both *cis* and *trans*-acting factors that may explain the rate of *chLEAP-2* gene expression in response to coccidiosis in the chicken.

2. The growth was significantly ($P \leq 0.05$) affected by the turkey genotype. The changes of BW and ADG in heritage varieties have a similar pattern compared to CC turkeys. CC turkeys have been highly selected for increased body weight and growth rate. Thus highest growth parameters were reported from CC turkeys. As expected, CC turkeys were superior to heritage birds in performance for most of the traits evaluated. NA males and females were heaviest while RP birds were lightest among heritage birds. The plasma melatonin concentration was also affected by the variety. The semen quality traits were specific for different turkey varieties. CC turkey reached the sexual maturity earlier than heritage turkey hens. MW turkey had the better egg laying performances while CC turkey had the highest average egg weight. When compared the male and female reproductive performances of turkeys, heritage turkeys showed better reproductive performances compared to CC turkeys in general. In conclusion, the phenotypic variation among different varieties of turkeys used in the

present study account for observed differences in the growth, reproductive parameters and plasma melatonin concentration suggesting that these differences could be used for selecting birds for future breeding programs.

3. In the DNA sequence of 24.6 kb including the *turClock* gene, 12 SNPs located in the introns 6, 9 and 16, were identified. The SNPs reported here were not in HWE ($P \leq 0.05$). D′ among the SNPs ranged from 0.05 to 1.00. The pairwise F_{ST} value ranged from 0.03 to 0.90. The haplogroups of the *turClock* gene were significantly associated with BW at 309 d of age, FCR for the periods of 34 - 68 d and 69 - 159 d, egg production and average egg weight ($P \leq 0.05$). Association among haplogroups in the *turClock* gene within each turkey variety showed that some haplogroups associated with some of growth and reproductive parameters suggesting that the differences within variety remain and would be valuable for further improvement of turkeys.

4. Seven SNPs, one each in the exon 18 and the intron 5, two in the exons 19 and three in the introns 6 were detected in the DNA region of 16.6 kb including the *turPeriod-3* gene. The SNPs detected in the exon 19 were non-synonymous, involved an amino acid change from methionine to threonine at 953aa while the other involves an amino acid change from serine to phenylalanine at 955aa positions. About 42 % the SNPs reported here were in HWE ($P \geq 0.05$). D′ among SNPs ranged from 0.03 to 1.00. The pairwise F_{ST} value estimated for the eight turkey varieties ranged from 0.01 to 0.43. The haplogroups of *turPeriod-3* gene were significantly associated with BW at 231 d of age, ADG for the period of 160 - 231 d of age, FCR for the periods of 69 - 159 d and 160 - 231 d, egg production, egg weight, semen quality traits and plasma

melatonin concentration ($P \leq 0.05$). In addition, some haplogroups in the *turPeriod-3* gene within turkey varieties associated with some of growth and reproductive parameters.

5. Seven SNPs including four and three in the introns 3 and 6 respectively were identified in the DNA region of 15.0 kb which included the *turCry1* gene. The SNPs reported here were not in HWE ($P \leq 0.05$). D′ among the SNPs ranged from 0.48 to 0.89. The pairwise F_{ST} value estimated for the eight turkey varieties ranged from 0.004 to 0.67. The haplogroups of *turCry1* gene were significantly associated with ADG for the period of 35 - 68 d, FCR for the periods of 160 - 231 d and 34 - 231 d, egg production and semen ejaculate volume ($P \leq 0.05$).

6. Fifteen SNPs, including two SNPs in the promoter region, five each in the exon 1 and the intron 1 and three in the intron 3, were identified in the DNA region of 16.6 kb that included the *turCry2* gene. Four of the exonic SNPs were non-synonymous, involved amino acid changes: leucine to proline, aspartic acid to asparagine, aspartic acid to glutamic acid and valine to glycine. The SNPs reported here were not in HWE ($P \leq 0.05$). Across all SNPs, D′ ranged from 0.01 to 1.00. The pairwise F_{ST} value estimated for the eight turkey varieties ranged from 0.01 to 0.30. The haplogroups of *turCry2* gene were significantly associated with BW at 34, 68 and 231 d of age, ADG for the period of 160 - 231 d, FCR for the period of 34 - 68 d, average egg weight ($P \leq 0.05$).

The practical significance of the current study is that information gathered from genetic and association analyses of the circadian clock genes would be useful for the breeder to select the

best birds at the early stage of their life according to the breeder's choice of interest. In the traditional breeding program, breeder has to wait until birds reach to sexual maturity which is more time consuming. However, in the present study, we tried to identify any association between haplogroups of circadian clock genes and performance traits. A total of 41 SNPs were identified from the four genes that screened and developed haplogroups. Of the haplogroups studied in the current study, it appeared that haplogroups of *turPeriod-3* and *turCry2* genes are associated with growth, reproductive parameters and plasma melatonin among some turkey varieties. This suggests that *turPeriod-3* and *turCry2* genes are important candidate genes for studying growth and reproduction of turkeys.

Future work:

1. In the present study, we scanned the different regions of the clock genes and developed the haplogroups based on the SNPs identified. Therefore, in future, specific investigation to cover the rest of the regions of the clock genes should be focused to detect other variants.
2. In this study, we only examined the role of *cis*-acting SNPs in circadian clock genes. However, further studies are needed to determine whether *trans*-acting factors are responsible for regulating the circadian genes.
3. We only selected four different circadian genes that are predominantly involved in the molecular mechanism of the circadian clock. The future research should be focused to examine the other genes that regulate the circadian clock.

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