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Association Of The Melatonin Receptor C Gene With Egg Production Traits In Local Iraqi Chicken

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ABSTRACT

melatonin receptors are G protein-coupled receptors (GPCR) that bind melatonin. Three types of melatonin receptors have been cloned. melatonin receptor subtype MTNRc has been identified in amphibians and birds. Previous research has shown that the three common melatonin receptors regulate physiological processes, including seasonal reproduction and ovarian physiology. However, whether or not any polymorphisms of the different melatonin receptor subtypes are associated with reproductive traits in chickens is not known. In this study, we performed candidate gene analysis to identify single-nucleotide polymorphisms (SNPs) in the MTNR1C gene. In local Iraqi Chicken population. SNP discovery was achieved by sequencing DNA samples.

PCR-SSCP/PCR-sequencing, and PCR-RFLP method were used to genotype the MTNRc gene, respectively. SPSS program 24 was used to estimate the statistical significance of association between genotypes at each locus and reproductive traits of chickens. In a sample of 200 chickens, polymorphisms (JQ249896: g.294G>A) were detected in the melatonin receptor MTNRc, statistically significant association ($P < 0.05$) was found between SNP (MTNRc SNP) and reproductive traits: egg number at 100 days of age after maturity (EN) after crossbreeding, Body weight at first egg (BWM) for both flocks before and after hybridization but before crossbreeding statistically significant association ($P < 0.05$) with First Egg weight (FEW) and Age Maturity (AM), we found an association between MTNRc gene and some egg production traits in local Iraqi chicken, that can be used for genetic breeding program as a marker assisted selection marker.

Keywords: Local Iraqi chicken, genetic polymorphism, Melatonin receptor c, egg production traits, Local chicken, crossbreeding, breeding poultry and improvement

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INTRODUCTION

Iraqi local chickens are valuable genetic resources due to their adaptability to harsh conditions when raised in rural area or when reared in outer system as free-range chickens, these chickens responded well to improve their environment conditions, especially, nutrition and exhibited improvement in body weight at sexual maturity and egg weight (1). In addition, it was found that they classified as a good performance for egg production (2), because of breeding program for local chickens in developing countries are still out of competition with commercial breeding company that has access to technology advantages and economics of scale (3). It was strongly needed to establish breeding programs that allows improving performance of local chickens (4). Iraqi chicken characterized by high viability to adapt to the prevailing environmental conditions such as their heat resistance and resistance to some of the endemic diseases (2). Also characterized by their efficiency to moderate productivity and their needed a simple diet, especially in the free-range system and rural breeding but the egg production chain has to be short and variable range from one series to another for the same individual (5).

Egg production is a polygenic inheritance trait with low to moderate heritability, which depends on the period-involved (6). Molecular markers were used to map QTLs related to chicken growth and reproduction such as body weight (BW), Egg number (EN), and Age at First Egg (AFE) in the past decade (7,8). So, the using of molecular marker in breeding has a huge advantage, for the time being, molecular markers are widely used in poultry breeding (9).

Melatonin (N-acetyl-5-methoxytryptamine) (ML) an indole hormone produced from serotonin in the pineal gland. It is an important hormone that is synthesized mainly in the pineal gland, and has a profound effect on serval physiology process including circadian rhythm and reproduction through its special receptors in birds (10). Three melatonin receptor subtypes, MTNR1A (alias MT1, Mel1a), MTNR1B (alias MT2, Mel1b), and MTNR1C (alias Mel1c), which belong to the superfamily of G protein-coupled receptors, have been cloned in birds (11).

In birds, Functionally, melatonin stimulates several receptors and signaling passageways, especially on theca and granulosa cells, in addition to influencing intermediate effects through binding to G-protein coupled receptors (12). Recently, ovarian melatonin receptor subtypes were characterized in chicken, and their expression showed a direct contribution to hen reproduction processes and were thus marked as potential candidate genes for QTLs in chicken reproduction (13).

Birds have a pineal gland that varies in size, shape and localization (14). In the pineal gland, ML, which can be modulated by light, darkness and temperature changes, is produced (15). However, ML secretion may, be influenced by additional factors. Heat induced by high ambient temperature and high relative humidity during hot dry season causes heat stress in birds. Animal studies have shown that melatonin has a thermoregulatory role. Furthermore, ML has a crucial role in circadian thermoregulatory adjustments of body temperature (16).

The production of ML in poultry is controlled by three main mechanisms: direct light reception, endogenous generator and noradrenergic transmission (17). There are 3 ML receptor subtypes in chickens; Mel1A, Mel1B and Mel1C. Each of these receptors has different affinities for ML (18). ML receptor subtypes are found at different concentrations and quantities in different parts of the

body (19). Mel1C is present in the ovary and in the crustacean affects the crustal thickness of the egg (13).
In poultry, the synthesis of ML is controlled by genes that produce molecular oscillations under the influence of the negative feedback mechanism, so ML has the ability to convert environmental information into appropriate endocrine signals (20). For this reason, ML allows the physiology, metabolism and behavior of animals to be synchronized with optimum environmental conditions (21), it was synchronized with light changes, plays an essential role in the regulation of neuroendocrine processes and in the biology of all cells, in addition to there affects on growth and health status and the concentrations of other hormones in poultry (22).
In chicken, melatonin receptor subtypes were identified in ovaries, suggesting that melatonin directly affects ovarian function through activating of multiple receptors (23). the relationship between melatonin receptor subtypes and egg production traits in different species made melatonin receptor genes potential candidate genes for Quantitative Trait Loci QTL (24), thus, The objectives of the present study were as follows: to identify a polymorphism of the chicken MTNRc gene in an F2 resource population crossed by ISA Brawn males and Local Iraqi females, to develop a PCR-RFLP method for genotyping, and to evaluate the association between the gene polymorphism and egg production traits.

The paper is derived from the doctoral thesis of the first researcher

MATERIALS AND METHODS

Hybridized local Iraqi Resource Flock

The cross between ISA Brawn males and Local Iraqi Flock females and we obtained 100 hybridized local Iraqi females. These birds were raised in the same chicken house and fed the same food for the duration of the experiment. Experiment were conducted in poultry farm in the Ministry of Science and Technology/ Agricultural Research Directorate / Animal resources and fisher's center/ poultry department and DNA laboratory of Biology department college of Science/ Babylon University during the period from 20/2/2019 until 15/8/2020. For each individual were measured: First egg weight, the age at first egg, and Body weight at first egg and total egg number after 100 days breeding period as egg production traits.

Sequence Analysis

To detect nonsynonymous substitutions of this gene, we attempted to determine the nucleotide-coding sequences of the *MTNRc* gene of females by using genomic DNA. Genomic DNA was isolated from blood samples by manual procedure method from peripheral blood leukocytes by the Distal Water based method (25), and the DNA concentration was adjusted to 20 ng/µL. The sequences of MTNRc were determined by PCR-RFLP and PCR-SSCP-sequencing with 2 primers sets ((F): GGTGTATCCGTATCCTCTAA (R): GACAGTGGGACAATGAAGT, (F): GGACTATGGGAAACATTCACTGC (R): TACTCACCGTCTGGGCAAAG) respectively which were designed based on the chicken *MTNRc* information and gene sequences on chromosome 4 in GenBank (accession no. NC_006091and JQ249896.1, respectively). The PCR was performed in a total volume of 50 µL, The PCR conditions were as follows: 94°C for 10 min followed by 30 cycles of 94°C for 30 s, 52-64°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. The SSCP product was sequenced with forward primer. The sequences of both gene fragments analyzed were aligned by multiple sequence alignment program according to waterman (2020), with the sequences published in the Gen Bank database taken as a reference to identify the polymorphisms.

PCR-RFLP and PCR-SSCP Analysis

Genomic DNA was extracted from blood samples of the local Iraqi and hybridized flocks by the same method as described earlier. An MboI recognition site was created by the MTNRc SNP. The PCR products of this locus were digested with the restriction enzyme MboI (New England BioLabs 2019) at 37°C for 20 minuets, ran on a 2% agarose gel, and stained with red safe to detect the SNP by cleavage of the MTNR1C amplicon. PCR-SSCP analysis of the MTNRc SNP was performed according to (26).

Traits and statistical analysis

Associations between genotype and reproductive traits were assessed using one–way ANOVA, were used to find the association between the categorical variables, P value ($P \leq 0.05$) was considered statistically significant. (SPSS, Statistical Analysis System, Version 24, 2019).
Significance of the least squares means was tested with the Duncan's Multiple Range test. Pearson's chi-square test was used to check for Hardy-Weinberg equilibrium of SNPs discovered in the sample population.

RESULTS

The present study includes (200) samples which grouped as local Iraqi chickens flock (IF) (100) sample and local Iraqi chickens flock after hybridization (HIF) (100) sample. The genetic positions of this study were detected genetically in order to investigate if there are any genetic relationship for these genetic loci with Egg production trait. The study of this gene (located at chromosome 4), which includes two genetic sites one of them selected from previous reference (13) evaluated by PCR-RFLP technology, and another site designed by aid with NCBI primer BLAST National Central Bank Information (NCBI) online site, which Positioned in coded region (exon 2) studied by PCR-SSCP and Sequence technology.The genotyping region using PCR-RFLP method was investigated after amplified the target site. The genomic DNA of local Iraqi chickens (IF) before and after hybridization (HIF) were amplified and accomplished by the Thermo-cycler device under the optimal conditions. The results revealed that the presence a single band (372 bp) of the target sequence of MTNRc genetic site in agarose gel for both flocks' study (Fig. 1).

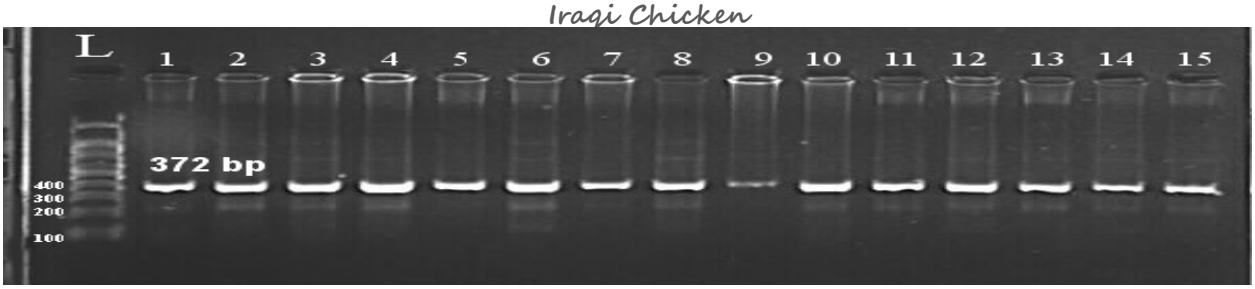
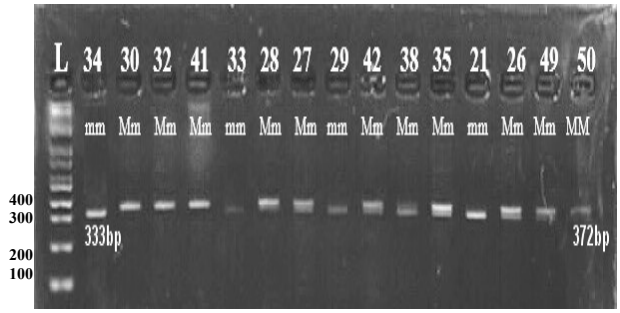


Figure (1): Agarose gel electrophoresis of MTNRc genetic locus. L; DNA size marker, lane (1-8) IF: Iraqi local chicken, lane (9-15) HIF: Hybridized local Iraqi chicken. After the amplification of the target site, the PCR products of the *MTNRc* target sequences were digested with *MboI* (5' G ↓ ATC 3') restriction enzyme to detect the JQ24989: g294 m>M SNP in 5'-flanking region of *MTNRc* gene (Fig.2)

Conformational polymorphism of the MTNRc genetic locus using PCR-RFLP method. The results showed that the presence of three different allelotypes of MTNRc (JQ24989: g294 SNP) were homozygous (MM) and (mm) and heterozygous (Mm) which occurred by single nucleotide substitution (M→ m) at MboI recognition site.



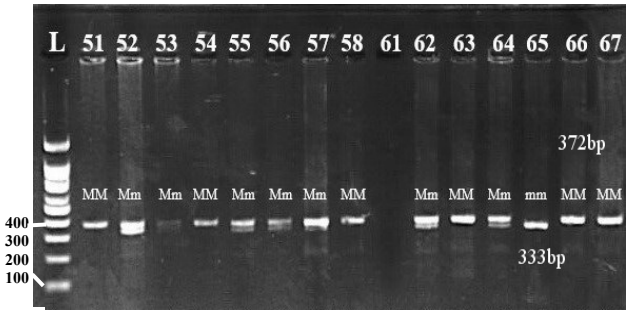


Figure (2): Agarose gel electrophoresis of MTNRc gene. L; DNA size marker, lane 34 to lane 50 RFLP- PCR (Iraqi chickens' sample), lane 51 to lane 67 RFLP- PCR (Hybridized Iraqi chickens' sample).

It is evident from Table (1) (A) that the percentages of phenotypic occurrences of the MTNRc gene in the IF before hybridization showed high significant differences ($p<0.05$) for the different genotypes, which amounted to 23%, 61% and 16% of the genotypes are MM, Mm and mm respectively, meaning that spread pronounced the heterozygous Mm genotype, followed by found the pure homozygous MM and mm genotype, but regarding the flock after hybridization, the percentages of the phenotypes of the same genetic site were as follows:

(A)

Genotype	Iraqi local chickens IF		hybridized flock local chickens HIF	
	number	Percentage (%)	number	Percentage (%)
MM	23	23%	15	15%
Mm	61	61%	63	63%
mm	16	16%	22	22%
Total	100	100%	100	100%
Chi-Square Test	35.180		40.340	
significant differences (P≤ 0.05)				

According to the results in Table (1) (B), we found that the allele frequency 0.54 for M allele and 0.47 for m allele in the hens of IF flock and in the flock after crossbreeding (HIF) found that the frequency 0.47 for M allele and 0.54 for m allele.

Table (2) shows polymorphism relationship of (MTNRc) gene at IF and HIF flocks' female local chickens for First 15% , 63 and 22% for the MM, Mm and mm genotypes, respectively, meaning that there is still clear prevalence for heterozygous Mm genotype and a raise of the homozygous individuals carrying the mm genotype, with descent of the MM genotype.

Table (1): (A) The number and the percentage for RFLP genotype melatonin receptor c
(MTNRc) gene at Iraqi and hybridized flock female local chickens.
(B) Allele frequency for RFLP genotype melatonin receptor c (MTNRc) gene at Iraqi and hybridized flock female local chickens.

Allele	Frequency	
	IF	HIF
P=M	0.54	0.47
q=m	0.47	0.54
Total	1(100%)	

egg weight (FEW), average of age maturity (AM), body weight at maturity, egg number for 100 days (AN).

And the results found that there is a significant difference by the mean weight of the first egg (FEW) 34.14 gm., age at sexual maturity (AM) 146.13 day and body weight at sexual maturity (BWM)1471.04 gm. in IF flock for the genotype (MM), but no significant differences among genotypes were identified at the number of egg production over a period of 100 days (EN).

In HIF flock we recorded significantly the highest value ($P<0.05$) (1648.36) gm and 61.5 egg for BWM and EN respectively by (mm) genotype, while there is no significant variation for other genotypes after hybridization.

Table 2: polymorphism relationship of melatonin receptor c (MTNRc) gene at Iraqi and hybridized flock female local chickens for First egg weight (FEW), average of age maturity (AM), body weight at maturity, egg number for 100 days (AN).

Trait	local Iraqi chickens			hybridized flock local chickens		
	Genotype (M± SE)			Genotype (M± SE)		
	MM	Mm	mm	MM	Mm	mm
First egg weight (FEW)	34.14±0.93 a	32.68±0.513 ab	31.23±0.902 b	46.28±1.005 a	46.72±0.997 a	42.57±1.387 a
average of age maturity (AM)	146.13±2.71 a	143.78±1.541 a	126±11.389 b	150.93±1.725 a	153.07±0.783 a	154.86±1.028 a
egg number for 100 days (AN)	45.78±3.462 a	53.03±2.066 a	46.18±2.633 a	40.2±3.552 b	55.74±2.614 a	61.5±4.133 a

Different letters per class indicates significant differences (P≤ 0.05), M: Mean, SE: Standard error .Within other locus, which designed by the aid of NCBI primer BLAST. The genotyping of this region using PCR-SSCP method was investigated after amplified the target site and sequence analysis for this genetic site. The results revealed that the presence a single band (276 bp) of the target sequence of Melatonin receptor c in agarose gel for both flocks' study (Fig. 3).

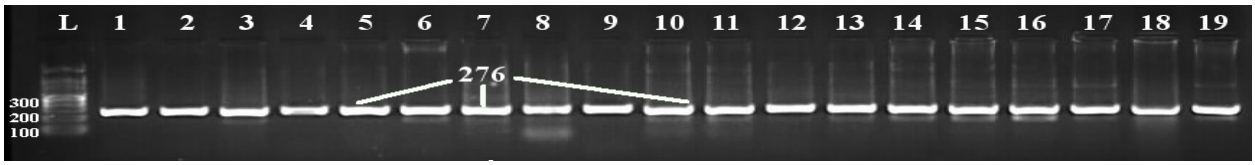


Figure (3): Agarose gel electrophoresis of Melatonin receptor c. L; DNA size marker, lane (1-10) IF: Iraqi local chicken, lane (11-19) HIF: Hybridized local Iraqi chicken. After the amplification of the target site, conformational polymorphism of the Melatonin receptor c using PCR-SSCP method. The results appeared that the presence of four different haplotype patterns named according to the number of bands were 4-bands, 3-bands, 2-bands and 1 band as shown in figure (4) for IF flock and HIF of PCR-SSCP gel electrophoresis. DNA bands (ssDNA) which located at the upper portion of the gel and the double stranded (dsDNA), which occupy the lower portion of the gel were observed. The variation of ssDNA in SSCP gels is relied to identify the genetic pattern of each amplified.

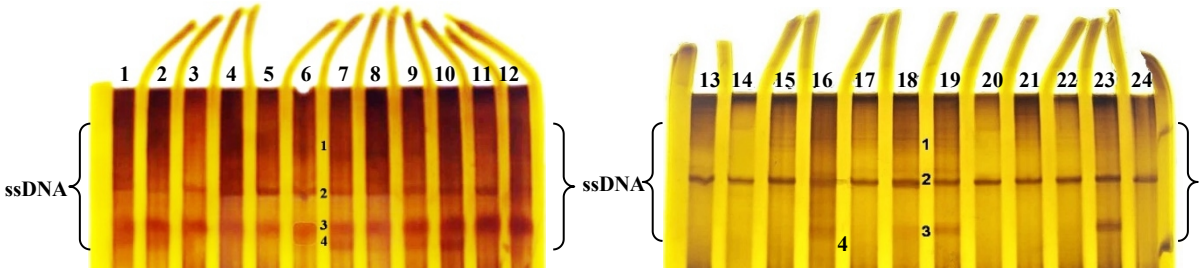


Figure (4): Polyacrylamide electrophoresis of Melatonin receptor c. Lane 1 to lane 12 IF chickens, Lane 13 to lane 24 HIF chickens. The PCR-SSCP of the Melatonin receptor c was performed to distinguish three (genotype) individuals 4-bands and 3-bands (heterozygous), 2-bands (homozygous) and 1-band (homozygous) in the Iraqi local chickens' flock (IF) samples and in hybridized Iraqi local chickens' flock (HIF) depending on the presence or absence the haplotype (Fig. 4), as we will see later when linking the genotypes with the phenotypic traits. The conformational variation among these haplotypes may be due to the presence of SNPs that caused a change in GC% when the single nucleotide substitution occurs like A→ G or C→A .... etc. that change the melting point (Tm) of the DNA fragments.All polymorphic sites identified by alignment of the sequences generated in the present study with the Jungle fowl (JF) genome sequence. Within 13 SNPs before crossbreeding (IF) but 45 SNPs after crossbreeding (HIF) by exons 2. The position of each variant is based on the May 2006 chicken genome build (WUGSC 2.1/galGal3) at UCSC (http:// genome.ucsc.edu). In addition, their exon locations affected exonic SNPs according to Human Genome Variation Society (HGVS) nomenclature standards. The distribution among the IF and HIF flocks studied of the polymorphic sites identified by sequencing, polymorphic sites were identified in study flocks (LL, LE, EE) genotypes indicating that the polymorphic sites identified in the flocks probably represent a large fraction of variation most common in local Iraqi chickens. However, EE and LL genotypes had 12, 13 unique variants SNPs respectively, in addition to LE genotype had 38 unique variants SNPs.

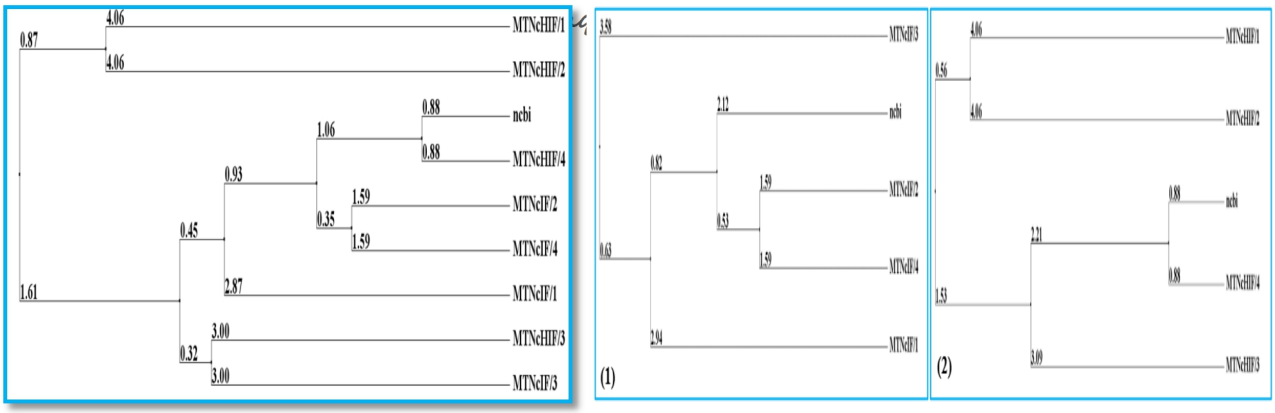


Figure (5): (A) phylogenetic tree for (1) IF and (2) HIF flocks.
(B) phylogenetic tree for both flocks' study (IF and HIF).

When aligning the results of the two study flocks with those of Jungle Fowl (JF) by Multiple Sequence Alignment (https://www.ebi.ac.uk/Tools/msa/clustalo/), it was found that the flock before crossbreeding was similar to Jungle Fowl chickens with an identical percentage of 92%, and this is evidence that local Iraqi chickens are wild type, non-hybrid and the phylogenetic tree illustrated what has been mentioned above figure (5, B, 1) and when crossed local Iraqi flock hen (IF) with males of the egg-laying breed (ISA Brawn), the results showed that the hybridized Iraqi flock (HIF) began to move away from JF with a match rate that decreased to 79%, Figure (5, *B, 2*), but despite this divergence Figure (5, A), the result reflected positively on egg quality and production traits and This will be clarified by the correlation between phenotypic and genetic characteristics later. Through the information of the sequences and correlation between the phenotypes with the genotypes.There were only 13 SNPs in the pre-crossbreeding (IF) flock, but 45 SNPs were identified after crossbreeding (HIF), 7 SNPs were preserved as before crossbreeding and two sites 139 and 140 were substituted by other nucleotide (139T>deletion, 140C>G) between the two flocks before and after crossbreeding.

Table 3: (A) The number and the percentage for sequence genotype melatonin receptor c (MTNRc) gene at Iraqi and hybridized flock female local chickens
(B): Allele frequency for Sequence genotype melatonin receptor c (MTNRc) gene at Iraqi and hybridized flock female local chickens.

(A)

Allele	Frequency	
	IF	HIF
P=L	0.72	0.33
q=E	0.29	0.67
Total	1	

(B)

Genotype	IF		HIF	
	number	Percentage (%)	number	Percentage (%)
LL	55	55%	30	30%
LE	33	33%	6	6%
EE	12	12%	64	64%
Total	100	100%	100	100%
Chi-Square Test	27.74		50.96	
	significant differences (P≤ 0.01)			

Table (4) shows polymorphism relationship of (MTNRc) gene at IF and HIF flocks' female local chickens for First egg weight (FEW), average of age maturity (AM), egg number for 100 days (AN), and the results found that there are no significant differences between the genotypes (LL, LE, EE) appeared when comparing with these parameters for two flocks IF and HIF flocks.When the two flocks were compared for each genotype before and after the crossbreed (Table 5), we found there is a significant difference with both genotypes (LL, EE) with First egg weight (FEW) (gm.) but the superior higher for the HIF flock over the IF flock for genotypes, LL genotype with average of age maturity (AM) (day) but the superior higher for the HIF flock over the IF flock for genotypes and LE genotype appeared with egg number for 100 days (AN) but the superior higher for the HIF flock over the IF flock.

Table 4: polymorphism relationship of melatonin receptor c (MTNRc) gene at Iraqi and hybridized flock female local chickens for First egg weight (FEW), average of age maturity (AM), body weight at maturity, egg number for 100 days (AN).

Trait	local Iraqi chickens	hybridized flock local chickens
	Genotype (M± SE)	Genotype (M± SE)

	LL	LE	EE	LL	LE	EE
(FEW)	32.09±0.531 a	34.05±0.810 a	32.5±0.713 a	40.6±1.529 a	37.73±4.641 a	41.05±1.196 a
(AM)	137±3.528 a	146.78±2.277 a	147.41±5.005 a	154.33±1.25 a	152.66±2.155 a	152.64±0.718 a
(AN)	50.94±2.119 a	47.66±2.751 a	54.33±4.586 a	53.53±3.73 a	64.3±2.660 a	54.312±2.66 a

Different letters per class indicates significant differences (P≤ 0.05), M: Mean, SE: Standard error

Table 5: Comparison between Iraqi and hybridized flock female local chickens for each sequenced genotype of melatonin receptor c (MTNRc) gene for First egg weight (FEW), average of age maturity (AM), egg number for 100 days (AN).

Trait	Flock	Genotype (M± SE)					
		LL	Sig.	LE	Sig.	EE	Sig.
(FEW)	IF	32.09±0.531	0.000	34.05±0.81	0.468	32.5±0.713	0.000
	HIF	40.6±1.529		37.73±4.641		41.05±1.196	
(AM)	IF	137±3.528	0.000	146.7±2.277	0.076	147.4±5.005	0.323
	HIF	154.3±1.254		152.6±2.155		152.6±0.718	
(AN)	IF	50.9±2.119	0.549	47.6±2.751	0.001	54.3±4.586	0.997
	HIF	53.5±3.723		64.3±2.660		54.3±2.66	

significant differences (P≤ 0.05), M: Mean, SE: Standard error

DISCUSSION

In the poultry industry, egg production is an important economic trait (27). our results similarly recent studies Li *et al* and Vu *et al* (13; 28) which found Birds with the AG (Mm at our study) genotype for the MTNRc SNP had shorter AM than those of AA (MM) genotype for the MTNRc SNP, lacking the MTNRc MboI restriction site, exhibited statistically significantly higher WFE (P < 0.05), they exhibited statistically significantly lower EN values (P < 0.01) than those with both the GG (mm) and AG (Mm) genotypes that were homozygous and heterozygous for the restriction site, respectively. these findings show in Table 1 an Essential rise in the percentage of m allele between the two flocks of the sample, which reflected on the productive and physical features studied for the PCR/RFLP genetic position by total egg number after 100 days after maturity, demonstrating that hens with the mm genotype produce their first egg at a lower age first egg (AFE) and produce more eggs at 100 days of age after maturity (EN) than those with the MM, and Mm genotypes (P < 0.05). This apparent production advantage to the mm genotype at MTNRc having a lower AFE and higher EN is consistent with the excess of heterozygous genotypes at this locus compared to equilibrium expectations, illustrated in Table 4, suggesting strong balancing selection. In contrast, the effect of allele E on EN is additive and may reflect the influence of positive selection. and the other significant production trait, age at first egg (AFE), an important indicator for sexual maturation in female chickens, is controlled by polygenes (29).

Due to crossbreeding, there were additional 36 SNPs, some of which reflected positively on the resulting flock by raising the quality and egg production traits, and others showed no significant differences between the phenotypes studied. The positive repercussions were as **disappearance SNPs in the flock after crossbreeding (HIF),** one of the positive effects of crossbreeding was the absence of a number of SNPs (54G>A, 252,258T>G, 263C>deletion) in order to return the flock to conformity with the JF by 100 per cent in those sites, which may have been one of the reasons for the decline in flock production before crossbreeding relative to the flock production ratio after crossbreeding, **the emergence of new SNPs in the resulting flock,** we noticed addition of new SNPs as a result of crossbreeding in the resulting flock (HIF), some of them didn't appeared any significant difference with the studied traits before and after crossbreeding, and others showed a significant difference on some productive traits for some breeding periods, these positive additional SNPs (241G>C, 225-226T>C, 156T>A, 145T>C, G) . It is evident from Table (3, A) that the percentages of phenotypic occurrences of the MTNRc gene in the IF before hybridization showed high significant differences (p<0.05) for the different genotypes, which amounted to 55%, 33% and 12% of the genotypes are LL, LE and EE respectively, meaning that there is a clear prevalence of the homozygous formations carrying the LL genotype, followed by heterozygous LE genotype and the lowest ratio for homozygous EE genotype. In the flock after hybridization, the percentages of the phenotypes of the same genetic site were as follows: 30%, 6% and 64% for the LL, LE and EE genotypes, respectively, meaning that there is a clear prevalence of the pure individuals carrying the EE genotype and an obvious drop of homozygous LL and scarcity of appearance heterozygous individuals carrying the LE genotype and by following the results in Table (3, B), we found that the frequency ratios for alleles were 0.72 for L haplotype allele and 0.29 for E haplotype allele in the hens of IF flock and in the flock after crossbreeding found

that the ratios were 0.33 for L haplotype allele and 0.67 for E haplotype allele. the findings show an essential rise in the percentage of E haplotype among the two flocks of the sample, which reflected in the productive and physical features studied for the genetic position as saw above.So when comparing genetic and phenotypic between IF and ,HIF for genetic loci of MTNRc studied to determine the success rate of crossbreeding in improving egg production and quality characteristics, it was found that there was a substantial difference ($p<0.05$) for the flock after crossbreeding for all the egg production characteristics studied, which implies hybridization success significantly, It also means that MTNRc gene could be used as a possible marker in Marker Assisted Selection (MAS) and genomic selection programs.

CONCLUSIONS

Due to crossbreeding, there were additional 36 SNPs with MTNRc gene sequencing, some of which reflected positively on the resulting flock by raising the quality and egg production traits, and others showed no significant differences between the phenotypes studied, The two flocks are responsive to the Hardy Weinberg equilibrium law before and after crossbreeding for MTNRc genetic location by PCR-RFLP, there was a substantial difference ($p<0.05$) for the flock after crossbreeding for all the egg production characteristics studied, which implies hybridization success significantly and finally MTNRc gene could be used as a possible marker in Marker Assisted Selection (MAS) and genomic selection programs.

Recommendations

Inbreeding for the Hybridization Iraqi Flock (HIF), Local Iraqi chickens (IF) and Hybridized local Iraqi chickens (HIF) should be used in main and reciprocal crossbreeding respectively with Isa Brown chicken to improve total quality and egg production traits and using the phenotypic variation of genotypes in the process of genetic improvement through the selection of genotypes that have a beneficial impact on economic traits for egg production trait

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